

**EFFECTS OF AGE ON KNEE ACTIVATION CHARACTERISTICS
DURING WEIGHT BEARING AND DIRECTIONAL LOADING**

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List of Acronyms

3D	Three-dimensional	MVIC	Maximum voluntary isometric contraction
Φ	Mean direction of muscle activation	OA	Osteoarthritis
ACL	Anterior cruciate ligament	UOMAM	University of Ottawa motion analysis model
ACL-D	Anterior cruciate ligament deficient	ANOVA	Analysis of variance
BF	Biceps femoris	MANOVA	Multiple analysis of variance
BMI	Body mass index	QUADS	Summed quadriceps muscle activation
CI	Coactivation Index	RF	Rectus femoris
EMG	Electromyography	RL	Resultant load
EMGi	Electromyography vector	SD	Standard deviation
EMG_{Max}	Maximum electromyography magnitude	SENIAM	Surface Electromyography for the Non-Invasive Assessment of Muscles
GASTROCS	Summed gastrocnemius muscle activation	SI	Specificity Index
HAMS	Summed hamstrings muscle activation	ST	Semitendinosus
LG	Lateral gastrocnemius	TFL	Tensor fascia lata
M	Mean	VL	Vastuslateralis
MG	Medial gastrocnemius	VM	Vastusmedialis
MU	Motor unit	X_{EMG}	Mean magnitude of muscle activation

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General Abstract

We developed a novel approach that requires subjects to produce and finely tune ground reaction forces (GRFs) while standing. Using this method we were able to identify specific contributions of individual muscles and how these contributions change with the effects of age. One of the aims of this investigation was to determine whether electromyographic data in our findings was due to random muscle activation or representative of a neuromuscular control strategy. Ten healthy young adults (5 male, 5 female) with their dominant foot fixed within a boot mounted to a force platform participated twice in a target matching protocol, requiring subjects to control both the direction and magnitude of GRF along the horizontal plane while maintaining constant inferior-superior loads of 50% body-weight. Subjects were asked to manoeuvre a cursor with their dominant leg to match a series of targets projected on a screen. Targets appeared at random one at-a-time, separated by 30° around a circular trajectory. Subjects applied loads to the force platform in various horizontal directions to move the cursor while also controlling body weight. A successful target match required subjects to maintain 50% body weight and 30% of their peak horizontal load for one second. Electromyography (EMG) of eight muscles that cross the knee joint, ground reaction forces, and kinematic data were recorded for each successful match. EMG was normalized to percent maximum voluntary isometric contractions collected on an isokinetic dynamometer. Each target matching session was separated by two-three days. A random model, single measures intra-class correlation analysed the reliability for both test-retest and intra-day results, in addition to intersubject reliability. We observed

moderate to high ICC values (0.60 – 0.993) for most muscles in most directions, indicating low within-subject variance. In addition, moderate to high between-subject reliability was observed in all eight muscle activation profiles, indicating subjects used similar neuromuscular control strategies to achieve the desired GRFs. Our findings support that groups who have undergone the same number of testing sessions can be compared, and that a single testing session is all that is required to compare neuromuscular control strategies used by a group to achieve target locations.

The second aim of this investigation was to evaluate age related differences in neuromuscular control about the knee joint using our target match protocol. Thirty-three healthy adults (17 younger 24 years ± 2 , 16 older 59 years ± 5), completed the same protocol evaluated above. The mean magnitude of muscle activity, specificity index, and mean direction of muscle activity were calculated in each target direction. Older adults presented with significantly lower strength in knee flexion and extension, hip abduction, and ankle plantar flexion. Significantly ($p < 0.05$) higher mean activation magnitudes in the rectus femoris, vastus lateralis, vastus medialis, biceps femoris, semitendinosus, medial gastrocnemius, and tensor fascia lata were also observed. Intraclass correlations (ICC) magnitudes indicate the percentage of global variance that can be explained by within subject and between trial variability. Muscle activation patterns were found to be similar in all muscles ($ICC \leq 0.82$). Similar patterns are supported by non-significant differences in mean direction of activation and muscle activation specificity. These results indicated that healthy older adults utilise different activation magnitudes for stabilising the knee while maintain similar muscle activation synergies in all muscles to younger adults.

Introduction

Given increased life expectancy and the prevalence of joint disorders, amongst this population (Badley, 2005). Continued investigation into age related changes to joint health is of crucial importance to our understanding of healthy ageing. Several investigations have thus been conducted into how joint and tissue biomechanics change with age, revealing a marked reduction in muscle strength (Hakkinen et al., 1998; Hortobagyi, Mizelle, Beam, & DeVita, 2003; Larsson, Grimby, & Karlsson, 1979; Lauretani et al., 2003; Rudolph, Schmitt, & Lewek, 2007) and neuromuscular control (DeVita & Hortobagyi, 2000; Hortobagyi & DeVita, 2000; Hortobagyi et al., 2005; Macaluso et al., 2002).

Come middle-age it is well established that there is a marked reduction of muscle strength (Vandervoort & McComas, 1986) that is not proportional to its change in cross sectional area. This reduction in strength, particularly of the quadriceps, appears to be unavoidable (Brooks & Faulkner, 1994; Frontera et al., 2000; Sipila & Suominen, 1993; Trappe, 2007) and has been suggested as a possible causal factor in the development of OA, since its impaired function would largely affect the way one stabilizes and loads the knee (Herzog & Longino, 2007; Rehan Youssef et al., 2009; Solomonow et al., 1987). The magnitude of muscle strength loss, however, is similar in adults regardless of whether one develops OA or not (Rudolph et al., 2007), suggesting an alternate paradigm is needed to explain why some older individuals develop OA while others do not. The ability to produce specific force in a controlled fashion through finely tuned muscle activity (neuromuscular control) is imperative to joint stability (Krishnan, Huston et al. 2008) however healthy older adults present with significantly reduced neuromuscular

control compared to younger adults. Rudolph et al., (2007) report that despite reductions in neuromuscular control in both OA symptomatic and asymptomatic subjects, only those with OA present with a “knee stiffening strategy” (increase coactivation of antagonist muscles) while older adults without OA present similar coactivation as healthy young and middle age controls. It may be that, for example maintenance of neuromuscular strategies despite muscle weakness could help modulate knee joint mechanics in such a way as to preserve the articulating surfaces of the knee. Conversely mal-adaption could be representative of reduced neuromuscular control and result in inappropriate joint mechanics and the initiation of OA (Herzog et al. 2007; Youssef et al. 2009). Contrary to Rudolph et al., (2007), others have found that even older healthy adults utilize similar stiffening strategies as older adults with OA (DeVita & Hortobagyi, 2000; Earles et al., 2001; Enoka et al., 2003; Hortobagyi & DeVita, 2000; Hortobagyi et al., 2004; Hortobagyi et al., 2003; Hortobagyi et al., 2005; Macaluso et al., 2002). The coactivation ratio of antagonistic muscle pairs utilised by healthy older adults were lower however, than those with OA and more consistent with younger healthy adults. With observed differences in percent activation of muscle, strength and neuromuscular control between healthy older adults and those who develop OA, it is plausible to suggest that age associated neuromuscular alterations that affect joint loading could be identified and used to identify those at risk of developing OA.

To date age associated changes in joint biomechanics have yet to be identified as either the precursor to, or the result of, OA. This makes it difficult to identify altered knee stabilisation strategies as either inappropriate neuromuscular adaptations or adaptations necessary for the individual to ensure mobility. Electromyography (EMG) driven

investigations into joint stabilisation during functional task assessments (i.e. walking, jumping, ascending, and descending) have already been conducted, where EMG recordings were analyzed to determine the role of muscles during a desired functional task (Hortobagyi & DeVita, 2000; Rudolph et al., 2007). Dynamic tasks however present a series of difficulties when trying to analyze joint stabilization, as it is difficult to separate biomechanical and neuromuscular contributions to movement. With this being the case, neuromuscular control strategies may be hidden by compensatory adaptations (Williams et al., 2003). The effect of which can enter a series of confounding variables including biomechanical factors, varying loads, and reciprocal effects of the adjacent limb (Williams, Barrance, Snyder-Mackler, Axe, & Buchanan, 2003). In this respect using a “simplified” force matching protocol under static conditions could be more appropriate for evaluating neuromuscular control strategies in respect to joint stability. The validity of this approach has been supported and used by several studies (Buchanan, Almdale, Lewis, & Rymer, 1986; Buchanan & Lloyd, 1997; Lloyd & Buchanan, 1996, 2001; Williams et al., 2003; Hortobagyi et al., 2004; Hortobagyi et al., 2001; Manini et al., 2005). Limiting dynamic movement provides a potential solution for controlling these confounding variables in respect to changes in joint kinematics and neuromuscular control. To date, however, the aforementioned isometric investigations into neuromuscular contributions to joint stability have only been conducted under open kinetic chain conditions, such that the foot is not fixed and segment is unloaded. However muscle recruitment and activation patterns about the knee show significant differences between seated and weight bearing conditions (Escamilla et al., 1998). As such, we believe that through a weight bearing, loading condition more applicable insight into the

contributions of the neuromuscular system to joint stability will be observed. With this insight, the measurement of neuromuscular control could thus be used to identify a subset population of older adults who present with altered joint biomechanics, subjecting them to an increased risk of developing a joint degenerative disorder. The classification of these changes in neuromuscular strategy supports the shift away from the traditional classification of quadriceps dysfunction and weakness to the identification of novel control properties of the quadriceps and other knee stabilising muscles amongst the aging and patients with OA (Hortobagyi et al., 2004).

Research objectives and hypotheses:

While age associated neuromuscular dysfunction and declining muscle strength have been suggested as contributors to altered joint loading and subsequent articular cartilage degradation (Herzog & Longino, 2007; Rehan Youssef, Longino, Seerattan, Leonard, & Herzog, 2009) No investigator has yet determined the level of interaction between these age-related declines, despite their dependence on one another to function properly over a lifetime (Delbono, 2003).. We believe that further investigation into healthy older adult's neuromuscular adaptations to muscle weakness will provide us with a foundation for understanding how individuals avoid developing OA despite age associated muscle weakness. Given that biomechanical factors, varying loads, reciprocal effects of the adjacent limb etc., (Williams, Barrance, Snyder-Mackler, Axe, & Buchanan, 2003) all present potential confounding variables when trying to investigate neuromuscular joint stabilisation strategies , a loaded static approach was used in an effort to control the influence of these parameters.

The purpose of this study is divided into two objectives: first objective aim is to validate and verify the reliability of a load bearing, force matching protocol designed to generate multidirectional ground reaction forces. The second objective is to examine age related differences in neuromuscular activity about the knee joint of populations of healthy young and older individuals performing the above force matching task. The above observations led us to postulate the following hypothesis:

1. Older healthy adults will present with greater muscle weakness and higher mean magnitude of muscle activation¹ compared to young healthy adults across all muscles.
2. Older healthy adults will present with significantly different muscle activation patterns (mean direction of muscle action², direction of skewness³) with lower specificity indices⁴ compared to young healthy adults.
3. Older adults will have significantly greater coactivations of the quadriceps and ham strings muscles (defined by a coactivation index) compared to young healthy adults.

¹The average of all the normalised EMG amplitudes at every target location.

² The mean direction of activation is the arctan of all EMG vectors summed x and y components.

³ The probability (p) of the resultant vector magnitude being greater in any of the 12 target directions.

⁴ A value describing that variability of muscle activation about its mean direction of muscle activation. Values are scalar between 0 (active in all direction) and 1 (highly specific in one direction)

Relevancy:

Knee joint instability is a common phenomenon associated with older adults and individuals with OA, speculated to be the result of reduced and altered muscular forces acting across the joint (Rudolph et al., 2007; Sharma et al., 1999). Compensation for muscle weakness in the prevention of joint instability may then be a major player in

maintenance of joint health. Observing healthy neuromuscular adaptations to age-associated reductions in muscle weakness and using them as a baseline to allow comparison with unhealthy populations could provide insight into how one may avoid developing a joint degenerative disorder by identifying differences in the way the joint is stabilised. It may be that these differences modulate knee joint mechanics in such a way as to help preserve the articulating surfaces of the knee. With aging considered as the prevailing causal factor in the development of idiopathic OA, we must first identify age related neuromuscular variations of healthy older adults in response to multi directional loading conditions.

Several studies have been conducted investigating knee stabilization strategies during force target matching tasks using highly controlled isometric joint loading strategies (Buchanan et al., 1986; Buchanan & Lloyd, 1997; Krishnan et al., 2008; Krishnan & Williams, 2009; Lloyd & Buchanan, 1996, 2001; Williams et al., 2003). To our knowledge however no study has been conducted where age related neuromuscular adaptations to multidirectional force loading has been investigated where the limb is loaded by both body weight and muscular stabilisation contributions. We believe the use of a weight bearing force matching task will provide more externally applicable evidence of joint stabilisation strategies.

With an aging population and the prevalence of joint disorders amongst the aging and elderly, the development of a simple early identification tool is necessary to effectively deal with the health needs of aging Canadians. If differences in joint stabilisation methods used by aging adults who do and who do not develop OA could be identified, then it could be possible to catalogue these differences and identify a healthy

and unhealthy adaptation to age-associated musculoskeletal and neuromuscular changes. Once these adaptations are identified, possible preventative strategies such as physiotherapy or strength and neuromuscular training could be used to aid individuals in returning to a healthy joint stabilising strategy and prevent the initiation of joint deterioration. This thesis is tasked with providing the healthy older adult population dataset that will become the basis of that comparison.

Review of Literature:

As individuals age, they undergo changes in their neuromuscular system which can and have been recognised to cause impairment of physical performance (Angulo-Kinzler et al., 1998; Burke & Kamen, 1995; Earles et al., 2001; Hortobagyi et al., 2006; Kido et al., 2004). Studies investigating neuromuscular impairments in old age have investigated concurrent muscle mass decline with decreased muscle fibres per cross sectional area, atrophy, and changes in fibre type (Herzog and Longino 2007; Youssef et al. 2009; Lexelle 1995; Lexelle et al. 1988; Brooks & Faulkner 1994; Doherty et al. 1993), decreased muscle strength (Hakkinen et al., 1998; Hortobagyi et al., 2003; Larsson et al., 1979; Rudolph et al., 2007; Lauretani, 2003), increased coactivation of antagonist muscles (Hortobagyi & DeVita, 2000; Hortobagyi et al., 2005; Lutz, Palmitier, An, & Chao, 1993; Macaluso et al., 2002) and impaired or altered ability to scale specific muscle force (Earles et al., 2001; Enoka et al., 2003; Hortobagyi et al., 2004; Hortobagyi et al., 2003). Though aging individuals undergo these related declines in motor control, it does not mean they will develop a joint degenerative disease; this often appears to occur idiopathically (Newman et al., 2003). What has been seen however, is that those who develop Osteoarthritis (OA) present in the early stages of the disease with significantly

higher levels of neuromuscular degradation compared to age matched controls (Hortobagyi et al., 2003); along with muscle wasting and diminished strength capacity per cross sectional area (Doherty et al., 1993; Hortobagyi et al., 2003; Overend et al., 1992; Young et al., 1984).

Joint Stabilisation

The nature of a task dictates muscles' roles as either agonist or antagonist; additionally the desired task dictates the neuromuscular control strategy required to complete it. Rapid movement of a limb can utilise the neuromuscular control method known as reciprocal activation: this method maximises the acceleration or deceleration of a limb by shutting off or inhibiting antagonist muscles upon the activation of the agonist, such as when removing a limb from a painful stimulus like a hot surface (Baratta et al., 1988). The control method switches to coactivation of both muscle groups when high levels of force are required to stabilise the joint. Flexors and extensors are also both recruited during moments of uncertainty (Hortobagyi & DeVita, 2000; Macaluso et al., 2002; Rudolph et al., 2007) like when on a slippery surface. These two methods are used interchangeably by a healthy adult during activities of daily living (ADL) like walking: just prior to heel strike during gait, the flexors and extensors of the lower limb co-activate to stabilise the limb to accept the load and translate it through stance phase; concurrently in the opposing limb, reciprocal activation is utilised, thus facilitating the rotation of the joint by allowing the agonist to act independently as the primary muscle responsible for rotation around the joint (Hortobagyi & Devita, 2006).

Coactivation of Antagonist with Age

A widely observed phenomenon associated with age is an apparent joint stiffening strategy. It appears as though disproportionate activation of antagonist muscles in comparison to agonist occurs to stabilise the joint during ADL or moments of destabilisation (Hortobagyi & DeVita, 2000; Macaluso et al., 2002; Rudolph et al., 2007). In a study by Hortobagyi and DeVita (2000) joint stiffness as a function of muscle co-activity of young and older women was investigated during downward stepping. This investigation revealed that elders stiffen their leg pre-touch-down with 120% more coactivation of the biceps femoris and tibialis anterior, thus leg stiffness duration was 136% longer, resulting in elders performing downward stepping with 64% more coactivation compared to younger adults.

Activation of these opposing muscle groups are likely controlled by a concurrent system of both cortical and spinal mediated inhibition (Hortobagyi et al., 2006; Hortobagyi & DeVita, 2000). If this is the case, age appears to alter this system such that it reduces antagonist inhibition control methods, resulting in increased coactivation of primary muscles during voluntary movement and times of joint stabilisation (Hortobagyi et al., 2006). Little is known about this coordination method and how it changes with age. Additionally, there is also a dearth of information surrounding the age associated increase of coactivation (Hortobagyi & Devita, 2006).

Muscle role in joint loading and stabilisation

Ligaments and muscle both play a role in maintaining joint stability. The properties of the two tissues separate their roles into passive and dynamic actions “preventing distraction of bones away from each other during loading” (Baratta et al.,

1988). Ligaments provide direction specific tension depending on their attachment and orientation over a limited range of motion as they do not generate or produce tension. Muscle can also act passively but can generate tension voluntarily over any range of motion (Baratta et al., 1988). Solomon et al. (1986) identified that often forces developed around a joint exceed the capabilities of the viscoelastic tissue of tendons in magnitude and direction, suggesting that in order to maintain equilibrium with agonist muscle action active muscle forces and torques in opposition to the desired action must be generated by the antagonist muscle in order to maintain joint stability (Solomonow, Baratta, Shoji, & D'Ambrosia, 1986; Solomonow et al., 1987). In 1988 Baratta et al. showed that during slow isokinetic contractions of the knee, antagonists provided a nearly constant opposing torque, suggesting that antagonistic muscle action is necessary to aid ligaments with distraction forces generated by the agonist muscle. The authors also speculated about the potential of focused stress points on the articulating surfaces of the joint with unequal agonist and antagonist activity, suggesting coactivation helps distribute the load equally over the articulating surface, thus reducing peak joint stress. Several other early investigations support the hypothesised role that antagonist muscles function in association with ligaments to actively stabilise the joint, citing their importance for physical conditioning and for those with strength offsets and ligament injuries. For example Solomon et al. (1987) identified the complex association between proprioceptive and kinaesthetic systems found within the ACL, and agonist and antagonist muscle during stabilisation actions, noting that stress (tension) on the ACL (during knee extension) resulted in moderate inhibitor effects on the quadriceps and a simultaneous excitation effect on the hamstrings. Finding similar results in patients with ACL

deficiencies, the authors propose that the antagonist muscle can take on the role of joint stabiliser during times of ACL stress or ligament deficiency. The proprioceptive sensors of muscle and ligaments are thus integrated to provide a neuromuscular protective mechanism that preserves the functional movement of the joint (Hurley, 1999).

Age-associated Neuromuscular Changes

Kido et al. (2004) and Hortobagyi and DeVita (2006) propose that there may be a decline in reciprocal activation, resulting in coactivation during voluntary movements. Kido et al. (2004) studied short latency inhibition by examining H-reflex EMG depression of the soleus and tibialis anterior muscles following a conditioning stimulus (1.5 times the MU threshold) on the common peroneal nerve finding an inverse relationship between the magnitude of inhibition of the H-reflex with age during quiet standing and walking. Kido et al (2004) suggested that a lack of modulation and a decrease in spinal reciprocal inhibition is proof of the existence of a spinal mediated inhibition method and that a decline in spinal reciprocal inhibition does occur with age. Additional evidence to corroborate the existence of age related cortical inhibition was found by Hortibagy et al. (2006) using transcranial magnetic stimulation (TMS): young and older subjects were administered a mild conditioning stimuli to the median nerve at the elbow. Following conditioning, a test stimulus was administered using TMS at 1-ms intervals, lasting in duration between 11 and 24 ms, evoking motor potentials in the extensor and flexors of the wrist. Young individuals presented conditioning interaction such that the motor potentials of the extensor carpi radialis were significantly depressed by 55% to 69% compared to older adults who showed no depression. Hotobagyi and

DeVita (2006) suggested that this confirms the existence of cortical reciprocal inhibition, and shows that like spinal reciprocal inhibition it too is reduced with age.

Presynaptic inhibition also plays a role in mediating agonist and antagonistic activation by inhibiting neurotransmitter release at the neuromuscular junction (Earles et al., 2001; Hortobagyi & Devita, 2006). It operates by increasing its effect at the afferent terminal of antagonist muscle prior to voluntary contraction and reducing its effect at the agonist, thus again maximising the extension or flexion acceleration-deceleration in much the same way that reciprocal inhibition works. This method of inhibition seems to diminish with age. A study conducted by Earls et al. (2001), again using a conditioned stimulus on the common peroneal nerve of 1.5 times that of the motor threshold, found that there was a significant depression in the test H-reflex administered 100ms later in the soleus muscles of young individuals (average age 22yrs) but not however, in older adults (average 73 yrs). Thus it appears as though age associated decreases in reciprocal and presynaptic inhibition change the control method with which the motoneuron pool is modulated by shifting control of voluntary movements to cortical mechanisms (Hortobagyi&DeVita 2006).Electroencephalogram (EEG; a measure of electrical activity of the brain) imaging studies have shown increased brain activation during precision motor tasks in older individuals in areas responsible for effective motor executions, planning, timing, and afferent feedback which could support increased cortical control methodology. These results also suggested that increased antagonistic muscle activity may be due to leaking motor commands to the adjacent areas of agonistic muscle control (Mattay et al., 2002).

Ageing Skeletal Muscle

It is well established that with increasing age comes a discernable loss of muscle mass known as sarcopenia (Brooks & Faulkner, 1994; Doherty et al., 1993; Frontera et al., 2000; Herzog & Longino, 2007; Lexell, 1995; Lexell, Taylor, & Sjostrom, 1988). Considered to be an important causal pathway to disability (Fried & Guralnik, 1997), its presence and commonality among the elderly suggest that it may play a significant role in the development of OA, as muscle plays a complex role in the stabilisation of the knee joint (Herzog & Longino, 2007; Rehan Youssef et al., 2009); again however, the cause of sarcopenia has yet to be determined.

As we age there appears to be changes in the size and number of fibres in elderly muscle. A widely accepted conclusion is that fast-twitch or type II muscle fibres undergo significant declines in size, while type I or slow-twitch fibres do not appear to change a great deal (Doherty et al., 1993; Frontera et al., 2000; Lexell et al., 1988). Reductions in size of type II motor units however has been found to be secondary to the reduction in fibre number, which has been reported to decrease as much as 40% by age 70 (Brooks & Faulkner, 1994; Lexell, 1995; Lexell & Downham, 1992). Therefore, the combined effect of the reduction in the number of fibres and atrophy of type II muscle fibres in part explains the discernable loss of contractile material associated with sarcopenia. This reduction contributes to the observed reduction in cross sectional area (CSA) and to the reduction in strength. The described age-associated muscle atrophy and fibre loss may in part be due to neurologic motor unit (MU) remodelling (Brooks & Faulkner, 1994; Doherty et al., 1993; Lexell, 1995; Lexell et al., 1988; Roos, Rice, & Vandervoort, 1997).

Motor Unit Remodelling

Motor unit remodelling theory attempts to describe the natural turnover of synaptic connections at the neuromuscular junction dictated by deinnervation, axon sprouting, and potential reinnervation of surviving MUs (Brooks & Faulkner, 1994). With age, it appears as though deinnervation exceeds axon sprouting and reinnervation of muscle fibres, in particular fast-twitch MU (Faulkner, Larkin, Claflin, & Brooks, 2007; Roos et al., 1997). In an experiment by Doherty et al. (1993) maximum voluntary contractions (MVC), maximum twitch contractions, and spike triggered averaging of the biceps brachii and brachialis muscles of 24 younger subjects (22-38) and 20 older subjects (60-81) were used to determine strength, single MU action potentials (S-MUAP) and MU number changes with age. Doherty and colleagues found that MU number was found to decrease by 50% in older adults and that older subjects' MVC and max twitch contractions were reduced up to one third that of young adults. Doherty suggested that strength reductions are secondary to the loss of MUs however, as reductions in MUs could explain the reduction in force and fibre number. Doherty and company also report significant enlargements of surviving MUs in older individuals represented by increases of mean S-MUAP amplitude, which also provides evidence of collateral sprouting and supports remodelling theory. As we age it appears as though a loss or a decline in the number of MU occurs before the atrophy of muscle fibres. Remaining fibres will either atrophy or become reinnervated in accordance with this theory, potentially resulting in enlarged innervation ratios of surviving MU.

As age related MU remodelling seems to favour type II fibres, a large proportion of the remaining contractile capability of aged muscle is controlled by slow twitch fibres

(Lexell et al. 1988). In an experiment conducted by Connelly et al. (1999), mean MU firing rates in the tibialis anterior of healthy active older men were significantly lower compared to younger men. This reduction in firing rate matches the characteristics of aged muscle and provides indirect support for age associated remodelling theory, as not only was aged muscle characteristically weaker by 26%, but also slower in firing rate, consistent with increased slow-twitch MU numbers. Additionally, lower mean firing rates observed in this study support that there are significantly fewer fast twitch MU contributing to force generation. The authors suggested that if you take these findings together, paralleled with remodelling theory, a slower motoneuron pool exists for older subjects to complete voluntary muscle contractions. As a consequence, the ability to scale or fine tune force output is also impaired as MU in tetanus cannot produce additional force or be recruited again until relaxed, thus limiting recruitment strategies (Connelly, Rice, Roos, & Vandervoort, 1999; Doherty et al., 1993; Roos et al., 1997).

As mentioned, in addition to muscle mass loss, a marked decrease in strength is also associated with age (Hakkinen et al., 1998; Hortobagyi et al., 2003; Larsson et al., 1979; Rudolph et al., 2007; Lauretani, 2003). It is predicted that this loss can translate to a 20-40% decline in muscle strength (Doherty et al., 1993; Hakkinen et al., 1998; Roos et al., 1997) and decline at a rate up to as much as 15% per decade following middle age (Vandervoort & McComas, 1986). This loss evidently is unavoidable, regardless of an individual's strength or training. Elite athletes, while testing higher for strength than untrained individuals at a given time, present similar deficits in strength independent of muscle CSA, as those who are untrained (Brooks & Faulkner, 1994; Frontera et al., 2000; Sipila & Suominen, 1993; Trappe, 2007). Frontera et al. (2000) confirmed that type I and

II fibres are weaker in older men and women compared to younger adults by examining individual muscle fibres immersed in an activating solution and measuring their maximal active force difference. As such, the reported reduction in force generation after normalising for CSA suggests that atrophy of muscle fibres can only account for a fraction of age-associated muscle weakness (Frontera et al., 2000; Roos et al., 1997). This suggestion was supported by various investigations into the effects of resistance training on muscle strength. According to a study conducted by Hakkinen et al. (1998) on elderly and middle aged healthy men and women, fibre atrophy only explains a small part of the muscle specific force loss seen with age. Following a six month strengthening protocol, Hakkinen and colleagues found that there was a disproportionate increase in voluntary strength and agonist muscle activation compared to CSA in both age groups, but to a greater extent amongst the elderly. Additionally, results from several studies also showed that older adults can fully activate: plantar and dorsiflexors (Vandervoort & McComas, 1986), tibialis anterior (Connelly et al., 1999), adductor pollicis (Phillips, Bruce, Newton, & Woledge, 1992), and quadriceps muscles (Roos et al., 1997). Therefore, age associated losses in strength are not the result of reduced central drive from the motor cortex. As such, it appears that strength and activation losses have less to do with activation, fibre number, hypertrophy and/or atrophy in non-fit individuals, but are affected to a greater extent by a form of possible neural reorganisation and adjusted recruitment strategies improving efficiency and precision of the task.

The Effects of Age on Activities of Daily Living

The ability for older adults to perform ADL declines with age (Grabiner & Enoka, 1995; Rudolph et al., 2007). Hortobagyi et al. (2003) suggested one possible reason for

this could be that elderly individuals perform daily activities such as walking, stair ascent/descent, and chair raises at a greater percentage of their maximal effort. This proposition suggests that older adults have difficulty performing ADL not because what the task demands but due to a reduced reserved capacity to perform the task at hand. The concern surrounding the required effort of the elderly is that if encountered with a task such as a sudden perturbation, or even during ADLs the task may exceed the maximal capacities of these individuals. Few studies have quantified the relative effort required by older adults to produce joint torques during ADLs. This information could provide valuable information about the neuromuscular systems of older adults and the quality of muscle action required to conduct everyday tasks (Hortobagyi et al., 2003). From the investigations that have been conducted the required effort to go from a seated to a standing position is a minimum of 1.53N.m/kg at the hip and the knee (Yoshioka, Nagano, Himeno, & Fukashiro, 2007), which equates to as much as 97% of available strength in frail individuals (Hughes, Myers, & Schenkman, 1996). Hortobagyi et al. (2003) expanded on this idea and on their previous study on the coactivity of muscle during downward stepping (Hortobagyi & Devita, 2000) by investigating the coactivation responses and level of effort required to complete ADL. They recorded EMG of the biceps femoris and vastus lateralis and used inverse dynamics calculations to compute the percentage of muscle activation and relative effort required during maximum isometric contractions and ADL. Their results supported previous findings of age associated isometric strength losses (60% decline) in association with a 27% decrease in lower limb moments during ADL; interestingly, the authors also found that the relative effort for community dwelling older adults to complete ADLs (82%) was almost twice that of

young adults (46%), with 1.6-fold increase in coactivation of antagonist muscles. The lower moments estimated for elders suggest that they utilise a different motor strategy to execute ADLs. The difficulty, however, with observing dynamic actions such as those conducted in this investigation is that identifying muscle strategies utilised by subject groups is difficult as older adults appear to distribute total available torques to other joints (DeVita & Hortobagyi, 2000). Additionally, only two muscles were reported on in these investigations, thus how individual muscles respond to age and contribute to stabilisation strategies cannot be reported. The possibility may exist that redistributed joint torques result in unloading of the knee and increased loading of the hip along with altered muscle activation to compensate for pain or muscle weakness (DeVita et al., 2001). This subsequent redistribution could alter the joint loading mechanics, thus altering cartilage nutrition, maintenance, and distribution - possibly resulting in the initiation of joint deterioration. With older individuals utilising near maximal efforts to conduct some ADL they place themselves at risk of increasing impact loads at the knee (Lafortune, Hennig, & Lake, 1996). It is plausible to suggest that when these individuals perform more functionally demanding tasks such as fall prevention strategies, they may exceed their maximal effort and subject their articulating surfaces to unstable and possibly damaging conditions (Luchies, Alexander, Schultz, & Ashton-Miller, 1994). Hortobagyi et al. (2003) concur with the findings of Rudolph et al. (2007) that increases in coactivation represent a stiffening strategy to prevent instances of destabilisation and as a compensation for neuromuscular impairment and loss of muscle strength. However, the authors add that older healthy adults execute ADLs at near maximal effort. These findings suggested increased neural drive to involved muscles and larger MU innervation

ratios related to age associated remodelling theory, both of which have been discussed above as possible mechanisms for the initiation of joint deterioration. However, various strategies utilised by subjects in the investigation are difficult to monitor and identify in a clinical setting; as such, alterations in muscle balance, strategy, and the resultant redistribution of specific joint torques could go unidentified as altered strategy. Identifying these strategies and the differences between young and older adults could provide insight into how significant alterations in knee loading and stabilisation strategies may predispose individuals to developing OA.

Impairments to the neuromuscular sensory systems discussed above, in association with the effects of age associated MU remodelling theory, would affect feedback mechanisms that are important to the control of submaximal forces (Hortobagyi et al., 2001). Aberrations in the way older adults recruit and regulate force have been suggested to predispose them to errors in joint proprioception, muscle force accuracy, steadiness and fatigue (Hortobagyi et al., 2004). In addition, pain effects like those associated with OA alter force production of the muscles surrounding a joint, influencing not only force magnitude and maximal strength, but also control (Fried & Guralnik, 1997; Grabiner & Owings, 2002). These impairments may thus contribute to an overall reduction in the control of submaximal force production important in an individual's ability to complete ADLs and stabilise the knee safely.

The control of movement as a measure of force variability or steadiness and force production error have been measured as a function of the standard deviation of average force exertion of involved muscle, and the ability to successfully match a specific force target respectively (Enoka et al., 2003). Age associated changes have been seen during

force matching protocols at low to moderate levels of effort (Hortobagyi et al., 2001; Tracy & Enoka, 2006) where subjects of advancing age exhibit a substantial increase in both force production error (consistently overestimating the required force to match the target) and force variability (Hortobagyi et al., 2001; Tracy & Enoka, 2006). Error and reduced stability in muscle force production thus may have significant implications for the functionality of individuals when performing ADL (Hortobagyi et al., 2004). Though studies investigating these issues have been conducted - some of which suggest altered strategy and muscular contributions to joint stability and may provide insight, either into who may develop OA or how the disease may progress - no investigation has yet been conducted that identifies altered strategies and then follow subjects longitudinally to see if they develop OA; therefore while age associated changes in joint loading and altered neuromuscular control can be identified, their contributions to OA can only be speculated upon.

Examining Force Control

When examining force production, previous investigations have required subjects to match for a certain period of time a pre-determined force level. Represented by a horizontal line on a computer monitor of an isokinetic dynamometer, force objectives have been set at specific levels during isometric, eccentric, and concentric contractions (Hortobagyi et al., 2004; Hortobagyi et al., 2001; Manini et al., 2005). While these investigations provide information on force control and variations between age groups, they are unable to provide evidence of how individuals compensate for knee joint instability. Additionally these investigations are limited only to the flexion and extension axis. As joint instability is not limited to a single axis, and several muscles have the

ability to produce varus or valgus moments at the knee, limiting investigations to one axis therefore limits the investigations ability to report on joint stabilisation strategies.

Target-matching protocols evaluating muscle activation patterns about the healthy knee joint using EMG have been developed to examine knee activation strategies in a combination of both the flexion-extension and varus-valgus axis (Buchanan & Lloyd, 1997; Lloyd & Buchanan, 2001). During these protocols, subjects are seated with their shank in a cylinder cast which is fixed to a 6-axis force transducer (Figure 1A).

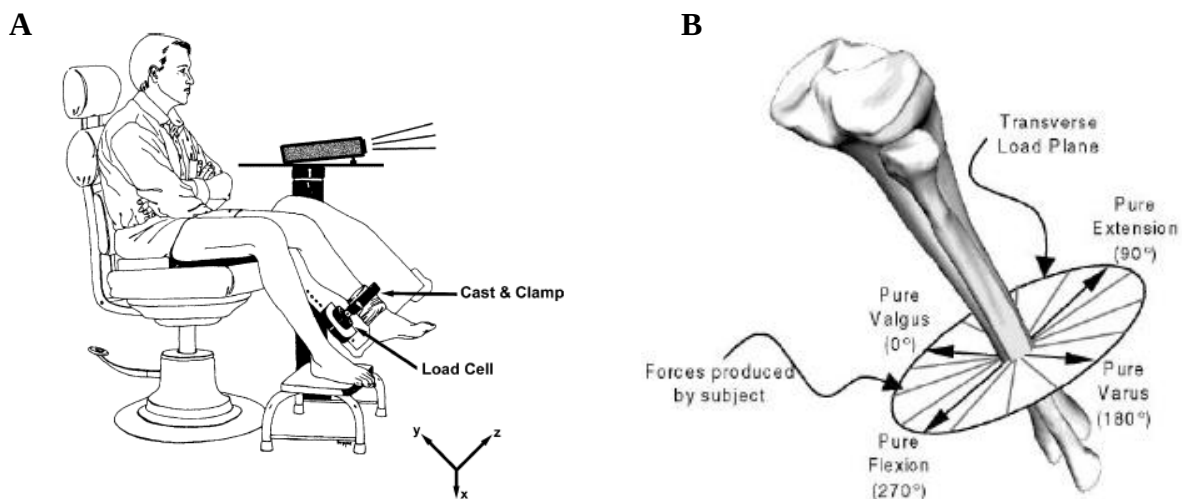


Figure 1: (A) Set up for force matching protocol from Buchanan et al., (1997). Seated subjects had their dominant leg fixed at the shank within a 6 axis force transducer (Buchanan & Lloyd, 1997). Directional forces in the transverse axis of the shank (B) applied against the transducer were scaled to a sub maximal contraction and used to control the projected cursor (Lloyd & Buchanan, 2001).

Forces applied to the fixed transducer are isometric and used to control a circular cursor displayed onscreen to the subject. The aim of this protocol - instead of attempting to match a horizontal line - is to navigate the cursor to various target locations on the visual display. The locations of the targets appear at random and are scaled to a normalised percentage of the subjects' pre-determined MVC in specific loading directions in the transverse plane of the shank. Cursor navigation was achieved by the application of force during knee extension and flexion, moving the cursor anteriorly and posteriorly and

during hip adduction and abduction moving the cursor medially or laterally (pure valgus or varus respectively Figure 1B) (Buchanan & Lloyd, 1997; Lloyd & Buchanan, 2001).

Using this protocol investigations have been conducted into how healthy individuals (Lloyd & Buchanan 1996; Buchanan & Lloyd, 1997; Lloyd & Buchanan, 2001) and those with anterior cruciate ligament deficiency (Williams et al., 2003) stabilise the knee. Muscle activations can then be broken up into polar coordinates. Each coordinate specific in direction and magnitude, specified by target locations using the above mentioned protocol. Thus each muscle mean activation magnitude can then be described as a vector. With this information, a principle direction of activation can then be determined (see methods section below). Using these techniques in a series of investigations, Lloyd and Buchanan (1996) report that the quadriceps and hamstrings act as the main stabilisers of the knee in flexion and extension and are well suited to support external moments generated around this axis. However when supporting pure varus or valgus moments, muscles capable of generating varus-valgus moments appear to compensate for a fraction of the external moment, leaving the soft tissues of the knee to support an average 83% of external load. The authors suggested that this may be representative of an acceptable level of loading in varus-valgus, and that a lack of compensation may be a neuromuscular control strategy to reduce or limit loads at the articulating surfaces of the knee. It is plausible to suggest that since coactivation results in larger contact forces at the knee (Markolf, Bargar, Shoemaker, & Amstutz, 1981) during varus or valgus moments the utilisation of a stiffening strategy may in fact apply damaging loads to the soft tissues of the knee. Thus a dual neuromuscular strategy may

exist to balance muscle the role of stabilising the joint while minimising the joint contact forces (Lloyd and Buchanan 1996). Buchanan and Lloyd (1997; 2001) continued their investigation into the dual role of muscle activation strategies and found that despite a smaller moment arm in the in the varus-valgus axis, the hamstrings and quadriceps appear to contribute up to 14% of the total 17% of muscle support of external moments in the varus-valgus axis. The remaining support contributed by the gracilis, sartorius and tensor fascia lata is a fraction of the support offered by the quadriceps and hamstrings despite having larger moment arms and higher relative activity in varus-valgus axis compared to flexion-extension (Buchanan and Lloyd 1997; 2001). With this dual strategy in mind, it remains unclear as to how individuals with reduced joint stability (like the aged population) stabilise the joint in the varus-valgus axis.

Anterior cruciate ligament deficiency (ACL-D) has been used in the past as an analogue for OA (Herzog et al., 1998; 2000) as these models disrupt joint integrity and induce similar OA responses such as joint instability (Herzog, Adams, Matyas, & Brooks, 1993). So while not a perfect analogue for ageing or OA, the results should provide some insight in to how joint individuals with reduced stability stabilise the knee joint. Using the same protocol as Buchanan and Lloyd (1997; 2001) Williams et al. (2003) investigated the neuromuscular control strategies of people with an ACL-D by assessing the specificity of muscle action using circular statistics (see data analysis) and comparing them to the subjects' contralateral limb and a healthy population. Muscle specificity is described by the degree of variability of a muscle with respects to its principle direction of action (Williams et al., 2003). Those with ACL-D described as "non-copers" suffer from joint instability and would do poorly without surgical intervention. It was found that

those with ACL-D had diminished specificity and increased global coactivation of knee stabilisers. The quadriceps were activated to a greater extent in nearly every direction, suggesting that subject with ACL-D had difficulty in regulating force production. While useful in completing the task at hand, it draws into question the effect this strategy may have on the health of articulating surfaces of the knee, if in fact this strategy occurs during daily activity. As already mentioned, Lloyd and Buchanan (1996) determined using a biomechanical knee model that supporting varus-valgus moments results in 83% of the load being supported by the soft tissues of the knee. Buchanan and Lloyd (1997; 2001) determined that the majority of the total muscular support in the varus-valgus axis is contributed by the hamstrings and quadriceps, suggesting that a dual strategy to maintain joint stability while limiting soft tissue loading. Thus if individuals with joint instability like those with ACL-D present with increased global coactivation, it is plausible to suggest that they too would present with increased contact forces at the knee. If this is the case, increased contact forces may predispose articular cartilage to injury and potentially joint degradation in particular if there is a large varus-valgus component involved in the desired task. If non-coping ACL-D individuals are an indication of how individuals with reduced or altered neuromuscular control and joint laxity respond, then it is plausible to suggest that aging individuals at risk of OA may altered neuromuscular control and stiffening strategies which thus could be distinguished from healthy young and older adults using a similar method.

Limitations of Previous Methodologies

The results in the previous investigations may in part be influenced by the nature of the experimental task. Knee joint stability is functionally dependent on the loading

environment, influenced by contact with the articulating surface and the applied load during muscular stabilisation, the soft tissues involved, and the effect of gravity (Markolf, Bargar, Shoemaker, & Amstutz, 1981; Williams et al., 2003). Seated force control or open kinetic chain tasks are thus unnatural when compared to ADL, which generally occur when the joint is loaded from weight bearing. It is thus possible that under open kinetic chain conditions the afferent feedback from both joint and muscle receptors differ from ADLs, influencing neuromuscular control and may not accurately reflect the conditions that occur during ADLs.

When defining whether a motion as either open or closed kinetic chain we consider whether or not the distal segment of an appendage is free to move (open) or is fixed in place (closed). It has been observed that muscular recruitment and joint motion are different when an appendage is met with fixed resistance as opposed unfixed conditions (Palmitier, An, Scott, & Chao, 1991). Investigations into the biomechanical differences of the two conditions have yielded significant changes in patellofemoral and tibiofemoral contact forces, anterior-posterior shear forces, and muscle activity; these differences would thus alter the environment in which the joint is stabilised (Escamilla et al., 1998; Stensdotter et al., 2003; Wilk et al., 1996). Past investigations reported that closed kinetic chain protocols present increased compressive forces at both the tibiofemoral and patelofemoral plateaus (Escamilla et al., 1998), likely a result of reported increases in muscle coactivation (Wilk et al., 1996). Increased coactivation is supported by increased muscle activation of involved muscles, in particular those of the hamstrings (Stensdotter et al., 2003). As a result of apparent increases in stabilising strategies used during closed kinetic chain activities, shear force in both the anterior and

posterior directions has consistently shown to be lower, reducing strains on the anterior and posterior cruciate ligaments (Lutz et al., 1993). Additionally, proprioceptive feedback differs between closed kinetic chain and open kinetic chain as joint receptors may become more apt to detect changes due to increased loading of the joint and decreased strain on ligaments (Kiefer et al., 1998).

With this in mind, it is plausible to suggest the quality of muscle force and the activation strategy may differ under weight bearing closed kinetic chain conditions, which would reflect more realistic compensation strategies used during ADLs by the elderly and those who may develop OA.

Purpose

This research is part of a long term research program designed to compare neuromuscular control strategies of young, old, and OA affected males and females. The goal is to identify neuromuscular alterations associated with aging and OA. The determination of neuromuscular deficits will provide valuable information to the development of evidence based training protocols designed to improve neuromuscular control in hopes of improving the quality of muscular control and joint stabilisation for patients with OA or those at risk of a joint degenerative disease. Additionally, this program could also be used as a measure of identifying those at risk of developing OA based on their functional ability.

This study is part of the first steps involved in a program aimed at identifying the quality of submaximal contractions and neuromuscular activation strategies used by healthy young adult males and females compared to those used by a healthy older (60±5yrs) population. Neuromuscular activation strategies used by subjects will be

investigated during a highly controlled, isometric, closed kinetic chain target matching protocol at various anterior-posterior-varus-valgus joint loads scaled to 30% maximum voluntary contractions for a period of five seconds.

The goals of this thesis are to first: determine the reliability of muscle activations produced during a load bearing, force matching protocol designed to generate multidirectional ground reaction forces. The second goal of this thesis is to identify neuromuscular alterations to joint stabilisation strategies associated with aging. The determination of strategic differences will provide valuable information to the development of evidence based training protocols designed to increase neuromuscular control in hopes of improving muscular control and joint stabilisation for patients and older adults at risk of developing a joint degenerative disease. Additionally, this program could also be used as a measure of identifying those at risk of developing OA based on their functional ability.

Methodology

This study is part of the first steps involved in a program aimed at identifying the quality of submaximal contractions and neuromuscular activation strategies used by healthy young adult males and females compared to those used by a healthy older (60 ± 5 yrs) population. An adapted target matching protocol was designed to collect subjects' ability to produce and modulate ground reaction forces (Flaxman, 2011). Force-related were set such that the subject were required to modulate and maintain forces at random in multiple directions and percent MVCs along a 360° transverse plane of the long axis of the shank. Subjects were required to match these specific targets under quasi-

isometric, weight bearing conditions in an effort to examine subjects muscle control strategies.

Study Design

This study compared electromyography (EMG) patterns and neuromuscular control strategies in healthy young adults (24 ± 2 yrs) with those used by healthy older adults (59 ± 5 yrs). The muscle activity of eight muscles that cross the knee joint of both the dominant limb and support limb was recorded with surface electrodes during the target matching tasks. This design allowed us to investigate age-associated differences in neuromuscular patterns involved with direction specific knee stabilisation in young and older healthy adults. A force plate was used to record the ground reaction forces of the target leg (dominant limb) required to control the cursor and identify age associated differences in neuromuscular control. Additionally the support limb's ground reaction forces were collected using a second force platform. Only the target limb data was analysed.

Participants

A power analysis of our primary outcome data (age differences across muscle activation patterns) was conducted using G*Power (3.1.0) software, based on previous data (Flaxman, 2011). The analysis determined that a sample size of 16 in each group was necessary to effectively test our hypotheses ($\alpha < 0.05$, power > 0.90).

Thirty-three subjects (17 young, 16 older) were recruited through posters and advertisements. Subjects had to be community dwelling, recreationally active (2 times per week), in good health, and had not been diagnosed with osteoarthritis (OA) by a physician to be eligible. Subjects were excluded if they had a history of: (1) chronic to

hip, knee or ankle, altered functional ability to either dominant or non-dominant limb, (2) muscular or ligament injury, or bone fracture within the last six months prior to testing, (3) nervous injury to the lower limb, joint effusion, non-age-associated muscle atrophy, diabetes, or any other medical complications that could have introduce error into the results of the investigation. As well subjects were asked to fill out a *Knee injury and osteoarthritis outcome score* KOOS (E. M. Roos, Roos, & Ekdahl, 2003) questionnaire as a qualitative assessment of knee function and pain (E. M. Roos & Toksvig-Larsen, 2003). Uncertainty about the integrity of a joint was settled by a basic functional evaluation of the lower limb by an experienced researcher.

Equipment and Data Collection

Muscle Activations

Subjects were fitted with bipolar single differential silver surface electrodes (SP-E04, DE 2.1, DelSys Inc., MA) 10mm in length, 1mm in diameter, and spaced 10mm apart. Electrodes were connected to a portable input module (SP-N05, Bagnoli-8, DelSys Inc., Boston, MA) attached to a custom harness worn by the participant. The grounding electrode (Dermatrode HE-R Farmado, BV, Nuland, Netherlands) was placed on the left clavicle. The system unit was amplified via a connection to the main amplifier unit (SP-B08, Bagnoli-16, DelSys Inc., Boston, MA). EMG was sampled at 1000Hz, amplified by 1000 and band-pass filtered between 20-450Hz using a 16 bit A/D conversion Board (NI PCI 6229, National Instrument Corp., Austin TX).

Force Platform

Ground reaction forces were recorded at 1000Hz using two force platforms (4060-08, Bertec Corporation, Columbus, OH, USA). To ensure the target matching was conducted under isometric conditions a semi rigid wakeboard boot was attached to the surface of the force platform.

VICON 3D Motion Imaging System

To ensure joint positions did not change a ten-camera infrared motion analysis system was used (VICON MX-13, Oxford Metrics, Oxford, UK) recording at 200Hz. Both a dynamic and static calibration was conducted prior to each trial. The dynamic calibration was conducted using a T-shaped wand (240mm) with three reflective markers. Each camera must record at least 3000 frames, using the three visible markers to identify the wands orientation in the recording volume. Following dynamic calibration, the static calibration using an L-shaped stationary frame (ErgoCal 14mm) was placed to define the origin of the collection volume. The calibrated capture volume was approximately 1.3m x 1.3m x 1.3m in volume. Support software for 3D motion analysis was Vicon Nexus (version 1.7).

Biodex Multi-Joint System 4 Pro

Maximal isometric forces were performed on an isokinetic dynamometer (BIODEX Multi-Joint System 4 Pro, 850-000, Biodex, New York). The analog signals of torque, position, and velocity were exported through the remote access port of the isokinetic dynamometer in real time at 1000Hz to an A/D conversion board (NI PCI

6229, National Instrument Corp., Austin TX) and collected using custom LabVIEW program.

Target Match Software

Target Match Software is a custom designed MATLAB 2007 version 7.5.0. 342 (MatWorks, Natick, MA, USA.) application designed to set the required task parameters of the knee stabilisation protocol, and to collect EMG and ground reaction force data. The purpose of this program is to promote direction specific, isometric muscle activations during weight bearing closed kinetic chain loading.

Protocol

Participant Set-up

Leg dominance was defined as the leg the subject would use to kick a ball as far as possible. Musculature activity of the dominant and non-dominant leg's rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), and tensor fascia lata (TFL) were recorded using surface EMG. Electrodes were placed over the muscle belly and the reference electrode on the left clavicle bone according to the SENIAM (Hermens and Preiks 1999) recommendations. Electrode placement sites were prepared by shaving if necessary, and abrasively rubbing and wiping of the contact area with an alcohol pad to maximise muscular activity conduction. Subjects were then instructed to perform a brief (5 minute) warm up on a stationary bike with zero resistance prior to MVC testing.

After performing the MVC testing (see below), subjects were asked to wear a tight fitting spandex suit and then fitted with 45 reflective markers in accordance to the

University of Ottawa's marker placement set (Appendix A – University of Ottawa's Marker Placement Set) to monitor and calculate hip, knee, and ankle joint angles during target matching. The purpose of this calculation was to identify variability in joint angle during target matching.

Maximum Voluntary Contractions

Maximal voluntary isometric contraction (MVIC) data was collected using an isokinetic dynamometer (BIODEX Multi-Joint System 4 Pro, 850-000, Biodex, New York) and analysed with custom made software (Labview 8.20; National Instruments Corp., Austin, TX, USA). Three 10 second repetitions of plantar flexion, knee extension, and knee flexion were recorded at relaxed hip, knee, and ankle joint angles of 30°, 30°, and 10°, respectively. Hip abduction was recorded in a standing position. Subjects were allotted 10 seconds to scale perceived force from a zero level to their maximal and hold it for approximately three seconds. Verbal encouragement and onscreen visual feedback was provided by researchers. EMG data was recorded from these trials for use in normalising EMG to maximal voluntary effort.

Establishing Target Locations

With the dominant foot in the boot fixed to the force platform, locations for the target match procedure were determined by asking the subject to produce maximal anterior (forward), posterior (backward), medial (inwards), and lateral (outwards) forces against the ground as well as maximal internal and external foot rotations; while producing these maximal forces subjects were to maintain 50% body weight on both feet. A visual scale was provided to assist the subject maintain this parameter. The maximum

resultant force (MRF) produced during each of these trials termed as maximal voluntary efforts (MVE) were used to standardise the subject's matching task to 30% of the MVE.

The trial targets are standardised to each subject using the following equation:

$$MRF = \sqrt{[(\cos\theta \cdot Fx \cdot \%maxF)^2 + (\cos(90 - \theta) \cdot Fy \cdot \%maxF)^2]} \quad (1)$$

Where the angle between the target and the positive axis is θ , Fx and Fy represents the peak force produced along the $\pm x$ and y axis (varus/valgus and anterior/posterior respectively) during MVE testing, $\%maxF$ is percent maximal voluntary isometric force recorded against the force platform. The value of the resultant vector is then also used to calculate force error in terms of magnitude. Deviation away from this value represents the absolute force error produce by the subject (Flaxman, 2011).

Target Matching

With their dominant leg secured to a force platform at the ankle via a semi ridged boot, subjects were positioned squarely in-front of a projection screen (Figure 2). The support limb or non dominant limb was be placed unsecured on the adjacent force platform, offset posteriorly 400mm. Foot position of the support limb was marked to ensure that all targets are reached in the same relative posture. Upon starting the trial, subjects were instructed to conduct a brief practice session to prevent a learning effect. Subjects were asked to match a minimum of 12 randomly assigned targets. Subjects were asked to maintain their body position throughout the protocol, however as no joint is restricted from movement small changes in joint angle were expected. Targets were projected onscreen at random along with a movable cursor that the subject could control in real time with varied directional force and percent body weight. The subjects were

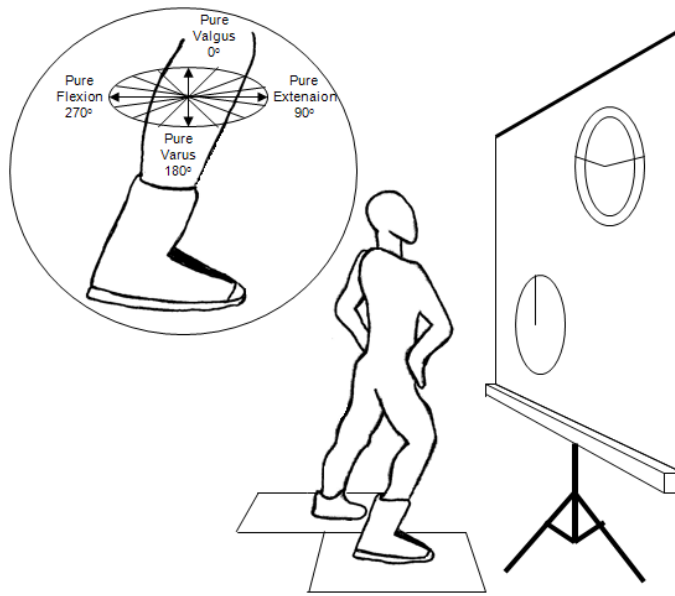
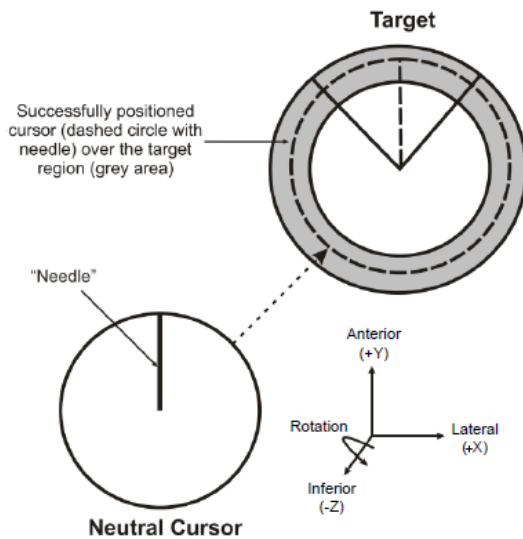


Figure 2: Set up for force matching protocol modified from Buchanan et al., (1997). Standing subjects had their dominant leg fixed at the foot to a force platform, with their supporting leg placed on adjacent force platform. Directional forces in the transverse axis of the shank applied against the force plate were scaled to a sub maximal contraction and used to control the projected cursor

required to hold the movable cursor over the desired target for one second before moving on to the next random target position. As the subject correctly matched a target, the target would turn green, indicating that the correct weight (50% body weight) reaction force had been achieved. Four seconds of EMG, force, and kinematic data was collected (three seconds prior to the match and the one second match). The cursor was operated in four degrees of freedom: anterior/posterior loads moved the cursor up and down on the screen; reflecting anterior/posterior loads upon the internal tibial plateau. Medial/lateral loads moved the cursor left and right on the screen; reflecting varus/valgus loads on the interior surface of the tibial plateau (Figure 2). Increasing or decreasing the weight on the control foot or dominant leg made the target larger and smaller, influencing the percent body weight experienced by the joint.

The trial began in the same manner as the practice session with the movable cursor in the middle of the screen. The targets appeared randomly on the screen corresponding to a desired direction of force, 30% MVE, and 50% body weight (See figure 3a for an example of a successful match). Following a successful match, subjects were prompted to relax, the next target appeared 30 s later. Targets randomly appeared, in 12 different locations evenly spaced by 30° on a circular trajectory (Figure 3b). Each target was to be matched three times, for a total of 36 targets. Marker position data was also recorded throughout the entire trial to be used in the estimation of joint kinematics. Subjects were allowed to pause at any time during trials if they begin feeling fatigued.

A



B

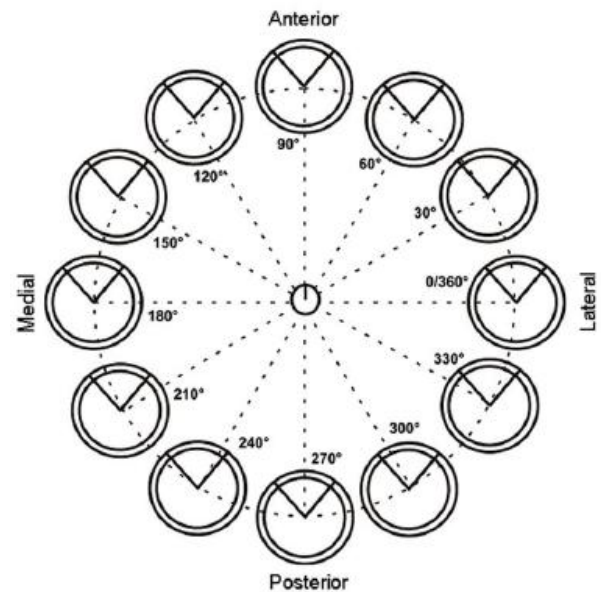


Figure 3:(A) Example of a successful match: subjects must match targets both in direction and magnitude equal to 30% of their maximum voluntary effort while maintaining 50% of their body weight on their dominant leg (Adapted from Williams et al., 2003). (B)The target locations separated by 30° on a circular trajectory (Flaxman, 2011)

Validation

The use of a weight bearing target match protocol is novel, and therefore its associated dependent variables reliability must be examined. As such ten healthy younger

adults were examined using a comparative analysis using a test re-test protocol. Subjects were asked to conduct the same protocol as above and to return within two or three days of their first trial to be re-tested

Reliability Analysis

Two-tailed paired T-tests were used to determine if differences in normalised GRFs (expressed in Newton's per kilogram of BW) and mean EMG magnitudes occurred between testing session 1 and 2. The significance level was set at alpha (α) $\leq$ 0.05. The reliability of our results was assessed with a random model, single measures intra-class correlation ($ICC_{(2,1)}$). ICCs were calculated for each muscle and each direction for: (1) session 1's multiple measurements reliability, (2) session 2's multiple measurements reliability, and (3) session 1 to session 2 (test-retest) reliability. The EMG values for test-retest reliability was the ensemble averaged EMG across the three repetitions.

To quantify the similarity between subjects, we again used an $ICC_{(2,k)}$ following recommendations of Shrout and Fleiss (Shrout & Fleiss, 1979) and Portney and Watkins (Portney & Watkins, 2000): we evaluated the within-target variance with subjects being the reliability measure by scaling the EMG to the maximum value recorded in the given muscle of each subject.

Data Analysis

Specificity Index

Only the one second required to successfully match the target was analysed. This was done using a custom LabVIEW program. The EMG data was filtered with a 4th order *Butterworth* dual low-pass filter (6Hz) to obtain linear envelopes and the average

amplitude was computed. MATLAB (2007b) was used for the remainder of EMG data analysis. The mean EMG amplitude of the three trials at each percent MVC in each target direction was then computed. The mean amplitude of each muscle was then normalised to the peak amplitude gathered from the 3 MVICs. Each muscle's normalised EMG value was represented as a vector (EMGi) where its direction and magnitude was specified by one of the 12 target orientations around the circular trajectory and by the normalised EMG value in that direction.

Traditional linear statistics cannot be used as each EMGi has a directional component between 0 and 360 degrees. Breaking EMGi into their Cartesian coordinates (x_i and y_i) allows for the addition of all normalised EMG at each target direction, and the calculation of the mean amplitude of activation (X_{EMG}) based on the number of targets (n) (Equation 2). The mean direction of muscle activity (Φ) for each muscle defined by Equation 3 is the arctan of the muscle's resultant vector, defined by the muscle's vector mean (refer to Appendix B for vector calculations) (Buchanan, 1986).

$$X_{EMG} = \frac{\sum EMG_i}{n} \quad (2)$$

$$\Phi = \arctan\left(\frac{\sum y_i}{\sum x_i}\right) \quad (3)$$

$$SI = \frac{\sqrt{\left(\frac{\sum x_i}{n}\right)^2 + \left(\frac{\sum y_i}{n}\right)^2}}{\sqrt{\left(\frac{\sum |x_i|}{n}\right)^2 + \left(\frac{\sum |y_i|}{n}\right)^2}} \quad (4)$$

As the mean cannot be calculated using traditional linear statistics, neither can muscle activation variance. Therefore, a specificity index (SI) (Equation 4), previously used by Williams et al. (2003), Krishnan et al. (2008), and Flaxman (2011) was calculated which describes the degree to which muscle activation was focused around its Φ . The SI is derived by the ratio of the muscle's resultant vector (X_{EMG} and Φ) to the sum

of all the absolute EMG magnitudes of all the vectors. The resultant value is scalar with a value ranging between 0 and 1.0. A value of 1.0 indicates a muscle that is solely activated in one direction, a value of 0 indicates a purely non-specific muscle with equal muscle activation in all directions (Figure 4).

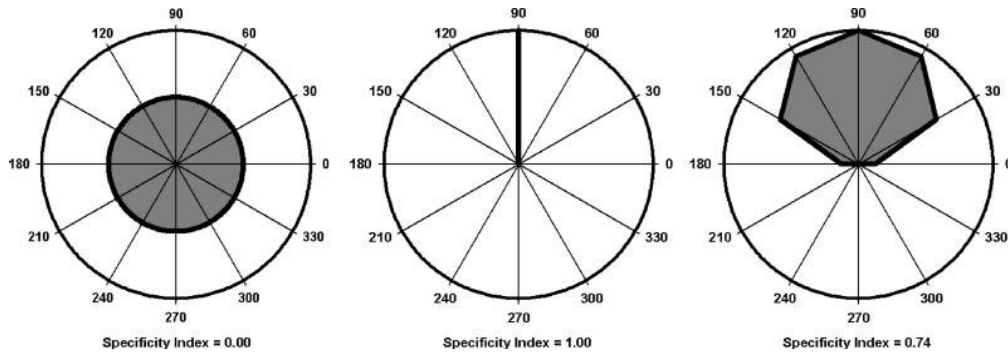


Figure 4: Polar plots demonstrating the specificity index (Adapted from Krishnana et al., 2008). A specificity index value of 0.00 indicates that the muscle was active in all directions (A). A specificity value of 1.00 indicates that the muscle was solely active in one direction (90°) (B). A muscle that has 74% of its muscle activity in its mean direction of action (C).

As an additional measure to identify whether muscle activation data was significantly skewed in the Φ , the probability (p) of the resultant vector magnitude being greater in any of the 12 directions was calculated using Equation 5:

$$p = e^{(-L2n)(10^{-4})} \quad (5)$$

Where: e = base of natural logarithm, L = mean vector magnitude (expressed as a percent of the sum of all observed vector magnitudes), and n = number of observations (Curry, 1956). A $p < 0.05$ indicates a muscle activation pattern that is asymmetrical; such that vector lengths are significantly longer (activated to a greater degree) in one general direction, and not so purely by chance.

Coactivation Index

In each target direction the degree of simultaneous coactivation of antagonistic muscles was defined using a coactivation index (CI) (Rudolph et al. 2001; Rudolph,

Schmitt, &Lewek, 2007). Antagonistic muscles involved in this study include the vastuslateralis and biceps femoris (VL-BF), vastusmedialis and semitendinosus (VM-ST), vastuslateralis and lateral gastrocnemius (VL-LG). Additionally an overall CI was calculated for the sum of the contributions of the hamstrings and quadriceps muscles (H-Q).

$$CI = \left(\frac{\text{lower EMGi}}{\text{higher EMGi}} \right) \bullet (\text{lower EMGi} + \text{higher EMGi}) \quad (6)$$

EMGi refers to the muscle or group of muscles contribution to overall muscle activity; the muscle group with the higher muscle activation magnitude acting as the divisor. A CI value closer to 0 represents no coactivation between the muscle pair such that the muscle(s) with the lower EMGi was/were not simultaneously activated as the higher muscle(s) represented by the higher EMGi activation. The upper limit of the CI is a value of 2, indicating that both muscles were simultaneously activated at 100%. The ratio of activation would then equal 1 and the sum of the activation would equal 2 resulting in a CI equal to 2.

Knee Joint Kinematics and Kinetics

The kinematic measure of the hip, knee, and ankle joints was calculated using Vicon's Nexus software (v1.3) and a modified version of the Plug-in-Gait model (Vicon, Oxford Metrics, Oxford, UK) referred to as the U of O Motion Analysis Model (UOMAM). This step was to ensure that subjects maintain a consistent position throughout the trial and between subjects. Any linear and angular displacement of each segment was calculated to ensure limb position remained constant.

Statistical analysis

To identify whether EMG magnitude varied between age groups as a function of direction, a mixed between within factorial (2x12) ANOVA was used. Muscles with significant main effects for age and direction were further analyzed with a post hoc analysis using independent t-tests to identified specific directions where EMG magnitude differed between age groups. An adjust alpha (0.025) was used to protect against type I error due to related variables when braking EMG activations into its x and y components. A mixed within-between factorial (2x12) ANOVA was also used to test for significant age related and directional effects on CI muscle pairs using the same post hoc analysis if significant main effects for age, direction, or if an interaction was found. An adjusted alpha (0.025) was also used.

Muscle strength, mean magnitude of activation (X_{EMG}) and specificity index (SI) were determined using independent t-tests (SPSS version 17.0, SPSS Inc., Chicago, USA). Traditional linear statistics cannot be used since Φ was expressed in polar coordinates. A circular analogue called the Watson-Williams two-sample test of the null hypotheses was used to determine if direction of muscle activity differed as an effect of age using *CircStat: A MATLAB Toolbox for Circular Statistics* (Berens, 2009).

To evaluate within-target variance or the similarity between age group activation patterns, an intra-class correlation coefficient ($ICC_{(2,k)}$) was used. Subject EMG activations were scaled to the maximum of a given muscle such that all activation ranged between 0 and 1 (Shrout & Fleiss, 1979; Portney & Walkins, 2000). Thus, as activation levels vary across targets, it can be seen if similar activations patterns are used for each muscle

**ARTICLE 1: RELIABILITY OF KNEE JOINT MUSCLE
ACTIVITY DURING WEIGHT BEARING FORCE
CONTROL**

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Running Title: Reliability of knee joint muscle activity during weight bearing force control

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Abstract

The aim of this study was to examine the reliability of electromyographic data recorded during these tasks. Healthy young adults stood with their dominant leg in a boot fixed to a force platform. A target matching protocol required subjects to control both the direction and magnitude of GRF along the horizontal plane while maintaining constant inferior-superior loads of 50% body-weight (BW). Each target matching task was repeated three times in a random order. Subjects were retested with the same protocol two-three days later. Normalised electromyography data of eight muscles crossing the knee joint was collected for each successful target match. A random model, single measures intra-class correlation analysed the reliability for both test-retest and intra-day results, in addition to intersubject reliability. The GRFs required to meet the targets were comparable to a range of activities of daily living, ranging from 0.48-58 N/kg of BW in the horizontal plane while maintaining 50%BW in the vertical plane. We observed moderate to high ICC values (0.60 – 0.993) for most muscles in most directions, indicating low within-subject variance. In addition, moderate to high between-subject reliability was observed in all eight muscle activation profiles, indicating subjects used similar neuromuscular control strategies to achieve the desired GRFs. In conclusion, our protocol identifies non-random weight-bearing motor control strategies. These results provide reliable insight into knee joint stabilisation strategies during weight bearing

Key words (5): Knee stability, muscle activity, weight bearing, electromyography, reliability

INTRODUCTION

Knee joint injuries and the development of knee joint osteoarthritis are serious burdens on our quality of life and our health care systems (Badley, 2005; Hartwick, Meeuwisse, Vandertuin, & Maitland, 2003; Srikanth et al., 2005). Joint stability defined as the ability of a joint to resist motion and a reduction in knee joint stability has been experimentally linked to knee joint injuries and the development of knee joint osteoarthritis (Gwinn, Wilckens, McDevitt, Herzog, Adams, Matyas, & Brooks, 1993; Hortobagyi et al., 2005). Knee joint stability arises from the integration of articular geometry, soft tissue restraints, and compressive loads from muscle action and bearing weight (Williams, Chmielewski, Rudolph, Buchanan, & Snyder-Mackler, 2001). Of these, muscles are the only active regulators of knee joint stability and recent studies have linked alterations of muscle activation with the development of osteoarthritis (Herzog & Longino, 2007; Hortobagyi, Tunnel, Moody, Beam, & Devita, 2001; Hortobagyi, Garry, Holbert, & Devita, 2004; Slemenda et al., 1997; Youssef, Longino, Seerattan, Leonard, & Herzog, 2009). Weakened muscle is subject to premature fatigue, and can lead to reduced limb loading joint instability, and disuse, altering normal loading of the knee. The overall effect could limit nutrition delivery to the cartilage and interfere with the maintenance and repair of the cartilage matrix resulting in cartilage degeneration (Solomonow et al., 1987, O'Hara, Urban, & Maroudas, 1990; Palmoski, Colyer, & Brandt, 1980; Youssef et al. 2009) As such, increasing our understanding of how individual muscles crossing the knee joint contribute to its stability is crucial. In particular, identifying situations or populations which demonstrate inefficient control strategies

during functional tasks could help explain the higher prevalence of joint instability in a given population or lead to the development of rehabilitation tools.

Electromyographic (EMG) recordings of knee joint musculature has been used as a reliable measure for evaluating voluntary muscle function in a variety of conditions (Bolga & Uhl, 2007; Claiborne, Timmons, & Pincivero, 2009; Staehli, Glatthorn, Casartelli, & Maffiuletti, 2010), especially isometric knee exercise (Mathur, Eng, & MacIntyre, 2005; Meskers, de Groot, Arwert, Rozendaal, & Rozing, 2004; A. Rainoldi et al., 1999; A. Rainoldi, Bullock-Saxton, Cavarretta, & Hogan, 2001). While the focus of these studies has been primarily on knee joint flexion-extension, Buchanan and Lloyd (T. S. Buchanan & Lloyd, 1997) have developed a force target matching protocol to evaluate muscle activation strategies while subjects isometrically produce force against a load cell in various combinations of knee flexion-extension and varus-valgus loading directions, as these would be more reflective of physiological loading conditions. A number of studies have used a similar protocol in order to (1) identify variations in knee muscle activation of different populations (Benoit, Barrance, Manal, & Buchanan, 2006; Krishnan, Huston, Amendola, & Williams, 2008; Krishnan & Williams, 2009; Williams, Barrance, Snyder-Mackler, Axe, & Buchanan, 2003), (2) explore strategies of knee joint stabilisation (i.e. coactivation) (T. S. Buchanan & Lloyd, 1997; Lloyd & Buchanan, 2001; Williams et al., 2003)[Buchanan and Lloyd, 1997; Lloyd and Buchanan, 2001; Williams et al, 2003], and (3) develop EMG driven models for predicting muscular force contribution to loading conditions (Benoit et al., 2006; T. S. Buchanan, Lloyd, Manal, & Besier, 2004; Lloyd & Buchanan, 1996; Lloyd, Buchanan, & Besier, 2005; Wang & Buchanan, 2002)(T. S.

Buchanan et al., 2004; Lloyd & Buchanan, 1996; Lloyd et al., 2005; Wang & Buchanan, 2002).

While these studies have shed light onto the role of knee joint muscles in supporting external, directional loads, subjects were seated during the tasks and the foot was free to move. As such, the ankle, knee and hip joints were compressed primarily by muscle contractions, and not body weight (BW) loading. These loading conditions affect several feedback mechanisms, altering muscular recruitment characteristics and patterns, as well as lower limb kinematics, compared to weight bearing conditions (Escamilla et al., 1998; Palmieri, An, Scott, & Chao, 1991; Shultz, Beynnon, & Schmitz, 2009; Stensdotter, Hodges, Mellor, Sundelin, & Hager-Ross, 2003; Wilk et al., 1996). As such, the external validity of the above mentioned studies with respect to closed-kinetic chain weight-bearing tasks, similar to what is generally seen in activities of daily living, may be limited.

For example, the primary roles of knee joint muscles in activities of daily living are to support BW and control mobility while opposing forces that would lead to non-physiological motion and injury. When a person steadies themselves on their feet while riding a bus or opening a door: a stable platform must be maintained allowing the person to complete the functional task and this is achieved through the modulation of ground reaction force (GRF) direction and magnitude. The resulting GRFs will be transmitted across the joints of the lower limb through shear and compressive loads. The person responds to these loads by contracting knee joint musculature to stabilise the joint and accommodate the applied loads. Evaluating neuromuscular functions with respect to knee

joint stabilisation during these tasks, and in particular the role individual muscles play in supporting these loads, seems warranted and is the focus of our study.

We have developed an approach that combines the benefits of isometric force matching tasks with the benefits of the physiologically relevant effects of gravity and joint compressive forces (Flaxman, 2011). Our protocol requires subjects to maintain approximately 50% BW while standing and actively producing and controlling GRF direction and magnitude. Our protocol therefore has the potential to provide insight into functional knee joint stabilisation strategies under physiological loading conditions. The purpose of this investigation was to evaluate the reliability of the protocol by measuring the intra-session, test-retest and inter-subject reliability of muscle activation in response to our novel force target matching tasks.

METHODS

Subjects

Ten healthy active young adults (five males, five females; mean age of $24.4.0 \pm 1.65$ years) participated in this study. Exclusion criteria included a history of: a significant lower limb injury (i.e. ligament rupture); a lower limb sprain, muscle or tendon injury, or fracture within six months of participation; a lower extremity motor nerve lesion; the presence of knee joint effusion; muscle atrophy, diabetes, or any other reported physical impairment that could affect the results of the study. The study was approved by the Institute Ethics Committee. The experimental procedure consisted of two repeated testing sessions (test and retest), separated by 2-3 days. At the beginning of the first session, all subjects were familiarized with the testing equipment and environment. Subjects were required to read and sign an informed consent form as well as fill out a

KOOS (E. M. Roos, Roos, & Ekdahl, 2003) questionnaire as a qualitative assessment of knee function and pain (E. M. Roos & Toksvig-Larsen, 2003).

Electromyography and maximum voluntary isometric contractions

For each testing session, 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), vastusmedialis (VM), vastuslateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), and tensor fascia lata (TFL) of each leg. Electrode placements followed the recommendations by SENIAM (Hermens, Freriks, & Merletti, 1999) and DeLuca (DeLuca, 1997) and were marked using a permanent marker such that the same configuration could be utilised in the retest session.

Subjects performed a five minute warm-up on a stationary bike (Monark AB, Sweden; 90 RPM with no resistance). Maximal voluntary isometric contraction (MVIC) data was collected using an isokinetic dynamometer (BIODEX Multi-Joint System 4 Pro, 850-000, Biodex, New York) and analysed with custom made software (Labview 8.20; National Instruments Corp., Austin, TX, USA). Three repetitions of plantar flexion, knee extension, and knee flexion were recorded at relaxed hip, knee, and ankle joint angles of 30°, 30°, and 10°, respectively. Hip abduction was recorded in a standing position. Subjects were allotted 10 s to scale perceived force from a zero level to their maximal and hold it for approximately three seconds. Constant verbal encouragement and onscreen visual feedback were provided by researchers. EMG signals for MVIC 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).

Weight-bearing, force control protocol

A force target matching protocol (T. S. Buchanan & Lloyd, 1997) was modified to assess the subject'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 while bearing weight. Subjects 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 and slightly posterior to the test plate (Figure 5a). The foot location of the support leg was marked with tape and

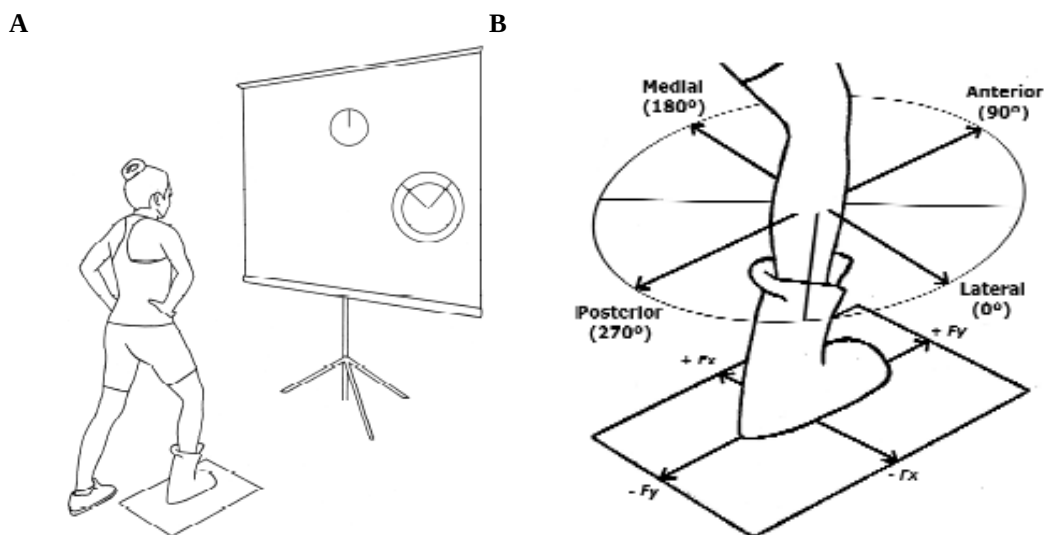


Figure 5: Laboratory setup: **A)** The subject was standing with the dominant foot in a boot fixed to a force platform and the opposite leg situated posterior and adjacently. A projector screen displaying biofeedback of ground reaction forces is placed in front. **B)** Forces acting at the tibial plateau in response to ground reaction forces applied by the subject in various combinations along the horizontal plane.

maintained throughout testing. Joint positions were recorded using a ten-camera infrared motion analysis system (Vicon MX-13, Oxford Metrics, Oxford, UK) sampling at 200

Hz. Nexus software (version 1.7, Oxford Metrics, Oxford, UK) was used to record and output the three-dimensional (3D) motion capture data. Subjects had 45 reflective markers (14mm diameter) placed on their body according to a modified Plug-in Gait marker set (Vicon, Oxford Metrics, Oxford, UK) (Beaulieu, Lamontagne, & Beaulé, 2010)

Subject weight and lower limb and pelvis anthropometric measurements were also recorded. Subjects were required to maintain 30° hip flexion, 30° knee flexion, and 10° ankle flexion; it was determined that joint position did not differ across subjects or groups and was not a covariate. As such, we consider subjects to be performing quasi-isometric tasks with these joint positions.

A video screen with a projected image of the cursor and target was placed in front of the subject (Figure 5a). This provided biofeedback on the direction and magnitude of GRFs applied to the force platform by the dominant leg (Figure 5b). These forces controlled a projected cursor during the target matching tests. The cursor moved with three degrees-of-freedom: 1) anterior/posterior loads (force along the $\pm y$ axis) moved the cursor upward/downward on the screen, 2) medial/lateral loads (force along the $\pm x$ axis) moved the cursor to the left/right, 3) inferior/superior loads (force along the $\pm z$ axis, corresponding to the long axis of the tibia) made the cursor smaller/larger (controlling the amount of BW applied to the ground). Successful matching of the cursor over the target (Figure 6a) for one second triggered data collection for this one second of target matching (during which forces in all directions had to match the target specifications) and three seconds prior to the target match (pre-match) using a custom made Matlab application (2007b, The Mathworks, Natick, MA, USA). Twelve target matching practice trials were

performed before testing to minimise the effects of learning and introduce the subjects to the testing procedures after which subjects were instrumented with the above mentioned EMG electrodes and surface markers. Following these procedures, 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 ($\pm y$) and medial/lateral ($\pm x$). Target locations were normalised to 30% of the peak load recorded during these maximal effort trials based on the circumference of a circle whose radius was scaled to these peak loads. Twelve different locations, evenly spaced by 30° about this circular trajectory were randomly displayed for matching (Figure 6b). The inferior-superior

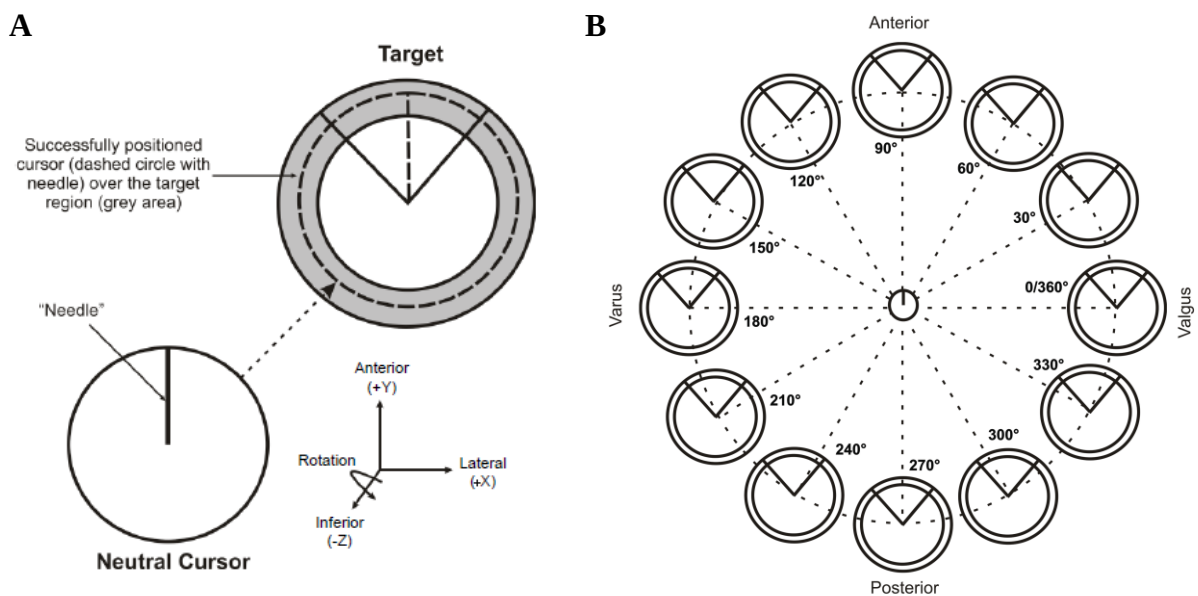


Figure 6: (A) Example of a successful match. To accomplish a successful match, subject must match targets both in direction and magnitude equal to 30% of their maximum voluntary effort while maintaining 50% of their body weight on their dominant leg(Adapted from Williams et al., 2003). (B)The target locations separated by 30° on a circular trajectory (Flaxman, 2011).

load required to maintain the correct cursor size over the target (i.e.: load along the long axis of the leg) was set at $50\% \pm 5\%$ of BW. Each target location had to be matched three times for a total of 36 trials. A minimum 30 relaxation period was provided between trials

and subjects were allowed to perform the trials at their own pace (see Flaxman, 2011 for full discussion of required force calculations and the target matching Matlab application).

Signal processing and statistical analysis

Custom Labview (v8.2, National Instruments Corp., Austin, TX) and Matlab applications were used to process the data: EMG signals were conditioned with a bias removal, full wave rectification and a 4th order dual pass Butterworth low-pass filter at 6 Hz. The one second of successful target match EMG data was isolated, normalised to the peak amplitude gathered from the 3 MVICs, and averaged over this second. Two-tailed paired T-tests were used to determine if differences in normalised GRFs (expressed in Newton's per kilogram of BW) and mean EMG magnitudes occurred between testing session 1 and 2. The significance level was set at alpha (α) $\leq$ 0.05. The reliability of our results was assessed with a random model, single measures intra-class correlation (ICC_(2,1)) - a method widely used to assess EMG reliability (Bolga & Uhl, 2007; Claiborne et al., 2009; Kollmitzer, Ebenbichler, & Kopf, 1999; Mathur et al., 2005; Meskers et al., 2004; A. Rainoldi et al., 1999; Staehli et al., 2010). ICCs were calculated for each muscle and each direction for: (1) session 1's multiple measurements reliability, (2) session 2's multiple measurements reliability, and (3) session 1 to session 2 (test-retest) reliability. The EMG values for test-retest reliability was the ensemble averaged EMG across the three repetitions. Note that the ICC must be regarded with caution since values may be small or negative when the inter-subject variation is limited, meaning the subjects belong to the same class since their scores are homogenous (Lahey, Downey, & Saal, 1983; A. Rainoldi et al., 1999) Variability among subjects must be significant otherwise the actual limits of the ICC do not match the theoretical limits of 0.0 – 1.0 and

it cannot be considered valid. In fact, it is possible for ratios to range from negative to positive infinity (Lahey et al., 1983). If this is the case, the alternative standard error of measurement (SEMs) was used to express the absolute reliability of the measure (i.e. the error of parameter estimation).

To quantify the similarity between subjects, we again used an $ICC_{(2,k)}$ following recommendations of Shrout and Fleiss (Shrout & Fleiss, 1979) and Portney and Watkins (Portney & Watkins, 2000): we evaluated the within-target variance with subjects being the reliability measure by scaling the EMG to the maximum value recorded in the given muscle of each subject. We thus evaluated if subjects' activation levels varied across targets (modulated) in a similar fashion, indicating whether they were using the same neuromuscular control strategy to achieve the desired forces.

RESULTS

Ground Reaction Forces

The mean normalised force expressed in Newton's per kilogram of BW required to match a target (i.e.: 30% of maximal effort) were 0.58 (± 0.18), 0.60 (± 0.21), 0.48 (± 0.19), and 0.57 (± 0.18) N/kg at 0°, 90°, 180°, and 270° respectively on testing session-1, and 0.57 (± 0.13), 0.57 (± 0.18), 0.48 (± 0.16), and 0.57 (± 0.13) N/kg, respectively, on testing session-2. There were no significant differences between testing sessions at any target location (two-tailed paired T-test; $p \geq 0.46$).

Activation Patterns

The group mean activations for both testing sessions are presented as EMG polar plots in Figure 7. The ST mean activation EMG magnitude (across all directions) was

significantly greater in session- 1 compared to session- 2 ($p = 0.005$) while the RF followed the same trend ($p=0.06$). All other muscles demonstrate similar activation magnitudes across testing sessions.

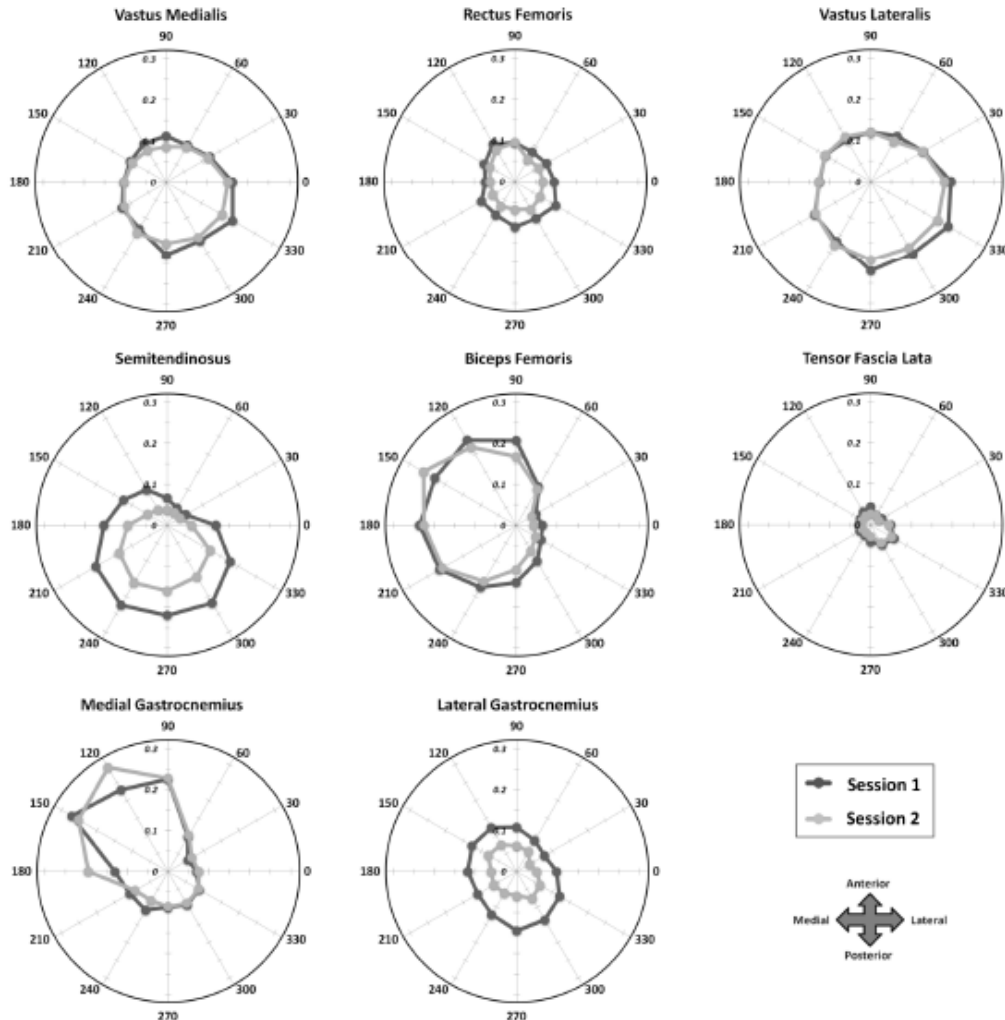


Figure 7: Group mean EMG polar plots for both testing sessions. Numbers about the circular trajectory correspond to the target locations in degrees. Where the pattern intersects the target location's radius represents the amount of activation needed to reach each target location. Plots are scaled from 0-0.3 (30% MVIC)

A qualitative evaluation of the activation patterns demonstrates that the quadriceps (VL, RF, and VM) and the LG have circular profiles, indicating similar activation magnitudes in every direction. In contrast, the ST, BF, and MG all show preferential activation, indicating relatively higher activity levels occurred in one general

loading direction (posterior, medial, and anterior-medial, respectively) compared to another.

Mean activation levels across all targets were greater than 0.1 (10% MVIC) in all muscles except for the TFL (in both testing sessions) and the LG (in the second session). Given that the task required the modulation of GRF vectors in different directions, at some target locations EMG levels for a given muscle were below 10% MVIC (see Tables 1 and 2). This is due to the sensitivity of ICC values when a low EMG activity is recorded and will be discussed below.

Intra-session (within day trial-to-trial) reliability

Intra-session reliability coefficients and SEMs for each muscle at each target location are presented in Table 1. In general, muscles displayed moderate to high ICC values in most loading directions for both testing sessions. The quadriceps muscles presented moderate to high ICC values (0.63-0.95) during both sessions and for most directions. The BF ICC's were also high (0.74-0.94) in most directions which elicited activation levels above 10% MVIC, except for session-2 at 60° (ICC = 0.56). The MG and the ST ICC values (0.56-0.97 and 0.73-0.98 respectively) were also very good, as were those of the TFL during session- 1 (excluding at the 90° target) (ICCs = 0.73-0.96).

In most instances with ICCs values <0.50 the muscle activation levels were at or below 0.1 (10%MVIC). In these instances the ICCs must be interpreted with caution as they become sensitive to noisy EMG or very small changes in the EMG signal. Furthermore, the validity of ICC data is questionable when the data lacks between-subject variance. An ANOVA was ran with all ICCs to identify instances of homogeneity and identified by a $p>0.05$. The ICCs calculated with homogenous data and low

Table 1: Intra-class correlation (ICC) coefficients of each muscle for each testing session displaying trial-to-trial (intra-session reliability). Standard error of measurements are displayed in brackets (units = ratio of MVIC with limit of 1.0)

Muscle	Target Location (°)											
	0	30	60	90	120	150	180	210	240	270	300	330
<i>Day 1</i>												
RF	0.83 (0.08)	0.94 (0.04)	0.48 (0.09)	0.46 (0.10)	0.61 (0.11)	0.51 (0.10)	0.61 † (0.08)	0.84 (0.09)	0.91 (0.06)	0.88 (0.08)	0.92 (0.06)	0.82 (0.10)
VL	0.79 (0.14)	0.81 (0.08)	0.63 (0.10)	0.76 (0.08)	0.78 (0.08)	0.75 (0.09)	0.34 (0.12)	0.75 (0.13)	0.84 (0.10)	0.80 (0.17)	0.74 (0.16)	0.72 (0.21)
VM	0.86 (0.14)	0.80 (0.10)	0.64 (0.11)	0.91 (0.05)	0.64 (0.09)	0.79 (0.06)	0.77 (0.09)	0.90 (0.07)	0.85 (0.09)	0.83 (0.20)	0.64 (0.21)	0.72 (0.27)
BF	0.37 † (0.08)	0.80 † (0.05)	0.86 (0.10)	0.91 (0.11)	0.89 (0.14)	0.94 (0.10)	0.96 (0.10)	0.88 (0.14)	0.84 (0.13)	0.80 (0.12)	0.74 (0.09)	0.68 † (0.07)
ST	0.14 * (0.14)	0.22 *† (0.09)	0.69 † (0.06)	0.35 (0.19)	0.66 (0.15)	0.69 (0.15)	0.70 (0.17)	0.86 (0.14)	0.56 (0.25)	0.62 (0.17)	0.59 (0.23)	0.41 (0.19)
LG	0.90 (0.10)	0.99 (0.03)	0.98 (0.04)	0.96 (0.06)	0.97 (0.05)	0.88 (0.11)	0.80 (0.13)	0.90 (0.09)	0.83 (0.14)	0.80 (0.15)	0.82 (0.15)	0.95 (0.02)
MG	0.79 (0.09)	0.95 † (0.02)	0.46 † (0.09)	0.59 (0.24)	0.05 * (0.20)	0.60 (0.29)	0.68 (0.14)	0.36 (0.12)	0.15 * (0.12)	0.23 *† (0.07)	0.19 * (0.09)	0.30 * (0.08)
TFL	0.73 † (0.05)	0.96 † (0.02)	0.88 † (0.03)	0.37 † (0.08)	0.73 † (0.04)	0.94 † (0.02)	0.93 † (0.02)	0.97 † (0.01)	0.96 † (0.01)	0.80 † (0.05)	0.77 † (0.05)	0.68 † (0.08)
<i>Day 2</i>												
RF	0.94 † (0.03)	0.96 † (0.02)	0.59 † (0.05)	0.59 (0.10)	0.69 (0.09)	0.35 † (0.09)	0.54 † (0.06)	0.91 † (0.03)	0.95 † (0.03)	0.94 † (0.03)	0.68 (0.08)	0.84 † (0.05)
VL	0.86 (0.09)	0.81 (0.09)	0.78 (0.06)	0.87 (0.06)	0.88 (0.06)	0.95 (0.04)	0.81 (0.07)	0.69 (0.11)	0.85 (0.11)	0.88 (0.13)	0.89 (0.08)	0.80 (0.11)
VM	0.85 (0.08)	0.68 (0.06)	0.00 * (0.06)	0.77 (0.05)	0.65 (0.06)	0.85 (0.04)	0.75 (0.05)	0.71 (0.09)	0.70 (0.09)	0.90 (0.09)	0.94 (0.06)	0.91 (0.07)
BF	0.85 † (0.02)	0.81 † (0.03)	0.56 (0.07)	0.81 (0.09)	0.89 (0.13)	0.94 (0.12)	0.96 (0.08)	0.83 (0.14)	0.75 (0.13)	0.70 (0.10)	0.60 † (0.05)	0.55 † (0.04)
ST	0.14 *† (0.11)	0.87 † (0.09)	0.88 (0.11)	0.97 (0.04)	0.85 (0.11)	0.98 (0.03)	0.73 (0.09)	0.82 (0.12)	0.87 (0.11)	0.88 (0.12)	0.93 (0.08)	0.86 (0.09)
LG	0.85 † (0.03)	0.62 † (0.04)	0.56 † (0.07)	0.90 † (0.03)	0.88 † (0.04)	0.85 (0.05)	0.78 † (0.04)	0.87 † (0.04)	0.74 † (0.06)	0.88 † (0.04)	0.81 (0.06)	0.97 † (0.03)
MG	0.67 (0.12)	0.93 † (0.06)	0.62 † (0.15)	0.56 (0.24)	0.73 (0.24)	0.61 (0.26)	0.67 (0.23)	0.81 (0.10)	0.97 (0.05)	0.89 (0.08)	0.83 (0.09)	0.90 (0.07)
TFL	0.43 † (0.07)	0.90 † (0.01)	0.17 *† (0.08)	0.23 *† (0.04)	0.52 † (0.03)	0.59 † (0.02)	0.62 † (0.02)	0.69 † (0.02)	0.88 † (0.01)	0.54 † (0.04)	0.84 † (0.05)	0.82 † (0.04)

* Instances of insignificant between-subjects variability ($p > 0.05$; $n = 6$).

† Instances of low levels of muscle activation ($< 10\%$ MVIC) across all subjects ($n = 12$).

*† Instances of low levels of muscle activation ($< 10\%$ MVIC) across all subjects and insignificant between-subjects variability ($p > 0.05$; $n = 5$).

activation levels are identified in Tables 1 and 2 and should be disregarded with respect to describing their reliability. The final tally indicates 156 of 192 target and muscle combinations with good intra-session reliability; 23 combinations with either homogeneous data, low activation levels or a combination of both indicating the reliability measures are inappropriate; while 13 target-muscle combinations displayed lower intra-session reliability.

Test-Retest Reliability

Test-retest reliability coefficients and SEMs for each muscle at each target location are presented in Table 2. The quadriceps and hamstring muscles generally demonstrated moderate to high ICC values between 0.60 – 0.94 in most loading directions. The gastrocnemius muscles demonstrated homogeneous variability in all target directions except for the LG at 330°. Their interpretation is therefore difficult and it may be more useful to use their SEM as an indicator of reliability: SEMs range from 0.06-0.19 (6-19% MVIC) for the MG and 0.10-0.12 (10-12% MVIC) for the LG. The final tally indicates 48 of 96 target and muscle combinations with good test-retest reliability; 40 combinations with either homogeneous data, low activation levels or a

Table 2: Intra-class correlation (ICC) coefficients of each muscle displaying the day-to-day (test-retest) reliability. Standard error of measurements are displayed in brackets (units = ratio of MVIC with limit of 1.0).

Muscle	Target Location (°)											
	0	30	60	90	120	150	180	210	240	270	300	330
RF	0.79 (0.05)	0.67 † (0.05)	0.52 *† (0.05)	0.74 (0.05)	0.67 (0.06)	0.79 (0.04)	0.66 † (0.04)	0.63 (0.06)	0.70 (0.06)	0.60 (0.07)	0.64 (0.07)	0.65 (0.07)
VL	0.65 (0.09)	0.53 (0.08)	0.42 (0.06)	0.82 (0.05)	0.69 (0.06)	0.75 (0.05)	0.75 (0.05)	0.66 (0.08)	0.67 (0.09)	0.67 (0.13)	0.75 (0.09)	0.58 (0.13)
VM	0.74 (0.09)	0.56 (0.07)	0.15 * (0.07)	0.42 * (0.06)	0.56 (0.05)	0.61 (0.05)	0.67 (0.05)	0.85 (0.05)	0.89 (0.04)	0.80 (0.11)	0.77 (0.09)	0.65 (0.15)
BF	0.23 *† (0.04)	0.67 † (0.03)	0.43 * (0.09)	0.66 (0.11)	0.84 (0.10)	0.94 (0.08)	0.90 (0.09)	0.86 (0.09)	0.85 (0.07)	0.71 (0.08)	0.42 * (0.06)	0.31 *† (0.04)
ST	0.41 * (0.06)	0.54 † (0.03)	0.67 † (0.03)	0.62 (0.04)	0.56 (0.07)	0.53 (0.08)	0.75 (0.07)	0.75 (0.10)	0.71 (0.13)	0.63 (0.12)	0.70 (0.11)	0.64 (0.09)
LG	-0.04 * (0.11)	-0.09 * (0.11)	-0.11 * (0.11)	-0.05 * (0.11)	0.14 * (0.11)	0.30 * (0.11)	0.01 * (0.10)	-0.07* (0.11)	0.18 * (0.11)	0.16 * (0.12)	0.10 * (0.12)	0.56 (0.06)
MG	0.47 * (0.06)	0.57 † (0.06)	0.27 *† (0.09)	0.21 * (0.17)	0.17 * (0.16)	0.13 * (0.19)	-0.09 * (0.15)	0.26 * (0.09)	0.06 * (0.10)	0.25 * (0.08)	0.27 * (0.07)	0.27 * (0.07)
TFL	0.60 † (0.04)	0.57 † (0.03)	0.51 *† (0.03)	0.54 † (0.03)	0.43 *† (0.03)	0.39 *† (0.02)	0.45 * † (0.02)	0.58 † (0.02)	0.63 † (0.02)	0.77 † (0.02)	0.76† (0.04)	0.55 *† (0.05)

* Instances of insignificant between-subjects variability ($p > 0.05$; $n = 6$).

† Instances of low levels of muscle activation ($< 10\%$ MVIC) across all subjects ($n = 12$).

*† Instances of low levels of muscle activation ($< 10\%$ MVIC) across all subjects and insignificant between-subjects variability ($p > 0.05$; $n = 5$).

combination of both, indicating the reliability measures are not appropriate; while eight target-muscle combinations displayed lower test-retest reliability.

Activation pattern reliability

By observing the variance between targets across all subjects we could determine the reliability of subject activation strategies (if subjects modulate activity in the same way across targets) for each muscle. Significant between-target variance was observed ($p < 0.001$) indicating that the ICC was a valid measure of reliability. ICCs for all muscles were moderate (RF = 0.63; MG = 0.63) to high (VL = 0.87; VM = 0.85; BF = 0.96; ST = 0.76; LG = 0.94; TFL = 0.78) indicating that subjects used similar activation strategies to achieve the GRF targets.

DISCUSSION

We developed a protocol that requires subjects to voluntarily produce and control isometric GRF magnitude and direction while standing and maintaining 50% BW on the test leg. The purpose of this investigation was to determine the intra-session, test-retest and inter-subject reliability of muscle activation in response to our novel force target matching protocol which required subjects to match targets equivalent to 30% of their maximal efforts in 12 directions in the horizontal plane. When normalized to BW, the horizontal GRF required to match the targets were equivalent to, or greater GRF during gait termination (0.5 N/kg, 0.1 N/kg respectively) and vertical jump landing (0.4 N/kg, 0.1 N/kg respectively) (McClay et al., 1994). We believe that the application of 50% BW in combination with horizontal forces generated by the test leg illustrates the external validity of our task, leading us to believe that we are observing physiologically relevant neuromuscular control strategies in our subjects.

To determine whether our findings are due to chance or are representative of a non-random neuromuscular control strategy, we assessed the reliability of our task with

ICCs and SEMs. Based on Rainoldi et al. (A. Rainoldi et al., 1999) who also analysed EMG of the lower limb, and thresholds defined by Portney and Watkins (Portney & Watkins, 2000)[Portney and Watkins, 2000], we classified good reliability to be results with ICCs greater than 0.75 and moderate reliability with ICCs of 0.60-0.74, low reliability was observed where ICCs were less than 0.60. However, to date, there is no general consensus on which ICC value classifies a given task to be reliable. Reported lower thresholds for high and moderate ICCs range from 0.70-0.9 and 0.5-0.70, respectively, whereas the upper thresholds for low ICC values are less defined with categorical cut-offs ranging from 0.25-0.0 (Bartko, 1966; Claiborne et al., 2009; Newell & Carlton, 1985; Portney & Watkins, 2000; A. Rainoldi et al., 1999; A. Rainoldi et al., 2001).

According to our criterion, our protocol produced reliable results yielding ICC values corresponding to high reliability (0.750 – 0.993) and moderate reliability (0.60 – 0.740) in 204 of a possible 288 muscles and targets, while 14 more were between 0.51 and 0.59; this in turn is accompanied by generally low SEMs. Sixty-four of the measures cannot be interpreted for reliability due to one of or a combination of the following conditions: the first condition concerns the homogeneity of our population such that significantly similar activation magnitudes were observed across subjects. When this occurs (7 intra-session and 26 test-retest measures respectively), the ICC cannot be considered a valid measure of reliability because there is not enough variability within the observed group: the between-subject variance is not sufficiently high with respect to the within-subject variance. This situation would seem to support our observation that subjects are using similar activation strategies to achieve the targets, as indicated by our

high activation pattern reliability. The polar plots in Figure 7 also support this observation as it is evident that the test-retest activation profiles are similar with all subjects.

Under certain conditions, for example LG (session-2) and TFL (sessions-1 and -2), muscles displayed relatively high SEMs. This may be explained by the relatively low activation levels (between 3-10% MVIC) and leads us to the second condition associated with low ICC values. This condition occurs in target directions which elicit low activation levels (i.e. <10% MVIC), resulting in a relatively more sensitive ICC and SEM, as seen with 12 intra-session and 4 test-retest measures. When muscle activation is low, relatively small changes in activation between repetitions and testing sessions can result in poor reliability such that low or even negative ICC values may be calculated. For example, the low ICC values BF are observed in the general lateral loading directions where activation levels are less than 5% MVIC. Therefore, when considering the ICC and SEM we see large variance about the means but our task repeatedly shows minimal “functional” activation. The positive relationship between activation level and reliability has been previously described (Bartko, 1966; Claiborne et al., 2009; Newell & Carlton, 1985; Portney & Watkins, 2000; A. Rainoldi et al., 1999; A. Rainoldi et al., 2001) and our data would support this observation. Given the reliability of our measures when the activation level is below 10% MVIC, we associate the lower ICC values of these measures with the low activation level and believe that in populations which would require a higher level of activation to achieve the target, the reliability would increase along with the increasing activation level.

In 20 of a possible 288 muscle and target combinations a third condition exists: when the subjects' scores do not agree and the measure is considered unreliable for a given muscle and target combination. Of these 20 measures, 14 ICC values were between 0.51-0.59, which though below our criterion, has been previously described as acceptable variability (Claiborne et al., 2009; A. Rainoldi et al., 2001). We observed higher trial-to-trial (Table 1) reliability compared to day-to-day (Table 2), which is to be expected given the nature of EMG measurement. We attempted to place the electrodes in the same location by marking the skin with a permanent marker; however the exact orientation of the electrode could not be verified and would affect signal reliability. Furthermore, physiological factors such as muscle fibre and motor unit discharge rates, along with the manner in which the skin was prepared and electrode placed will also influence the reliability our results (Farina, Merletti, & Enoka, 2004; Kankaanpaa, Taimela, Laaksonen, Hanninen, & Airaksinen, 1998). While the influences of these parameters were minimized to the best of our ability, they may be a source of underestimation in the test-retest reliability of our protocol. We also accounted for potential learning effects by providing practice trials, randomising our trials, and taking a mean of repeated measures. Higher levels of reliability are however observed in the second testing session compared to the first which is also reflected in the test-retest ICC values. The learning of a new motor task presents limitations in itself: a subject's ability to cognitively perceive and understand the task can influence their performance (Kollmitzer et al., 1999) and subjects were aware of our testing requirements come the second session. Research shows that coordination of muscle activation while executing a given task increases with practice such that coactivation of antagonist muscle pairs and variability of muscle activation is

minimised (Nyland, Brosky, Currier, Nitz, & Caborn, 1994). This concept is reflected when analysing differences between skilled athletes and beginners (da Fonseca, Vaz, de Aquino, & Bricio, 2006). Nevertheless, the patterns of the subjects (i.e.: the muscle synergies used to achieve the targets) did not change from session 1 to session- 2 and the within-subject variability was also similar from session 1 to session 2. From this we conclude that comparisons between groups can be made so long as the groups being compared have had the same exposure to the test (i.e.: same number of testing sessions).

Many studies have found EMG at maximal and submaximal efforts to be reliable for both between and within testing sessions (Farrell & Richards, 1986; Merletti, Rainoldi, & Farina, 2001). Specifically, a number of studies have been conducted during voluntary torque generation at the knee from a variety of patients under various conditions. Staehli et al. (Staehli et al., 2010) whom investigated test-retest reliability of the quadriceps of patients with OA found high retest reliability of the RF and VL (ICC = 0.93 and 0.94 respectively) during MVICs. Mathur et al. (Mathur et al., 2005) found good to excellent EMG reliability during sustained contractions at 80% and 20% with ICCs ranging between 0.65-0.84 and 0.85-0.92 respectively for the VL, VM and RF. However, these studies are limited to the use of an isokinetic dynamometer and restrict force generation to one functional axis (Claiborne et al., 2009; A. Rainoldi et al., 2001). Under these conditions the knee is not compressed by BW, thus affecting several feedback mechanisms (Escamilla et al., 1998; Shultz et al., 2009; Stensdotter et al., 2003), and movement is generally limited to a single degree of freedom about a single joint. Hence, extending the result of these findings to functional tasks may be difficult. To our knowledge, previous target-matching studies of the lower-leg have not reported the

reliability of their measures. As such, we believe that ours is the first study to report on the reliability of the EMG of knee joint musculature during a weight bearing task, in addition to that of a target matching task that requires subject to actively modulate and control GRFs.

Previously, a muscle's activation was thought to be dependent on its moment arm orientation (Andriacchi, Andersson, Ortengren, & Mikosz, 1984; T. S. Buchanan & Lloyd, 1997) such that greatest activation would occur about its functional axis. Accordingly, former force target match studies (Benoit et al., 2006; T. S. Buchanan & Lloyd, 1997; Krishnan et al., 2008; Lloyd & Buchanan, 2001; Williams et al., 2003)(T. S. Buchanan & Lloyd, 1997; Krishnan et al., 2008; Lloyd & Buchanan, 2001; Williams et al., 2003) report their results according to this principle, although the reliability of these results has, to our knowledge, not been reported. In contrast, we observed that a muscle could present reliable activation profiles within-subjects and between-subjects in general directions opposite of its functional axis (i.e. BF and MG). We attribute this discrepancy between the seated tests and our standing tests to the inclusion of BW loading: our task involves the coordination of the entire lower limb, meaning contribution from the hip and ankle is also present and these joints are compressed with BW. To reach a targeted force direction and magnitude, GRFs in abduction or adduction must be modulated simultaneously with anterior-posterior shear forces and compressive forces along the long axis of the limb. A moment about the hip must be generated to achieve the task and the knee is then stabilised to create a rigid segment in order for that moment to elicit the required GRF through a stiffened ankle joint and foot fixed to the ground. This situation occurs during many activities of daily living such as opening a heavy door during which

the foot is ‘rigidly’ fixed to the ground due to the frictional forces acting between the foot and ground (otherwise the foot slips, for example). Given the relatively high horizontal GRF needed to reach the targets, we consider our protocol and the resulting activation patterns to be both functionally relevant and reflective of knee joint stabilisation strategies.

In our study the muscles which showed lower reliability scores were two joint muscles (LG, MG and TFL). For example, the MG day-to-day measures displayed insufficient variability at 90, 120, and 150° along with activations exceeding 20% MVIC. The SEM in these directions was also relatively high. Further investigation into the role of these, and other observed two joint muscles, may provide greater insight into their contribution with regards to matching the target force and direction.

Conclusion

We have developed a protocol that requires subjects to modulate GRF direction and magnitude while maintaining 50% BW. The forces generated are comparable to those of activities of daily living and we can identify the individual muscular contribution associated with each direction of force. We found our measures to be reliable between trials and testing sessions. We observed that subjects used similar activation synergies to achieve the required GRFs and modulated these synergies in a similar fashion across target directions, as evidenced by their similar muscle activation profiles observed across subjects and across days. Our mean EMG polar plots are therefore valid representations of how individual muscles contribute to GRFs under isometric weight bearing conditions and are not due to randomness. These results also indicate that groups who have undergone the same number of testing sessions can be compared and that a single testing

session is all that is required to determine the neuromuscular control strategy used by a group to achieve the target locations. Future investigation into these muscle activation characteristics may provide valuable insight into knee joint injury neuromuscular stabilisation strategies in healthy and pathological populations.

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**ARTICLE 2:EFFECTS of AGE ON KNEE ACTIVATION
CHARACTERISTICS DURING WEIGHT BEARING
DIRECTIONAL LOADING**

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Running Title: Ages Effect on Knee Activation During Weight Bearing Directional
Loading

Abstract

The ability to recruit and modulate muscle activity is imperative to knee stability. To investigate age-related differences in neuromuscular activity of muscles that cross the knee joint, a highly controlled, voluntary, quasi-isometric, weight bearing target matching protocol was used. With their dominant foot in a boot fixed to a force platform thirty-nine healthy adults, 19 younger (age 24 years ± 2) and 20 older subjects (age 59 years ± 5) manoeuvred a cursor with their dominant leg to match a series of random targets projected on a screen around a circular trajectory separated by 30° . Subjects applied loads to the force platform in various horizontal directions manoeuvring the cursor while also controlling body weight. Successful target matches required maintained body weight (50%) and 30% of their peak horizontal load for one second. Electromyography (EMG), ground reaction forces, and kinetic data were recorded. Normalized to percent maximum voluntary isometric contractions the: mean magnitude of muscle activity, specificity index, and mean directions of muscle activity were calculated in each target direction. Older adults presented significantly higher mean activation magnitudes in the rectus femoris, vastuslateralis, vastusmedialis, biceps femoris, semitendinosus, medial gastrocnemius, and tensor fascia lata ($p < 0.025$) were observed. Intraclass correlations ($ICC_{(2,k)}$) indicated activation patterns were similar in all muscles ($ICC \leq 0.82$). Similar patterns are supported by non-significant differences in mean direction of activation and specificity. Results indicated that healthy older adults utilise different activation magnitudes for

stabilising the knee while maintain similar muscle activation synergies in all muscles to younger adults.

Key words: Age differences, knee, muscle activity, joint stability

INTRODUCTION

Recently, considerable attention has been given to ageing knee joint muscle function (Hortobagyi, Garry, Holbert, &Devita, 2004; Hortobagyi et al., 2005; Rudolph, Schmitt, &Lewek, 2007) as weakness has been identified as an important predictor of functional ability, (Fried &Guralnik, 1997; Lauretani et al., 2003) joint instability (Solomonow et al., 1987) and the onset of osteoarthritis (OA) (Slemenda et al., 1997). The ability to produce specific force in a controlled fashion through finely tuned muscle activity (neuromuscular control) fundamentally affects an individual's ability to stabilize the knee. It is well established that with increasing age comes a reduction of muscle performance in the form of discernable loss of muscle mass known as sarcopenia (Brooks & Faulkner, 1994; Doherty, Vandervoort, & Brown, 1993; Frontera et al., 2000; Herzog &Longino, 2007; Lexell, Taylor, &Sjostrom, 1988) affecting maximal strength but also the sensory system. Studies investigating neuromuscular impairments in old age have found aberrations in muscle fibre type, motor unit size, and number affect the way older adults recruit muscle and regulate submaximal force(Brooks & Faulkner, 1994; Doherty et al., 1993; Lexell et al., 1988; Lexell, 1995).. Simulated age associated neurologic dysfunctional and muscle weakness investigations have been conducted by Herzog et al. (2007) and Youssef et al. (2009) using a botulinum toxin and have evoked detrimental changes in knee cartilage of rabbits. The authors suggested that weakened muscle was subject to premature fatigue, and caused reduced limb loading and disuse, altering normal

loading of the knee. The overall effect could weaken nutrition delivery to the cartilage and interfere with the maintenance and repair of the cartilage matrix resulting in cartilage degeneration (O'Hara, Urban, & Maroudas, 1990; Palmoski, Colyer, & Brandt, 1980).

Despite these impairments, many older adults are able to stabilize and support their joints during daily activities without developing OA and therefore must compensate for reduced muscle performance in some way. Many strategies have been identified showing that older adults compensate for reduced strength and sensory feedback by utilizing a variety of neuromuscular adaptations. Hortobagyi and colleagues (Hortobagyi&DeVita, 2000; Hortobagyi, Mizelle, Beam, &DeVita, 2003) found older adults performed activities of daily living (stair ascent-descent and sit-to-stand activities) at a greater percent of their functional capacity (ratio of functional joint moments to maximal joint moments) (82%), nearly double that of younger adults (46%), and they did so with greater coactivation of quadriceps and antagonistic muscles (coactivation). Additionally, older adults may change the location of function in regards to motor performance during gait by altering biomechanical parameters to increase hip range of motion, and to redistribute joint torques and powers to emphasize hip extensors while de-emphasizing knee extensor and ankle plantar flexors during gait (DeVita&Hortobagyi, 2000). These adaptations suggest that, compared to young adults, older adults require a higher level of effort, increased muscle activation, and use different motor patterns to complete a similar task.

When evaluating neuromuscular function as it relates to knee joint stability the use of dynamic tasks like those above make it difficult to uncouple biomechanical and neuromuscular contributions to joint stability (Williams, Chmielewski, Rudolph,

Buchanan, & Snyder-Mackler, 2001). Limiting contributing biomechanical factors may allow for clearer interpretation of muscle activation patterns under measurable loading conditions. Voluntary torque generations and corresponding electromyographic recordings of knee joint musculature have widely been used and are known to be a reliable measure for evaluating muscle function (Bolgla&Uhl, 2007; Claiborne, Timmons, &Pincivero, 2009; Staehli, Glatthorn, Casartelli, &Maffiuletti, 2010). When examining neuromuscular control of older adults during force stabilization at the knee, previous investigations have required subjects to match a pre-determined target force for a certain period of time. Represented by a horizontal line on a computer monitor of an isokinetic dynamometer, targets have been set at specific levels during isometric, eccentric, and concentric contractions (25 N, 50 N, and 100 N) (DeVita et al., 2001; Hortobagyi et al., 2004; Manini, Cook, Ordway, Ploutz-Snyder, &Ploutz-Snyder, 2005). While these studies limited dynamic movement to simplified isokinetic tasks in flexion and extension, the tasks are not the same for all subjects. Setting a specified force value may represent varying relative efforts depending on the subject. Observed variability during force matching tasks, therefore, may be due in part to the task itself, and not totally reflective of a reduced ability to stabilise. Additionally, muscles supporting shear forces across the knee are required to do so not only in the anterior posterior directions, but in medial and lateral directions as well. Not including these components limits the external application of these results.

A number of studies have identified the role of individual knee joint muscles in supporting loads and distributing these loads between muscle and soft tissues at the knee joint (DeVita et al., 2001; Hortobagyi et al., 2004; Manini, Cook, Ordway, Ploutz-

Snyder, &Ploutz-Snyder, 2005). While results from these investigations have elucidated meaningful interpretations of joint support strategies, they were collected under conditions where the subject was seated, with the limb unloaded and the foot free from restriction. Joint compression forces across the hip, knee, and ankle are contributed largely by muscle contractions with no body weight loading. This limitation affects several feedback mechanisms important to knee joint stabilization and is known to affect muscle recruitment amplitude, strategies, and kinematics (Escamilla et al., 1998; Palmitier, An, Scott, & Chao, 1991; Shultz, Beynnon, & Schmitz, 2009; Stensdotter, Hodges, Mellor, Sundelin, & Hager-Ross, 2003; Wilk et al., 1996). The use of a weight bearing static force matching protocol would provide more physiologically relevant insight into functional knee joint stabilisation strategies.

The purpose of this investigation was to examine age related changes in neuromuscular control of the muscles crossing the knee during weight bearing joint stabilisation tests. Based on the findings of Hortobagyi and colleagues (Hortobagyi&DeVita, 2000; Hortobagyi, Tunnel, Moody, Beam, &DeVita, 2001; Hortobagyi et al., 2003; Hortobagyi et al., 2005; Hortobagyi&Devita, 2006; Williams et al., 2003) it is hypothesised that older, healthy adults would present significantly less specificity, with significantly greater activation magnitudes, and consequently, significantly greater levels of coactivation.

METHODS

Participants

A total of 19 young (10 women, mean age 24 ± 2 years) and 20 older (10 women, mean age $59 \text{ years} \pm 5 \text{ years}$) healthy active adults who participate in sport and exercise

on a regular basis were recruited for this study. Young and older adults were similar in height (young 1.72 ± 0.07 m; older 1.70 ± 0.08 m) mass (young 70.4 ± 12.2 kg; older 71.1 ± 8.9 kg) and were all right foot dominant (defined by which leg they would use to kick a ball). Selection criteria required subjects to be free of: life altering injury to hip, knee or ankle; altered functional ability to either dominant or non-dominant limb; muscular or ligament injury; bone fracture within six months prior to testing, nervous injury to the lower limb; joint effusion; non-age-associated muscle atrophy; diabetes; or any other medical complications that could introduce error into the results of the investigation. Five subjects (two women, one young; three men, one young) had to withdraw from the study following non-completion of the practice trials. It was discovered following or during practice trials that these subjects had a condition not divulged during subject screening which would have nevertheless disqualified them from participation (injury or joint condition).

Table 3: Young and older adult participant demographics

Variables	Sex	Mean	SD	pValue
Age	Young	24	2	0.000*
	Older	59	5	
Height (m)	Young	1.72	0.07	0.536
	Older	1.7	0.08	
Weight (kg)	Young	70.4	12.2	0.849
	Older	71.1	8.9	
BMI (kg/m²)	Young	23.8	2.8	
	Older	24.6	2.5	

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

This study was approved by the University of Ottawa Ethics Committee. All subjects were provided with a thorough introduction to the testing procedure and equipment. Subjects were also required to fill out a *Knee injury and Osteoarthritis Outcome Score*

(KOOS) (E. M. Roos, Roos, & Ekdahl, 2003) questionnaire as a qualitative assessment of knee function and pain (E. M. Roos & Toksvig-Larsen, 2003).

Experimental Protocol

The same, validated protocol utilized by Flaxman (2011), an adapted protocol from Buchanan and colleagues (Buchanan, Almdale, Lewis, & Rymer, 1986), was used to assess different age populations' ability to stabilize the knee joint while applying specific horizontal GRFs against a force platform in multiple directions. After subjects had read and provided informed consent as approved by the University Ethics committee, subjects performed a series of 12 practice target matching trials. Specific attention was given to ensure all subjects received equal and adequate instruction to ensure participants could perform the task and to minimize learning effects.

To determine the amount of force required to reach each target location, the target match software was calibrated to each subject: with the subject's dominant foot in the boot under the same position conditions discussed above, subjects were to produce maximal anterior/posterior ($\pm y$) and medial/lateral ($\pm x$) GRF, while maintaining 50% BW on the dominant leg. Visual feedback was provided to the subject to assist them in maintaining 50% BW.

Following practice trials, the remaining 17 young and 16 older subjects were asked to wear spandex shorts and shirt and anthropometric data were then taken of the lower limb and pelvis. Bipolar surface electromyography (EMG) electrodes (SP-E04, DE 2.1, Delsys Inc., Boston, MA) connected to a portable input module (SP-N05, Bagnoli-8, DelSys Inc., Boston, MA) using a 16-channel EMG system (DS-B04, Bagnoli-16, Delsys Inc., Boston, MA) were placed over the muscle bellies of the rectus femoris (RF),

vastusmedialis (VM), vastuslateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), and tensor fascia lata (TFL) of each leg. Electrode placements followed the recommendations by SENIAM (Hermens et al. 1999) and De Luca, (1997).

A five-minute warm-up on a stationary bike (Monark AB, Sweden; 90RPM) with no resistance was provided for each subject prior to collection of maximal voluntary isometric contraction (MVIC) data. MVIC data were collected using an isokinetic dynamometer (BIODEX Multi-Joint System 4 Pro, 850-000, Biodex, New York and analysed with custom-made software (Labview 8.20, National Instruments Corp., Austin, TX, USA). Three 10 second repetitions of plantar flexion, knee extension, and knee flexion were recorded while seated at relaxed hip, knee, and ankle joint angles of 90°, 30°, and 10°, respectively. Hip abduction was collected with subjects standing: they were allotted 10 seconds to perform each MVIC, and instructed hold each maximum for at least three seconds. All EMG signals were amplified at the same gain of 1000 and sampled at 1000Hz, band-pass filtered at 20-450Hz using a 16 bit A/D Conversion Board (NI PCI 6229, National Instrument Corp., Austin TX).

Participants then stood with their dominant and non-dominant feet atop two force platforms (FP4060-08, Bertec Corporation, Columbus, OH, USA). The dominant foot (defined by the leg with which they would use to kick a ball as far as possible) was secured in a water-ski boot (Bio, O'Brien, Redmond, WA, USA) attached to a force platform adjacent and 0.28 m anterior to the force platform with which the subjects placed the support leg (Figure 8A). The desired joint angles were as followed: Hip, 30°;

A



B

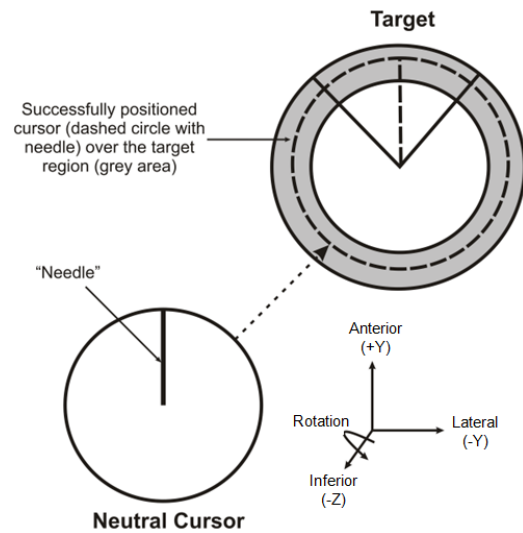


Figure 8: A) Laboratory setup **B)** Depiction of the target and cursor (Adapted from Williams et al., 2003). A successful match was achieved when the cursor was positioned over the target such that its circumference fits within the grey area of allowable error in the z-axis ($50\% \text{ body weight} \pm 5\%$). Allowable error calculates to 3.9% .

Knee, 30° ; Ankle 10° . The support foot position was outlined with tape to ensure subjects returned to the same position for the full duration of the trial.

To monitor joint positions a ten-camera infrared motion analysis system was used (Vicon MX-13, Oxford Metrics, Oxford, UK) sampling at 200Hz. Nexus software (version 1.7, Oxford Metrics, Oxford, UK) was used to record and output the three-dimensional (3D) motion capture data. Subjects had 45 reflective markers (14mm diameter) placed on their body according to a modified Plug-in Gait marker set (Vicon, Oxford Metrics, Oxford, UK) (Beaulieu, Lamontagne, & Beaulé, 2010). To provide visual feedback to the subjects about required and applied GRFs of the dominant foot both targets and cursor were projected on a screen placed in front of the participant at an equal distance for all subjects. Cursor size and movement was controlled within three degrees-of-freedom: 1) Force along the $\pm y$ axis (anterior/posterior) moved the projected cursor up and down, 2) force along the $\pm x$ axis (medial/lateral) moved the

cursor left and right, and 3) force loads that were inferior/superior along the $\pm z$ axis (decreased or increased the radius of the circular cursor). When subjects held the GRF and direction parameters for a target (Figure 8b) for one second the custom made Matlab target match application (The Mathworks, Natick, MA, USA) triggered the recording of the previous four seconds of EMG, GRF, and kinematic data. Input parameters into the custom Matlab target match application defined the number of targets and location (12, spaced by 30° about a circular trajectory) and the number of repetitions (three) for a total of 36 targets. Following each successful match, a minimum 30 second relax period was initiated where feedback projections were frozen to encourage the subject to relax. Subjects were also allowed to match targets at their own pace to avoid fatigue.

Data Processing

Matlab and Labview (v8.2, National Instruments Corp., Austin, TX) custom applications were used to process EMG signals: signals underwent bias removal; full wave rectification; and a 4th order dual pass Butterworth low-pass filter at a cut off of 6 Hz. Torque data had limb weight removed and was filtered using a 4th order dual pass Butterworth low-pass filter at a cut off of 6 Hz. Only EMG data of the final second when subjects were matching target parameters was analysed in this study and was normalized to the maximums obtained on the isokinetic dynamometer. EMG amplitude during the matched second was normalised to EMG peaks achieved during MVIC and then ensemble averaged with the three repetitions at each target location for each muscle.

Each muscle's ensemble averaged normalised EMG values were represented as a vector (EMGi), where its direction is specified by one of the 12 target orientations around the circular trajectory and its magnitude equal to the percent maximum EMG value in

that direction. Values were then graphed on a polar plot to visually represent muscle activation synergies and direction.

Traditional linear statistics cannot be used as each EMGi has a directional component between 0 and 360 degrees. Breaking EMGi into their Cartesian coordinates (x_i and y_i) allows for the addition of all normalised EMG at each target direction, and the calculation of the mean amplitude of activation (X_{EMG}) based on the number of targets (n) (Equation 1). The mean direction of muscle activity (Φ) for each muscle defined by Equation 2 is the arctan of the muscle's resultant vector, defined by the muscle's vector mean (refer to Appendix B for vector calculations) (Buchanan, 1986).

$$X_{EMG} = \frac{\sum EMGi}{n} \quad (1)$$

$$\Phi = \arctan\left(\frac{\sum y_i}{\sum x_i}\right) \quad (2)$$

$$SI = \frac{\sqrt{\left(\frac{\sum |x_i|}{n}\right)^2 + \left(\frac{\sum |y_i|}{n}\right)^2}}{\sqrt{\left(\frac{\sum |x_i|}{n}\right)^2 + \left(\frac{\sum |y_i|}{n}\right)^2}} \quad (3)$$

A specificity index (SI) (Equation 3), previously used by Williams et al. (2003), Krishnan et al. (2008), and Flaxman (2011) was calculated which describes the degree to which muscle activation is focused around its Φ . The SI is derived by the ratio of the muscle's resultant vector (X_{EMG} and Φ) to the sum of all the absolute EMG magnitudes of all the vectors. The resultant value is scalar with a value ranging between 0 and 1.0. A value of 1.0 indicates a muscle that is solely activated in one direction, a value of 0 indicates a purely non-specific muscle with equal muscle activation in all directions (refer to Appendix B for SI equation and calculations). As an additional measure to identify whether muscle activation data were significantly skewed in the Φ , the probability (p) of

the resultant vector magnitude being greater in any of the 12 directions was calculated using Equation 4

$$p = e^{(-L2n)(10^{-4})} \quad (4)$$

Where; e = base of natural logarithm, L = mean vector magnitude (expressed as a percent of the sum of all observed vector magnitudes), and n = number of observations (Curry, 1956). A $p < 0.05$ indicates a muscle activation pattern that is asymmetrical; such that vector lengths are significantly longer (activated to a greater degree) in one general direction, and not so purely by chance (Curry, 1956).

To quantify the level of contraction of antagonistic muscle pairs at each target location, a coactivation index (CI) was used. As used previously by Rudolph et al, (2007) and Lewek, Ramsey et al, (2005) the CI defines the ratio of summed antagonist's activation and the summed agonist's activation at each target direction, multiplied by the summed activity of both muscles:

$$CI = \left(\frac{\text{lower EMGi}}{\text{higher EMGi}} \right) \cdot (\text{lower EMGi} + \text{higher EMGi}) \quad (6)$$

The muscle or muscle groups (EMGi) with the greater activation was defined as the divisor and the lesser value as the numerator. A CI value closer to 0 suggests that the lower EMGi numerator was not simultaneously with higher muscle represented by the higher EMGi activation. The upper limit of the CI is 2, indicating that both muscles are activated at 100% simultaneously. The ratio of activation would then equal 1 and the sum of the activation would equal 2. Antagonistic muscle pairs under investigation were: the vastuslateralis and biceps femoris (VL-BF), vastusmedialis and semitendinosus (VM-ST), vastuslateralis and lateral gastrocnemius (VL-LG), vastusmedialis and medial gastrocnemius (VM-MG). A general quadriceps and hamstrings coactivation (QUADS-

HAMS) and a general quadriceps and gastrocnemius (QUADS-GASTROC) muscle grouping were also used to describe muscles acting as a functional group.

Statistical Analysis

To identify whether EMG magnitude varied between age groups as a function of direction, a mixed within-between factorial (2x12) ANOVA was used. Muscles with significant main effects for age and direction were further analyzed with a post hoc analysis using independent t-tests to identified specific directions where EMG magnitude differed between age groups. An adjust alpha (0.025) was used to protect against type I error due to related variables when braking EMG activations into its x and y components. A mixed within-between factorial (2x12) ANOVA was also used to test for significant age related and directional effects on CI muscle pairs using the same post hoc analysis if significant main effects for age, direction, or if an interaction was found. An adjusted alpha (0.025) was also used.

Muscle strength, mean magnitude of activation (X_{EMG}) and specificity index (SI) were determined using independent t-tests (SPSS version 17.0, SPSS Inc., Chicago, USA). Traditional linear statistics cannot be used since Φ was expressed in polar coordinates. A circular analogue called the Watson-Williams two-sample test of the null hypotheses was used to determine if direction of muscle activity differed as an effect of age using *CircStat: A MATLAB Toolbox for Circular Statistics*(Berens, 2009).

To evaluate within-target variance or the similarity between age group activation patterns, an intra-class correlation coefficient ($ICC_{(2,k)}$) was used. Subject EMG activations were scaled to the maximum of a given muscle such that all activation ranged between 0 and 1(Shrout& Fleiss, 1979; Portney&Walkins, 2000). Thus, as activation

levels vary across targets, it can be seen if similar activations patterns are used for each muscle.

RESULTS

Subject demographics are presented in table 3. KOOS scores for both groups indicate that out of a possible score of 100 indicating “no problems” young and older adults were free from: symptoms of OA (Mean=99.1±2.4, Mean=94.6± 6.5), Pain (Mean=99.8±0.7, Mean=97.8±3.5), difficulty performing: ADL (Mean=100.0±0.0, Mean=97.0±3.7) sports and recreation (Mean=99.8±0.7, Mean=98.5) and have good quality of life (Mean=99.6±1.6, 96.1±7.5) with respects to knee joint health. Thus none of these factors act as a possible covariate in terms of joint healthy. Older adults were found to be significantly weaker during MVIC in extension, flexion, and plantar flexion ($p=0.001$, $p=0.001$, and $p=0.002$ respectively) by 30%. While not significant, older adults were also found to be 18% weaker in abduction.

Polar plots depicting muscle activation patterns of healthy young and older participants, as well as the Φ for each muscle which presented asymmetrical data (p), are presented in Figure 9. If muscle activation polar plots are symmetrical, assigning a Φ may be misleading, as vector magnitudes are relatively equal in all directions. Asymmetry was only found in the BF, ST, and MG for both young and older adults; these results corresponded with higher SI (values ≥ 0.3) where activation was primarily directed in the lateral, posterior, and anterior-lateral directions, respectively. Muscles with symmetrical muscle patterns (RF, VL, VM, LG and TFL) had lower SI (≤ 0.3) and more circular muscle activation patterns. No significant differences were found between young and older adults for these muscles (Figure 10).

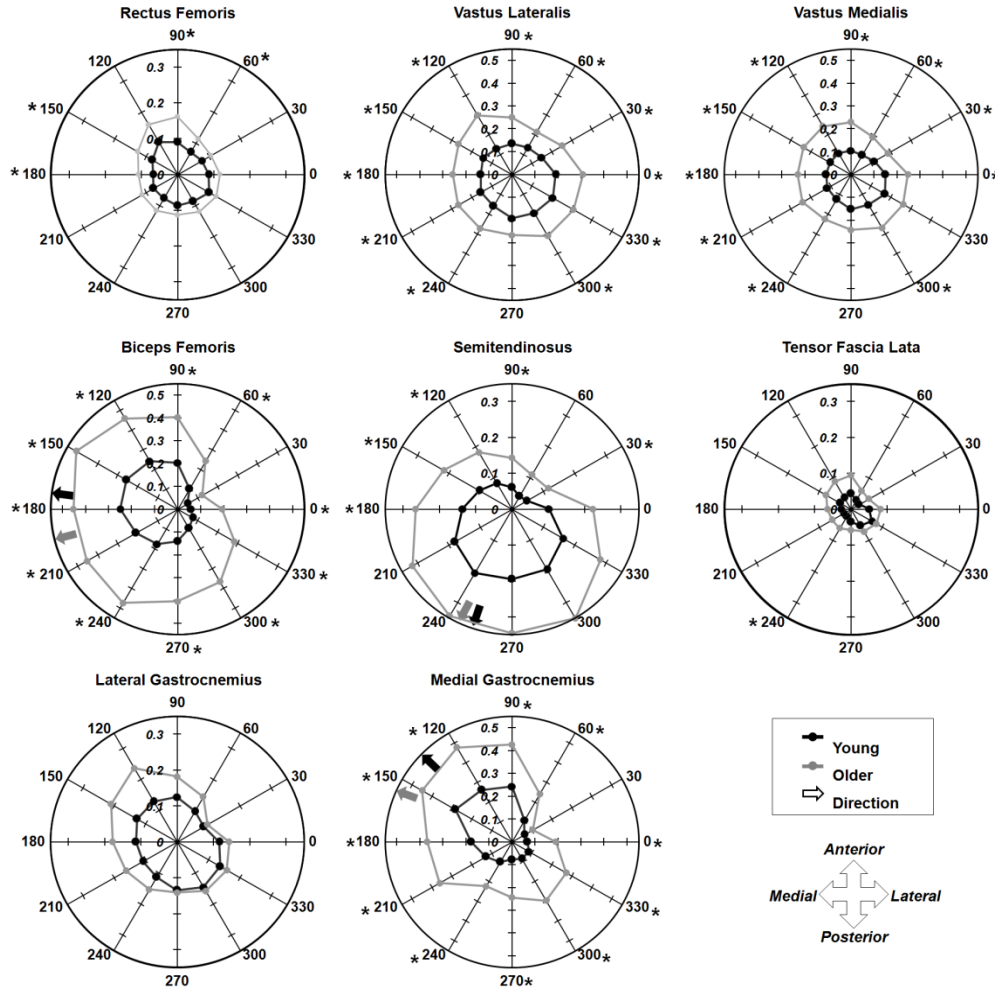


Figure 9: EMG polar plots of young and older adult mean activation patterns. Inner numbers along each target direction axis (represent normalized percent EMG. Locations where the activation pattern intersects each target direction axis represents the mean EMG activation to match that target. Plots are scaled to either 0.35 or 0.55 (35 of 55% MVIC EMG level). Mean direction of muscle activation (Φ) of muscles with significant asymmetry ($p < 0.05$) of young and older adults are identified by arrows for the: BF (Young: Mean=176.9°, SD=29.1°, $p=0.000$; Older: Mean=195°, SD=30.5°, $p=0.000$), ST (Young: Mean=252.6°, SD=27.8°, $p=0.000$; Older: Mean=244.3°, SD=29.5°, $p=0.00$), and MG (Young: Mean=139°, SD=38.7°, $p=0.000$; Older: Mean=156.0°, SD=27.4°). * beside target directions around circular trajectory identifies significant differences in EMG magnitude in that direction ($p < 0.025$) between young and older adults.

Significant variance between-targets was observed ($p < 0.000$) for all muscles indicating sufficient variability existed to conduct an $ICC_{(2,k)}$. ICCs (table 4) for all muscles were high (RF=0.84; VL=0.90; VM=0.91; BF=0.98; ST=0.974; LG=0.88; MG=0.97; TFL=0.87), indicating that young and older subjects used similar activation strategies to match targets.

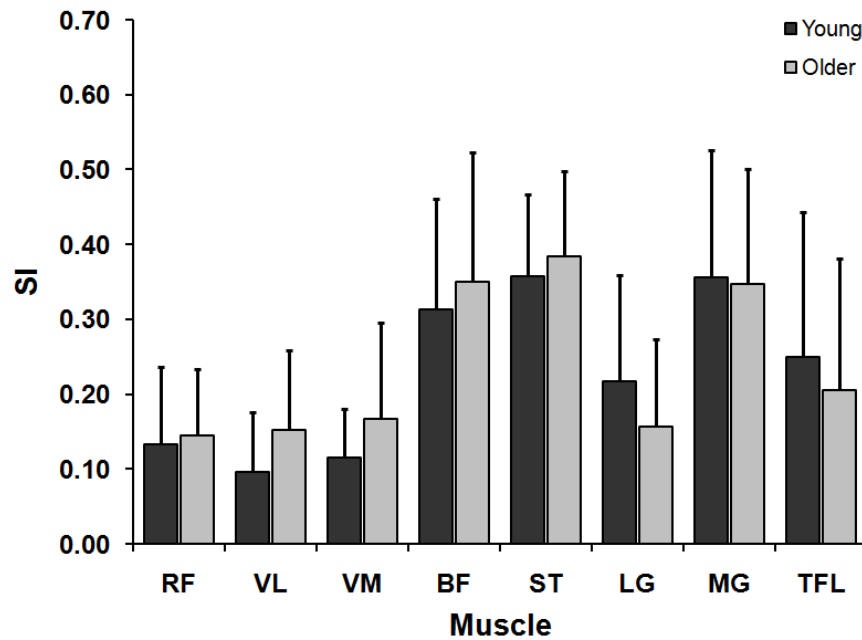


Figure 10: Mean specificity indices (SI) of young and older adults. * indicates muscle with significantly different SI between age groups ($p < 0.05$). Error bars indicate standard deviation.

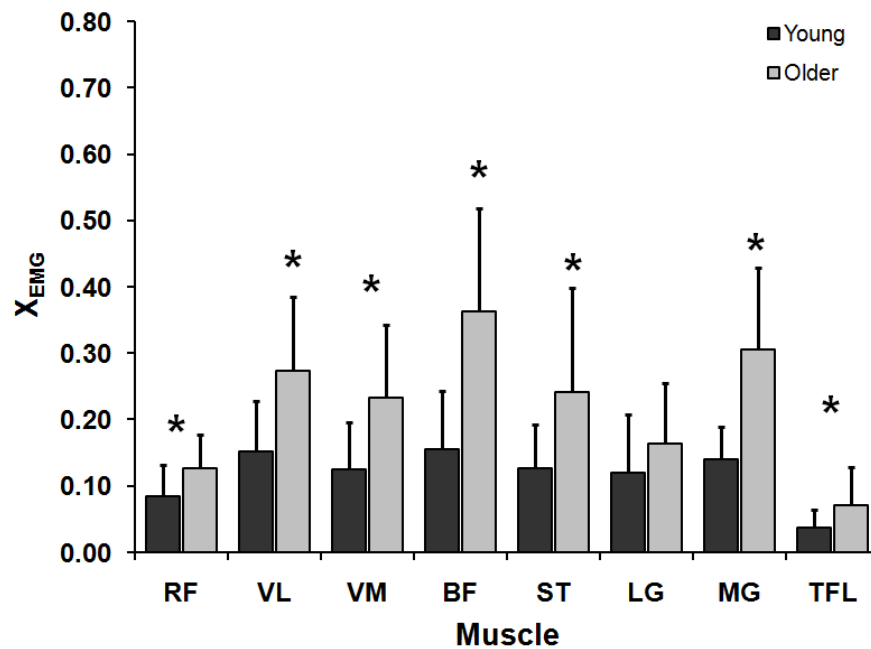


Figure 11: Mean resulting magnitude (X_{EMG}) of young and older adults. EMG activations are normalized to their max voluntary isometric contraction so all values range between 0 and 1. * indicates significance between age groups X_{EMG} ($p < 0.05$).

All muscles except LG showed significant main effects of age on EMG magnitude: RF ($p = 0.020$); VL ($p = 0.001$); VM ($p = 0.002$); BF ($p = 0.000$); ST ($p =$

0.010); MG ($p = 0.000$); TFL ($p = 0.034$) (Figure 11). Post hoc tests indicate that muscle activation in all muscles are significantly higher for older adults ($p < 0.025$) in nearly all directions for the VL, VM, BF, MG and primarily in the anterior directions between 30° - 180° for the RF and ST.

Table 4: Intra-class correlations ($ICC_{(2,k)}$) indicating the variability between all young and older subject's muscle activation patterns compared to the mean activation profile.

Muscle	RF	VL	VM	BF	ST	LG	MG	TFL
ICC	0.838	0.897	0.906	0.95	0.974	0.878	0.971	0.869
Upper CI	0.944	0.964	0.968	0.950	0.991	0.958	0.990	0.955
Lower CI	0.672	0.791	0.809	0.992	0.947	0.751	0.941	0.734

Abbreviations: Rectus femoris (RF); vastuslateralis (VL); vastusmedialis (VM); biceps femoris (BF); semitendinosus (ST); lateral gastrocnemius (LG); medial gastrocnemius (MG); tensor fascia lata (TFL).

Coactivation patterns were similar between age groups (Figure 12). However, significant main effects were found between QUADS-HAMS, QUADS-GASTROCS muscle groups and VM-ST VL-BF, muscle pairs ($p < 0.05$). Post hoc analyses showed that as a group, older adults' QUADS-HAMS and QUADS-GASTROCS muscle pairs presented significantly more coactivation in nearly all directions ($p < 0.025$). Older adults' VL-BF and VM-ST also presented significantly more coactivation at: 60° ($p = 0.024$); 180° ($p = 0.023$); 270° ($p = 0.024$), 300° ($p = 0.013$), 330° ($p = 0.13$), 360° ($p = 0.012$) (primarily posterior-lateral) and 30° ($p = 0.12$), 180° ($p = 0.13$), 360° ($p = 0.24$), (medial-lateral) respectively (Figure 12). A trend towards significance for both VL-BF and VM-ST in nearly all directions may be present however ($p < 0.05$).

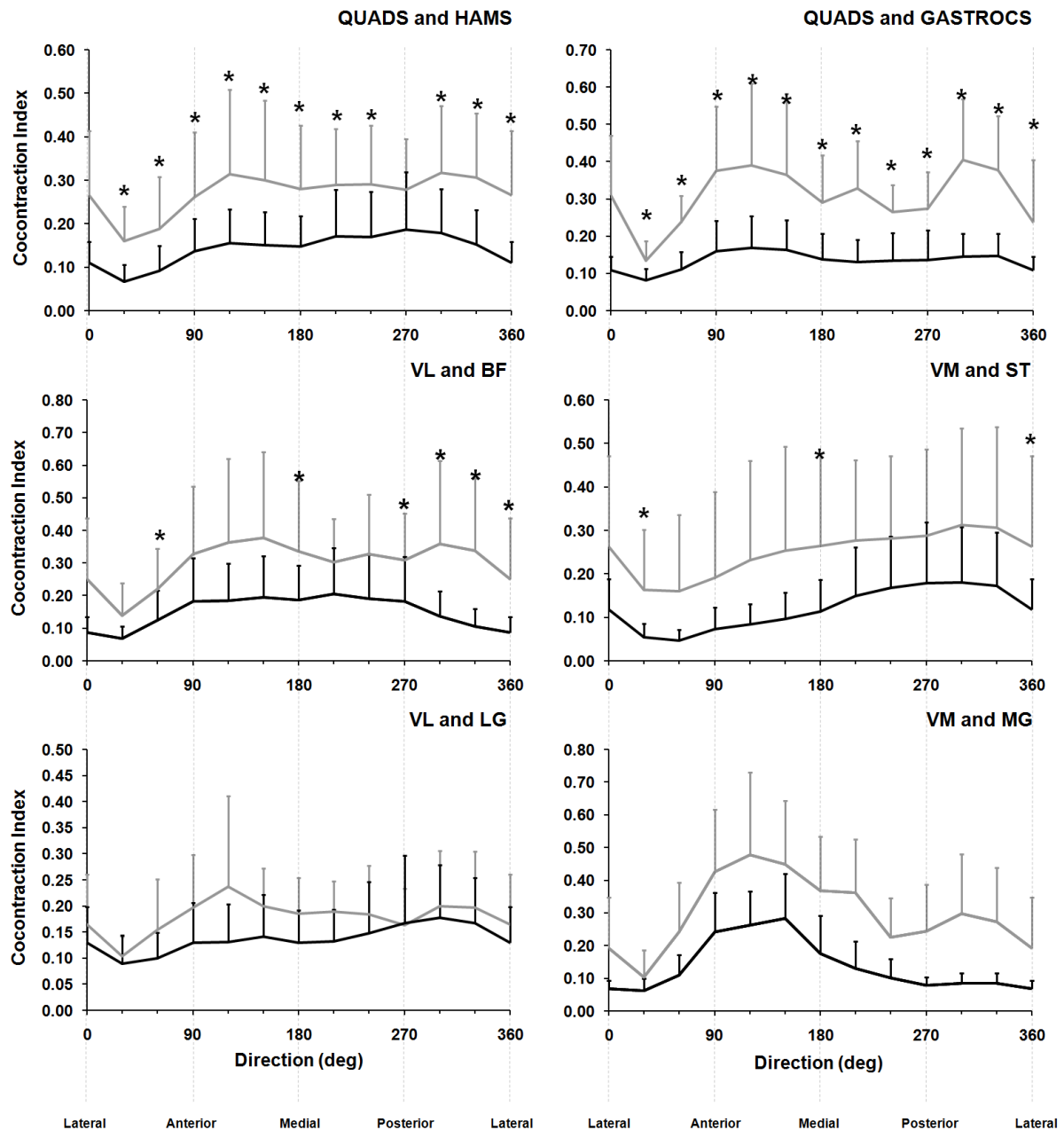


Figure 12: Antagonistic muscle pair coactivation index (CI), including post hoc analysis results for young (black) and older adults (grey). Target locations are along the x-axis (0-360), 0 and 360 represent the same target location. * indicates targets with significant differences in CI levels between age groups. Bars represent group standard deviations.

DISCUSSION

The aim of this study was to compare quasi-isometric voluntary control strategies of muscles which cross the knee joint of both young and older healthy adults during a

target matching protocol under physiologically relevant weight bearing conditions. The GRFs needed to match a target in this study were equivalent to, or greater than, medial-lateral GRF seen during running initiation at 3.8 m/s (0.3 N/kg and 0.4 N/kg respectively) or those seen anterior-posteriorly during gait termination (0.5 N/kg, 0.1 N/kg respectively) and vertical jump landing (0.4 N/kg, 0.1 N/kg respectfully) (McClay et al., 1994), indicating the external validity of this study (i.e.: that the forces generated at the knee joint reflect those of daily living). Mean ensemble activations and mean activations in each target direction were compared to determine whether particular muscles had differing activation characteristics. It was hypothesised that older healthy adults would have presented significantly less specificity, with significantly greater activation magnitudes and coactivation. Present muscle activation magnitudes indicate that as expected, older adults recruit significantly more muscle than younger adults to match identical force targets.

The presence and commonality of muscle weakness among the elderly suggest that as muscle plays a complex role in the stabilization of the knee joint, weakness may then play a significant role in the development of OA (Herzog & Longino, 2007; Rehan Youssef, Longino, Seerattan, Leonard, & Herzog, 2009). Force control variability, however, is not related to muscle strength (Hortobagyi et al., 2001). This observation is important as tests for strength are often used to identify those at risk of reduced functional ability (Lauretani et al., 2003). Our tests for activation variability in respect to Φ reveal no significant differences in muscle SI, or in Φ when asymmetrical activation patterns are found, suggesting that healthy older adults use similar activation synergies to reach targets as young adults. High $ICC_{(2,k)}$ values ranging between 0.84 and 0.98,

deemed to be excellent as they exceed 0.80 (Rainoldi et al., 1999; Rainoldi, Bullock-Saxton, Cavarretta, & Hogan, 2001; Portney& Watkins, 2000), identify that young and older adults have highly comparable activation patterns, indicating the use of similar activation strategies. Our results do however support our hypothesis that older adults would utilize greater coactivation defined by a CI for the quadriceps-hamstring and quadriceps-gastrocnemius grouped pairs as well as the VL-BF and VM-ST muscle pairs.

The use of a multi directional force matching protocol to investigate neuromuscular control of an older population is novel. Thus, our hypotheses were based on non-weight bearing, isometric, and isokinetic force control observations (DeVita et al., 2001; Hortobagyi et al., 2004; Williams et al., 2003), as well as dynamic observations during ADL between young and older adults (DeVita&Hortobagyi, 2000; Hortobagyi et al., 2003; Hortobagyi et al., 2004; Hortobagyi et al., 2005; Hortobagyi&Devita, 2006).

In previous investigations conducted by Hortobagyi et al. (2003; 2005), percent EMG of the VL, was as high as twofold greater during chair raises, stair ascent, and stair descent in older adults. This is consistent with our findings where the VL $X_{(EMG)}$ was $27\% \pm 11$ for older adults and $16\% \pm 8$ for younger subjects. This nearly twofold increase was seen in all muscles we tested except the LG (Figure 11). It has also been observed that increased activation ratios used by older adults during ADL had a moderate correlation with increased relative effort (ratio of functional joint moments to maximal joint moments), accounting for nearly 50% of observed variance between age populations (Hortobagyi et al., 2005). It has been suggested that this may be due to a concomitant effect of coactivation where increased antagonist contributions would have to be overcome to complete a given task (Ebenbichler et al., 1998; Hortobagyi et al., 2005).

Thus, as antagonist activity increased, agonist contributions would have to as well in order to overcome the opposing force, thus increasing relative effort. The contribution of coactivation to increased relative effort is under debate (Heiden, Lloyd, & Ackland, 2009); however, as target positions were standardized to a relative effort of 30% MVIC, observed variability in muscle activations in our investigation cannot be due to coactivation affecting relative effort. Instead it may be evidence of an altered neuromuscular strategy used to stabilize the knee. It also supports that even healthy older adults require up to, and more than, double the muscle activation magnitudes required by younger adults to complete an identical task.

When interpreting coactivation strategies used by older adults, global increases in all directions were observed for the QUADS-HAMS and QUADS-GASTROCS muscle groups. VL-BF and VM-ST muscle pairs also showed older adults had significantly higher CIs, limited to target directions of: 60°; 180°; 270°; 300°; 330°; 360/0°, and: 30°; 180°; 360/0°, respectively ($p < 0.025$). However, trends towards significance ($p < 0.05$) were found in all remaining target directions for the VM-ST and VL-BF, excluding at 210° for the latter pair. The commonality of increased coactivity directionally and during ADL (Hortobagyi et al., 2003; Hortobagyi et al., 2005; Hortobagyi et al., 2005) combined with similarity between coactivation patterns (Figure 12), suggests that increased antagonist activation may represent a required compensation to increased limb and joint instability, which is known to be a concomitant effect of muscle weakness (Solomonow et al., 1987). Currently, it is unknown at what age muscle activation patterns change, but it is interesting that our healthy subjects present such dramatic differences in muscle activation magnitudes and coactivity at a relatively young age (59+/-5 years. While

increased muscle recruitment and coactivation have been suggested as precursors to the development of OA, their presence clearly does not necessarily indicate a maladaptation to aging since our population had healthy knees. Hortobagyi et al. (2005) suggested that the source of the coactivation is primary to the presence of coactivity, and that specific reduction in quadriceps activation and increased hamstrings muscle activity are a better indicator of a maladaptation to muscle weakness. This suggestion is consistent with previous investigations that found antagonistic muscle pair imbalance (quadriceps, not hamstring weakness) was prevalent amongst OA populations (Hortobagyi et al., 2004; Slemenda et al., 1997).. Quadriceps weakness relative to the hamstrings could represent a muscular imbalance and loss of normal coactivation patterns which may alter normal joint mechanics and contact pressure distribution on articulating surfaces (Fitzgerald, Piva, Irrgang, Bouzubar, & Starz, 2004; Li, DeFrate, Zayontz, Park, & Gill, 2004).

Muscle Activation Patterns

In previous OKC studies, older adults were found to possess a diminished capacity to stabilize force accurately or steadily during isokinetic force control tasks (DeVita et al., 2001; Hortobagyi et al., 2004). These studies, combined with observed coactivation strategies (Hortobagyi&DeVita, 2000; Hortobagyi et al., 2003; Hortobagyi et al., 2005; Williams et al., 2003) and known variability in older adults ability to scale or fine tune force output (Connelly, Rice, Roos, & Vandervoort, 1999; Doherty et al., 1993; Roos et al., 1997), prompted our hypothesis that muscle activation patterns would be less specific around one activation direction and more circular around the polar plot origin. Our results, however, do not support these hypotheses and indicate the importance of including weight bearing when measuring neuromuscular control.

Since subjects were required to maintain equal weight on both feet, more physiologically relevant data was collected since the shear and compressive loads on the joints were more similar to those occurring during ADL: thus improving the external validity of the study. As such, we believe our results to be more representative of functional conditions, and believe that observed variability in muscle activation between groups to be reflective of altered neuromuscular strategy, and due less to inherent variability within the task itself.

The addition of body weight also resulted in muscle activation patterns with lower specificity and directions of activation that were not reflective of their previous role as knee flexor or extensor. For example, according to Buchanan & Lloyd (1997), the moment arm with which the BF and MG should be primarily active is in the posterolateral (330°) and posteromedial (225°) axes, respectively. Conversely, Flaxman (2011) found group Φ for the BF and MG to be in the medial (176°) and anterior-medial (135°) loading directions. Muscles yielding significant asymmetry ($p=0.000$) (BF, ST, and MG) with no significant differences in mean Φ coincide with higher specificity values ranging between 0.314 and 0.380. Interestingly observed specificity of both young and older subjects are low to those of previous work; supporting that muscle are less specific when supporting body weight (Flaxman 2011).

Contradictory to Buchanan and Lloyd (1997) Φ of the BF and MG counteract their primary functional moment arm. In support of observations made by Flaxman, (2011) the BF and MG act as specific stabilisers for valgus moments generated by hip flexors and knee extension moments. Observed ST activation profiles coincided with activation patterns observed previously (Williams et al. 2003; Flaxman,

2011). Predominant posterior activation (Buchanan & Lloyd, 1997) implies the ST's role is to create force in its primary functional axis as a specific moment generator (Flaxman 2011).

The TFL presented low X_{EMG} activations in all directions ($<10\%$), allowing for relatively small changes (that may not be functional) to result in large variability. Low activation may explain why TFL asymmetry and Φ were significantly different between young and older adults. Activation patterns between the two groups are relatively similar however, ($ICC_{(2,k)}=0.87$) thus, we believe that despite identified asymmetry, the TFL activation patterns between young and older adults are similar but play a minimal role in respect to this task. Remaining muscles (RF, VL, VM, and LG) presented relatively lower SIs between 0.11-0.24, and non-significant asymmetry ($p>0.05$).

Interestingly, despite significant increases in muscle activation and coactivation, muscle specificity between groups is remarkably similar. Similar vasti activation patterns across age ($ICC_{(2,k)} VL=0.90$, $VL=0.91$ respectively), is an important observation as quadriceps dysfunction is an indicator of functional ability linked to OA (Hortobagyi et al., 2005; Lewek et al., 2005). These similarities between groups support that quadriceps dysfunction is not a direct effect of age (Heiden et al., 2009; Phillips, Bruce, Newton, & Woledge, 1992).

This study is not, however, without limitations. Subjects recruited for this study were to be free of a history of knee problems and lower leg injuries. Thus, as with any study with exclusion criteria, we may have entered a selection bias towards individuals who have successfully avoided knee pathologies and were physically active. Therefore, the application of our results to the understanding of what protects the knee from

developing OA may be specific to an active population. That being said, if used as a control for future comparison studies with populations with joint instability, such as those with OA and who are active, the similarities between young and older active adults may provide valuable insight into the development of OA

Finally, the effects of gender specific knee stabilization methods were not controlled in this investigation. Despite no difference in healthy older men's and women's ability to fully activate musculature (Heiden et al., 2009; Kent-Braun & Ng, 1999; Phillips et al., 1992), women are found to be weaker relative to body weight, experiencing greater losses in muscle mass (Kent-Braun & Ng, 1999) while also having greater varus-valgus joint laxity. Thus, you might expect women to have greater muscle activation magnitudes and coactivity to stabilize the knee with advancing age. It is possible observed age and gender differences may act as a source of error in our investigation. As women have a higher incidence of developing OA (Srikanth et al., 2005), the effect of gender and age merits further investigation.

Conclusion

The analysis of healthy age related differences in neuromuscular control provides insight into how control strategies evolve over time. We have demonstrated that despite older adults having muscle weakness, increased muscle activation, and increased coactivation of antagonistic muscle pairs, muscles' functional roles are maintained. The omni-presence and similar coactivation patterns of the quadriceps-hamstrings and quadriceps-gastrocnemius muscle groups amongst healthy older adults with no signs or symptoms of OA suggest that rather than coactivation being considered a maladaptation to aging, it may in fact be a necessary adaptation to muscle weakness. Results in this

study further support the need to rethink knee joint muscle roles in support of findings made by Flaxman (2011): similar activation patterns confirm that the VL, VM, and LG act as omni-directional joint stabilisers, the BF and MG as specific stabilisers, and the ST as a directionally specific moment generator. These roles were maintained by both younger and older subjects, contrary to our hypothesis. This suggests that healthy older adults utilise similar stabilisation synergies as younger adults, albeit at higher activation magnitudes, to increase compressive forces at the knee. The net effect creates a stable environment for directionally specific moment generators to control ground reaction forces. While speculative, it seems that if muscle activation synergies needed to stabilize the knee are maintained with age, healthy knee joint mechanics and soft tissue loading will be as well. These similarities may contribute to protecting articulating surfaces of the knee from the associated degenerative effects of reduced and altered loading, joint laxity, muscle weakness, and altered neuromuscular control associated with developing OA (Herzog & Longino, 2007; Rehan Youssef et al., 2009).

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General Discussion and Conclusions

Given that the protocol used in this study was novel, it was important to establish its reliability. The first article explored and evaluated the reliability of this protocol by measuring the intra-session, test-retest and inter-subject reliability of muscle activations of 10 individuals in response to two sessions of our weight-bearing force target matching task. Results from these analyses reveal intra-session (within day trial-to-trial) muscle activation reliability to have moderate to high ICC values in most loading directions. Similarly, for test-retest reliability between days for the quadriceps and hamstrings ICC values ranged between 0.60-0.94. Finally activation pattern reliability indicated that subjects utilised similar activation strategies to achieve targets indicated by moderate to high ICCs (0.63-0.96) between testing days. Overall we found our protocol had strong correlations between weight bearing motor control strategies and GRFs.

There were instances where lower reliability was found, specifically including biauriculate muscle (LG, MG, and TFL). In particular were the gastrocnemius muscles, where for example the MG presented insufficient variability to conduct an ICC day-to-day in target directions: 90, 120, and 150°, with muscle activation exceeding 20% MVIC. This example leaves the SEM to provide reliability information, however in these directions SEM were also found to be high compared to their activation magnitudes. Further investigation into the role of these muscles may provide greater insight into their contributions with regards to matching directional force targets.

This observation and other discrepancies with previous findings in terms of joint specificity, and mean direction of activation could be attributed to our test being

conducted while standing and thus the inclusion of BW. Unlike seated tasks, our task involves the coordination of the entire lower limb where contributions from the hip, knee, and ankle joints are present and compressed by the effects of BW. As our protocol is tangent on Newton's third law, dictating that as subjects' plant their foot during target matching, the force exerted by the subject to match the target is equal and opposite to the force exerted by the ground to the person. The resulting GRFs are transmitted across the joints in the form of shear and compressive loads. The subject responds to these loads by contracting knee joint musculature to stabilise the joint as to maintain a rigid body to control the target. Moments at each target direction about the hip, knee, and ankle are presented in Appendix E. These results were not statistically analysed as they go beyond the scope of this thesis but are present to allow a better interpretation of where forces are generated and stabilised during force matching.

Prevailing hip moments in all functional axes were observed over knee and ankle moments for both older and younger adults. Moments about the knee were greatest during anterior targets, and were minimal in posterior directions, demonstrating flexion moments <0.05 Nm/kg. Large hip extension moments were however observed in medial, posterior, and lateral directions. Moments for both the hip and knee were greatest in the y-axis, peaking medially and reducing nearly to zero in lateral target directions.

Dominant hip moments in all functional axes likely act as actuators, driving cursor navigation. Knee musculature must then act to maintain static equilibrium, allowing forces to be transmitted through the segment. This reality is supported by the vasti muscles non-specific activation profiles with omnidirectional activation magnitudes.

Supporting activation profiles of the MG and BF, too demonstrate the stabilization role of knee joint musculature as stabilizers to dominant hip moments.

The second purpose of this thesis was to evaluate age related differences in neuromuscular control about the knee joint during our target matching protocol. Using this method we were able to identify specific contributions of individual muscles and how these contributions change with the effects of age. Mean ensemble activation and mean activations in each target direction were compared to determine whether particular muscles had differing activation characteristics. It was hypothesised that older healthy adults would have presented significantly less specificity, with significantly greater muscle activation magnitudes and coactivation.

Our results indicated that activation magnitudes amongst the older subject group were two folds greater compared to younger subjects in all investigated muscle excluding the LG. It was too observed that older subjects utilised greater coactivation between quadriceps and hamstrings and quadriceps gastrocnemius muscle pairs. Results contrary to our hypotheses however revealed that tests for activation variability in respect to Φ were not significantly different, nor was Φ when asymmetrical activation patterns were found. Similar results regarding the BF, ST, and MG muscle activation patterns are particularly interesting, as our hypothesis was that older adult's activation profiles would be less specific and circular in pattern around the polar plot origin. Thus circular results for the vastii muscles were not necessarily surprising and fit our hypotheses. Similarly skewed results and non significant differences in activation patters between the BF, ST, and MG indicate that older adults' specificity is similar to younger adults', suggesting they utilise similar muscle synergies to stabilise the knee and generate joint moments.

Within-target variance or the similarity between age group activation patterns were supported by high ICC values ranging between 0.84 and 0.98.

This study was the second step of a longer and larger research program. Our results here can act as a baseline for future comparisons against other symptomatic populations like active older adults with OA. There are some limitations however which require further discussion. The effects of gender specific knee stabilization methods were not controlled in this investigation. This is a potential source of error as it has been observed that neuromuscular control patterns of young females when stabilizing the knee can differ to males (Wojtys et al. 782-789; Wojtys, Ashton-Miller and Huston 10-16; Kanehisa, Ikegawa and Fukunaga 148-154). In an attempt to determine if gender acted as a covariate, the effects of gender and age were statistically analysed. Results from this analysis are reported in Appendix F. Young women presented with significant main effects of gender for greater quadriceps and gastrocnemius coactivation ($p=0.037$; power=0.573) between the VM & MG ($p=0.021$; power=0.673) determined using a mixed between within factorial (2x12) ANOVA for gender and target direction. A potential trend was also observed between VL & MG ($p=0.057$; power=0.489), consistent with previous observations made by Flaxman (2011). Additionally younger women also presented with less MG specificity ($p=0.026$; Men: Mean= 0.449, SD=0.098; Women: Mean=0.273, SD=0.181). Older women also present with significantly higher VM & MG coactivation ($p=0.021$; power=0.681), however no significant differences were observed for MG specificity or for VL & LG coactivation. Increased coactivation amongst older and younger women may suggest that a coactivation strategy is conserved with age, and may act as a covariate in our investigation. Younger women have also been found to

utilise greater quadriceps and hamstring activation during static and dynamic tasks (Krishnan et al. 2008; Pauda et al. 2006; Sigward & Powers, 2006). This observation was not present in our investigation, nor was it observed by Flaxman 2011. Older women also do not appear to utilise greater muscle activation, evident by no significant difference in X_{EMG} , contrary to what might be expected, as older women have increased joint laxity (Kent-Braun & Ng, 1999) and are perceived to experience greater losses in muscle mass compared to men. Investigations into gender differences and age with respects to muscle activation and strength have found healthy older men's and women's ability to fully activate musculature to be equal (Heiden, Lloyd and Ackland 807-815; Kent-Braun and Ng 22-29; Phillips et al. M45-9) and that the relationship between quadriceps size and strength in older men is very similar to those reported for older women (Young, Stokes, & Crowe, 1984; Young, Stokes, & Crowe, 1985). Thus unless some other form of pathological condition like OA is present, it may be that muscle weakness can be compensated for by using an alternate strategy like selective increases in coactivation to compensate for muscle weakness and associated joint laxity. Since women have a higher incidence of developing OA (Srikanth et al. 769-781) the effect of gender and age merits further investigation.

Finally although subjects were asked to maintain the same sagittal hip, knee, and ankle joint positions throughout target matching, we observed older subjects to do so with significantly less hip flexion (Appendix C). Our primary objective in terms of controlling for joint angle was at the knee in the sagittal plain. As no significant difference was observed, we did not conduct a covariate test for this variable. Lesser hip flexion angles have been seen to result in reduced RF (Maffiuletti & Lepers, 2003) and greater hamstring

activations (Lunnen et al., 1981). Thus because hip angles were less among older adults, it may be that RF muscle activations for this population may have been under-estimated, and hamstring activations over-estimated. Observations where EMG activation differences were observed due to flexion angle, occurred at angle increments greater than or equal to 45° (Lunnen et al., 1981; Maffiuletti & Lepers, 2003). The mean difference in hip flexion between age groups in our investigation was 6.64° , thus it is unlikely to have affected our results.

In conclusion, we have developed a protocol that requires subjects to modulate GRF direction and magnitude while maintaining 50% BW. Reliability analyses showed that subjects utilised similar activation synergies to modulate GRF to match targets between trials and testing sessions. Our findings support that groups who have undergone the same number of testing sessions can be compared, and that a single testing session is all that is required to compare neuromuscular control strategies used by a group to achieve target locations. It has also been observed that similar activation synergies used by active young adults are conserved by active adults of an age of 59 ± 5 years. These similarities were conserved despite evidence of an altered neuromuscular control strategy presenting significant increases in muscle activation and coactivation of antagonistic muscle pairs. Continued research into activation characteristics of those with a knee joint pathology may provide valuable insight into knee joint injury neuromuscular strategies, or potential maladaptions to age which may inevitably result in injury or OA without intervention.

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Appendix A – University of Ottawa’s Marker Placement Set

Figure 13: Schematic representation of University of Ottawa’s marker placement set.

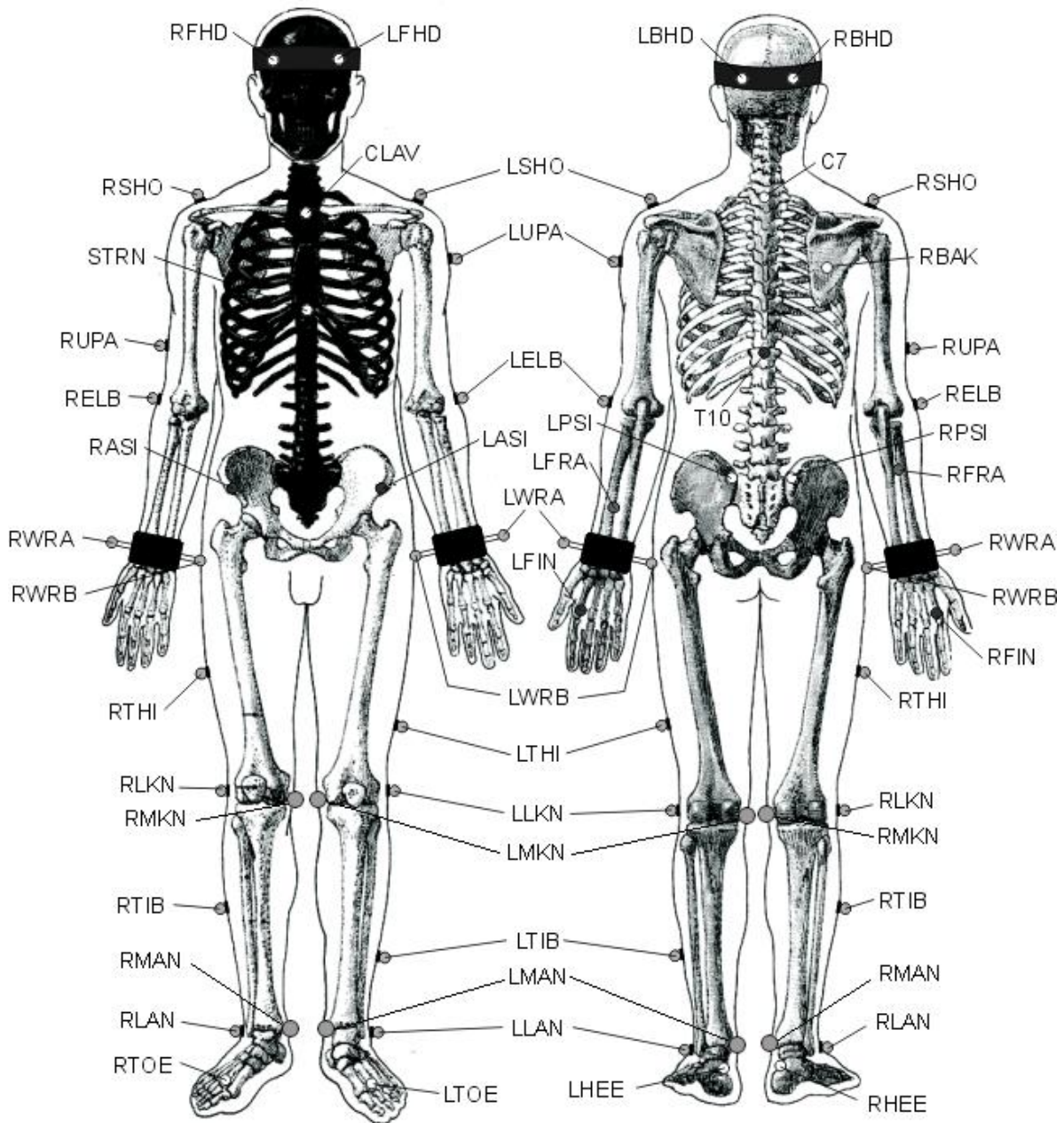


Table 5: Markers and their respective acronym descriptions that were used in this study. Each description corresponds to where the markers should be placed on the subject. Acronyms beginning with an “L” or “R” correspond to the left and right sides, respectively.

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 – Specificity Index (Flaxman et al., 2011)

Variance of Muscle Activation: Specificity Index

The specificity index (SI), representing the variance of muscle activation about its “mean direction of muscle activity” or resultant vector (REMG). To define the SI the mean *absolute* horizontal (Rx) and vertical components (Ry) are calculated by summing all the *absolute* x or y components of each vector respectively then dividing the result by the number of summated vectors components (N):

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

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

Pythagorean Theorem using the mean *absolute* horizontal and vertical components is used to determine the *absolute* resultant EMG (or summed EMG magnitudes of all the vectors) ($|\Sigma EMG_i|$):

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

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 the dominant leg of young and older subjects. 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 (*).

Age		Hip:X	Hip:Y	Hip:Z	Knee:X	Knee:Y	Knee:Z	Ankle:X	Ankle:Y	Ankle:Z
Young	<i>Mean</i>	30.66	-4.98	-0.70	20.87	-0.39	-0.52	-0.97	-0.12	4.75
	<i>SD</i>	8.18	4.62	12.21	7.26	4.24	15.65	7.46	2.81	10.36
Older	<i>Mean</i>	24.02	-4.36	-5.31	18.03	-2.15	9.31	-0.16	0.31	-0.11
	<i>SD</i>	8.51	4.13	11.92	6.76	6.70	11.58	5.27	0.77	7.52
p-Value		0.021*	0.779	0.293	0.247	0.379	0.051	0.699	0.485	0.132

* See Table 7 for descriptors.

Table 7: 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 – 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 (X_{EMG}), or specificity index (SI) for each muscle differed significantly across age (Table 8). Older adults TFL Φ was significantly different compared to younger adults ($M=180.9$, $SD=60.5$ vs $M=221.7$, $SD=69.4$, $t(32)$, $p<0.020$). Older adults displayed significantly greater X_{EMG} than younger adults for all muscle excluding the LG: RF ($M=0.13$, $SD=0.05$ vs. $M=0.08$, $SD=0.05$, $t(30.0)=-2.45$, $p<0.020$), VL ($M=0.27$, $SD=0.11$ vs. $M=0.15$, $SD=0.08$, $t(31.0)=-0.365$, $p<0.001$), VM ($M=0.23$, $SD=0.11$ vs $M=0.13$, $SD=0.07$, $t(23.6)=-3.36$, $p<0.003$), BF ($M=0.36$, $SD=0.15$ vs $M=0.15$, $SD=0.09$, $t(21.7)=-4.61$, $p<0.000$), ST ($M=0.24$, $SD=0.16$ vs $M=0.13$, $SD=0.07$, $t(18.46)=-2.65$, $p<0.016$), MG ($M=0.30$, $SD=0.12$ vs $M=0.14$, $SD=0.05$, $t(17.88)=-4.81$, $p<0.000$), and TFL ($M=0.07$, $SD=0.06$ vs $M=0.04$, $SD=0.03$, $t(19.70)=-2.14$, $p<0.045$).

A two way within-between factorial (2×12) analysis of variance (ANOVA) was utilised to examine each muscles EMG activity to see if it significantly differed as a result of age and target direction (Table 9). Sample data means of EMG magnitude were compared simultaneously to the effects of age and target direction allowing 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 ($p<0.05$) in all the tests; Huynh-Feldt adjusted values were therefore examined. The results displayed significant main effect for target location at the $p<0.05$ level and on EMG magnitude for all muscles. All muscles excluding LG showed significant main effects of age on EMG

magnitude: RF ($F(1, 30) = 5.993$, $p = 0.020$, partial $\eta^2 = 0.167$, power = 0.659), VL ($F(1, 30) = 12.860$, $p = 0.001$, partial $\eta^2 = 0.300$, power = 0.934), VM ($F(1, 30) = 11.445$, $p = 0.002$, partial $\eta^2 = 0.276$, power = 0.905), BF ($F(1, 30) = 22.643$, $p = 0.000$, partial $\eta^2 = 0.430$, power = 0.996), ST ($F(1, 30) = 7.675$, $p = 0.010$, partial $\eta^2 = 0.204$, power = 0.764), MG ($F(1, 30) = 26.468$, $p = 0.000$, partial $\eta^2 = 0.459$, power = 0.998), TFL ($F(1, 30) = 4.952$, $p = 0.034$, partial $\eta^2 = 0.142$, power = 0.577). 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 for all muscles excluding LG between young and older adults at the $p < 0.025$ level (Bonferonni adjustment for repeated measures: $\alpha = 0.05/25 = 0.025$) (Table 10). For the RF, older adults had significantly greater EMG magnitude at the target locations of: 60° ($M=0.114$, $SD=0.047$ vs. $M=0.073$, $SD=0.038$, $t(31)=-2.821$, $p < 0.008$), 90° ($M=0.161$, $SD=0.077$ vs. $M=0.090$, $SD=0.052$, $t(30)=-3.058$, $p < 0.005$), 150° ($M=0.127$, $SD=0.034$ vs. $M=0.081$, $SD=0.048$, $t(31)=-2.376$, $p < 0.024$), and 180° ($M=0.109$, $SD=0.037$ vs. $M=0.0109$, $SD=0.058$, $t(31)=-2.448$, $p < 0.020$). For the VL, older adults had significantly greater EMG magnitude at the target locations of: 0° ($M=0.313$, $SD=0.136$ vs $M=0.179$, $SD=0.095$, $t(30)=-3.27$, $p < 0.003$), 30° ($M=0.252$, $SD=0.106$ vs $M=0.143$, $SD=0.076$, $t(30)=-3.366$, $p < 0.002$), 60° ($M=0.215$, $SD=0.104$ vs $M=0.128$, $SD=0.069$, $t(30)=-2.828$, $p < 0.008$), 90° ($M=0.251$, $SD=0.125$ vs $M=0.129$, $SD=0.075$, $t(30)=-3.396$, $p < 0.002$), 120° ($M=0.304$, $SD=0.185$ vs $M=0.128$, $SD=0.057$, $t(16.3)=-3.542$, $p < 0.003$), 150° ($M=0.268$, $SD=0.141$ vs $M=0.136$, $SD=0.077$, $t(21.1)=-3.237$, $p < 0.004$), 180° ($M=0.255$, $SD=0.134$ vs $M=0.127$, $SD=0.067$, $t(20.1)=-3.34$, $p < 0.003$), 210° ($M=0.268$, $SD=0.134$ vs $M=0.147$, $SD=0.078$, $t(30)=-3.169$, $p < 0.004$),

240° (M=0.276, SD=0.152 vs M=0.149, SD=0.087, $t(30)=-2.954$, $p<0.006$), 300° (M=0.317, SD=0.127 vs M=0.182, SD=0.096, $t(30)=-3.399$, $p<0.002$), and 330° (M=0.314, SD=0.137 vs M=0.193, SD=0.112, $t(30)=-2.742$, $p<0.01$). For the VM, older adults had significantly greater EMG magnitude at the target locations of: 0° (M=0.247, SD=0.087 vs M=0.15, SD=0.104, $t(30)=-2.852$, $p<0.008$), 30° (M=0.185, SD=0.074 vs M=0.117, SD=0.068, $t(30)=-2.7$, $p<0.011$), 60° (M=0.189, SD=0.1 vs M=0.098, SD=0.057, $t(21.696)=-3.09$, $p<0.005$), 90° (M=0.23, SD=0.132 vs M=0.104, SD=0.053, $t(18.025)=-3.441$, $p<0.003$), 120° (M=0.243, SD=0.167 vs M=0.107, SD=0.05, $t(16.234)=-3.028$, $p<0.008$), 150° (M=0.237, SD=0.16 vs M=0.105, SD=0.053, $t(16.71)=-3.045$, $p<0.007$), 180° (M=0.23, SD=0.153 vs M=0.107, SD=0.058, $t(17.537)=-2.941$, $p<0.009$), 210° (M=0.242, SD=0.124 vs M=0.118, SD=0.07, $t(21.374)=-3.416$, $p<0.003$), 240° (M=0.226, SD=0.118 vs M=0.125, SD=0.069, $t(30)=-3.001$, $p<0.005$), and 300° (M=0.27, SD=0.115 vs M=0.153, SD=0.096, $t(30)=-3.146$, $p<0.004$). For the BF, older adults had significantly greater EMG magnitude at the target locations of: 0° (M=0.192, SD=0.12 vs M=0.058, SD=0.03, $t(15.506)=-4.209$, $p<0.001$), 60° (M=0.243, SD=0.156 vs M=0.102, SD=0.085, $t(30)=-3.229$, $p<0.003$), 90° (M=0.402, SD=0.244 vs M=0.202, SD=0.137, $t(30)=-2.893$, $p<0.007$), 120° (M=0.458, SD=0.261 vs M=0.241, SD=0.15, $t(30)=-2.925$, $p<0.006$), 150° (M=0.507, SD=0.254 vs M=0.257, SD=0.202, $t(30)=-3.108$, $p<0.004$), 180° (M=0.451, SD=0.219 vs M=0.249, SD=0.181, $t(30)=-2.87$, $p<0.007$), 210° (M=0.454, SD=0.215 vs M=0.209, SD=0.138, $t(30)=-3.891$, $p<0.001$), 240° (M=0.474, SD=0.259 vs M=0.177, SD=0.117, $t(18.965)=-4.085$, $p<0.001$), 270° (M=0.404, SD=0.216 vs M=0.141, SD=0.088, $t(18.025)=-4.398$, $p<0.000$), 300° (M=0.368, SD=0.234 vs M=0.097, SD=0.054, $t(15.33)=-4.371$, $p<0.001$), and 330°

($M=0.284$, $SD=0.17$ vs $M=0.076$, $SD=0.048$, $t(15.992)=-4.581$, $p<0.000$). For the ST, older adults had significantly greater EMG magnitude at the target locations of: 30° ($M=0.116$, $SD=0.1$ vs $M=0.046$, $SD=0.033$, $t(16.73)=-2.573$, $p<0.02$), 90° ($M=0.143$, $SD=0.108$ vs $M=0.061$, $SD=0.047$, $t(18.697)=-2.712$, $p<0.014$), 120° ($M=0.182$, $SD=0.118$ vs $M=0.082$, $SD=0.067$, $t(21.55)=-2.896$, $p<0.008$), 150° ($M=0.216$, $SD=0.142$ vs $M=0.104$, $SD=0.077$, $t(21.001)=-2.73$, $p<0.013$), and 180° ($M=0.265$, $SD=0.16$ vs $M=0.136$, $SD=0.09$, $t(21.426)=-2.778$, $p<0.011$). For the MG, older adults had significantly greater EMG magnitude at the target locations of: 0° ($M=0.192$, $SD=0.155$ vs $M=0.067$, $SD=0.025$, $t(14.652)=-3.066$, $p<0.008$), 60° ($M=0.242$, $SD=0.151$ vs $M=0.11$, $SD=0.063$, $t(18.224)=-3.173$, $p<0.005$), 90° ($M=0.425$, $SD=0.192$ vs $M=0.242$, $SD=0.12$, $t(30)=-3.274$, $p<0.003$), 120° ($M=0.477$, $SD=0.253$ vs $M=0.262$, $SD=0.103$, $t(18.057)=-3.064$, $p<0.007$), 150° ($M=0.449$, $SD=0.193$ vs $M=0.283$, $SD=0.135$, $t(30)=-2.832$, $p<0.008$), 180° ($M=0.367$, $SD=0.166$ vs $M=0.177$, $SD=0.115$, $t(30)=-3.809$, $p<0.001$), 210° ($M=0.361$, $SD=0.165$ vs $M=0.13$, $SD=0.082$, $t(19.925)=-4.91$, $p<0$), 240° ($M=0.225$, $SD=0.121$ vs $M=0.101$, $SD=0.057$, $t(19.397)=-3.634$, $p<0.002$), 270° ($M=0.244$, $SD=0.142$ vs $M=0.078$, $SD=0.025$, $t(14.775)=-4.479$, $p<0$), 300° ($M=0.298$, $SD=0.181$ vs $M=0.086$, $SD=0.03$, $t(14.7)=-4.481$, $p<0$), and 330° ($M=0.273$, $SD=0.165$ vs $M=0.085$, $SD=0.031$, $t(14.892)=-4.339$, $p<0.001$). For the ST, older adults had significantly greater EMG magnitude at the target location of 240° ($M=0.062$, $SD=0.058$ vs $M=0.022$, $SD=0.023$, $t(17.781)=-2.497$, $p<0.023$).

Table 8: Descriptive statistics of the muscle activation characteristics for each muscle of young and older subjects. Significant differences between ages was determined with independent T tests

Muscle	Sex	XEMG			Φ			SI		
		Mean	SD	pValue	Mean	SD	pValue	Mean	SD	pValue
RF	Young	0.084	0.047	0.020*	168.7	57.9	0.226	0.133	0.102	0.730
	Older	0.126	0.050		128.6	77.7		0.145	0.088	
VL	Young	0.152	0.075	0.001*	255.5	65.3	0.445	0.095	0.080	0.090
	Older	0.273	0.113		233.3	50.6		0.152	0.106	
VM	Young	0.126	0.071	0.003*	274.0	53.1	0.603	0.115	0.066	0.177
	Older	0.234	0.108		207.3	58.7		0.166	0.129	
BF	Young	0.155	0.089	0.000*	176.9	29.1	0.970	0.314	0.148	0.520
	Older	0.363	0.154		195.5	30.5		0.350	0.172	
ST	Young	0.126	0.067	0.016*	252.6	27.8	0.790	0.357	0.109	0.514
	Older	0.241	0.156		244.3	29.5		0.383	0.115	
LG	Young	0.120	0.087	0.172	200.9	67.9	0.835	0.218	0.141	0.190
	Older	0.164	0.091		189.2	62.1		0.156	0.118	
MG	Young	0.140	0.049	0.000*	139.9	38.7	0.535	0.356	0.170	0.888
	Older	0.305	0.124		156.0	27.4		0.348	0.153	
TFL	Young	0.037	0.028	0.045*	221.7	60.5	0.020	0.249	0.195	0.428
	Older	0.072	0.057		180.9	69.4		0.198	0.172	

Abbreviations: Rectus femoris (RF); vastuslateralis (VL); vastusmedialis (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 9: Statistical significance of main effects of age and target direction on EMG amplitude. A two way within-between factorial ANOVA was used to determine the reported p values.

Muscle	Main Effect of Direction	Direction *Age	Main Effect of Age
RF	0.000*	0.164	0.020*
VL	0.000*	0.193	0.001*
VM	0.002*	0.351	0.002*
BF	0.000*	0.079	0.000*
ST	0.000*	0.286	0.010*
LG	0.016*	0.174	0.172
MG	0.000*	0.064	0.000*
TFL	0.007*	0.363	0.034*

.Asterisks (*) identifies significant differences at the $p < 0.05$ level.

Table 10: Post hoc evaluation of mean EMG amplitude differences at each target location of young and older adults. Independent T tests were used to determine significance.

Direction	Sex	RF			VL			VM			TFL		
		Mean	SD	pValue	Mean	SD	pValue	Mean	SD	pValue	Mean	SD	pValue
0°	Young	0.086	0.054	0.082	0.179	0.095	0.003*	0.150	0.104	0.008*	0.051	0.048	0.166
	Older	0.117	0.043		0.313	0.136		0.247	0.087		0.082	0.073	
30°	Young	0.077	0.043	0.050	0.143	0.076	0.002*	0.117	0.068	0.011*	0.026	0.024	0.065
	Older	0.106	0.038		0.252	0.106		0.185	0.074		0.057	0.056	
60°	Young	0.073	0.038	0.008	0.128	0.069	0.008*	0.098	0.057	0.005*	0.028	0.029	0.079
	Older	0.114	0.047		0.215	0.104		0.189	0.100		0.059	0.058	
90°	Young	0.090	0.052	0.005*	0.129	0.075	0.002*	0.104	0.053	0.003*	0.044	0.037	0.059
	Older	0.161	0.077		0.251	0.125		0.230	0.132		0.095	0.092	
120°	Young	0.104	0.065	0.027	0.128	0.057	0.003*	0.107	0.050	0.008*	0.036	0.026	0.052
	Older	0.160	0.073		0.304	0.185		0.243	0.167		0.088	0.092	
150°	Young	0.081	0.048	0.024*	0.136	0.077	0.004*	0.105	0.053	0.007*	0.033	0.031	0.042
	Older	0.127	0.064		0.268	0.141		0.237	0.160		0.079	0.075	
180°	Young	0.068	0.037	0.020*	0.127	0.067	0.003*	0.107	0.058	0.009*	0.027	0.024	0.038
	Older	0.109	0.058		0.255	0.134		0.230	0.153		0.065	0.061	
210°	Young	0.077	0.059	0.076	0.147	0.078	0.004*	0.118	0.070	0.003*	0.023	0.023	0.033
	Older	0.113	0.053		0.268	0.134		0.242	0.124		0.060	0.058	
240°	Young	0.077	0.057	0.063	0.149	0.087	0.006*	0.125	0.069	0.005*	0.022	0.023	0.023*
	Older	0.116	0.060		0.276	0.152		0.226	0.118		0.062	0.058	
270°	Young	0.087	0.070	0.257	0.180	0.109	0.038	0.152	0.126	0.052	0.036	0.036	0.174
	Older	0.113	0.060		0.261	0.102		0.243	0.126		0.059	0.055	
300°	Young	0.086	0.061	0.091	0.182	0.096	0.002*	0.153	0.096	0.004*	0.053	0.047	0.305
	Older	0.120	0.047		0.317	0.127		0.270	0.115		0.073	0.057	
330°	Young	0.100	0.069	0.269	0.193	0.112	0.010*	0.170	0.138	0.045*	0.067	0.058	0.531
	Older	0.123	0.047		0.314	0.137		0.262	0.108		0.081	0.071	

Abbreviations: Rectus Femorus (RF); vastuslateralis (VL); vastusmedialis (VM); tensor fascia lata (TFL) standard deviation (SD). Asterisks (*) identifies significant differences at the $p < 0.025$ level (alpha was adjusted for repeated measures).

Direction	Sex	BF			ST			MG		
		Mean	SD	pValue	Mean	SD	pValue	Mean	SD	pValue
0°	Young	0.058	0.030	0.001*	0.103	0.058	0.032 [‡]	0.067	0.025	0.008*
	Older	0.192	0.120		0.224	0.193		0.192	0.155	
30°	Young	0.050	0.033	0.041	0.046	0.033	0.020*	0.062	0.036	0.080
	Older	0.122	0.122		0.116	0.100		0.104	0.081	
60°	Young	0.102	0.085	0.003*	0.041	0.034	0.026	0.110	0.063	0.005*
	Older	0.243	0.156		0.110	0.104		0.242	0.151	
90°	Young	0.202	0.137	0.007*	0.061	0.047	0.014*	0.242	0.120	0.003*
	Older	0.402	0.244		0.143	0.108		0.425	0.192	
120°	Young	0.241	0.150	0.006*	0.082	0.067	0.008*	0.262	0.103	0.007*
	Older	0.458	0.261		0.182	0.118		0.477	0.253	
150°	Young	0.257	0.202	0.004*	0.104	0.077	0.013*	0.283	0.135	0.008*
	Older	0.507	0.254		0.216	0.142		0.449	0.193	
180°	Young	0.249	0.181	0.007*	0.136	0.090	0.011*	0.177	0.115	0.001*
	Older	0.451	0.219		0.265	0.160		0.367	0.166	
210°	Young	0.209	0.138	0.001*	0.182	0.113	0.030	0.130	0.082	0.000*
	Older	0.454	0.215		0.317	0.214		0.361	0.165	
240°	Young	0.177	0.117	0.001*	0.206	0.131	0.045	0.101	0.057	0.002*
	Older	0.474	0.259		0.344	0.220		0.225	0.121	
270°	Young	0.141	0.088	0.000*	0.195	0.094	0.044	0.078	0.025	0.000*
	Older	0.404	0.216		0.346	0.255		0.244	0.142	
300°	Young	0.097	0.054	0.001*	0.194	0.107	0.038	0.086	0.030	0.000*
	Older	0.368	0.234		0.351	0.251		0.298	0.181	
330°	Young	0.076	0.048	0.000*	0.163	0.084	0.040	0.085	0.031	0.001*
	Older	0.284	0.170		0.282	0.192		0.273	0.165	

Abbreviations: Biceps femoris (BF); semitendinosus (ST); medial gastrocnemius; standard deviation (SD). Asterisks (*) identifies significant differences at the $p < 0.025$ level (alpha was adjusted for repeated measures).

Coactivation Indices

A two way within-between factorial (2x12) analysis of variance (ANOVA) for each antagonistic muscle pair was used to examine whether the coactivation index (CI) value significantly differed as a result of age and target direction (Table 11)

Simultaneously CI sample data means were compared to independent factors of age 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 ($p < 0.05$) 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. All muscles excluding VL & LG showed significant main effects for age on CI: QUADS and HAMS ($F(1, 29) = 15.778$, $p = 0.000$, partial $\eta^2 = 0.352$, power = 0.970), QUADS and GASTROCS ($F(1, 29) = 35.294$, $p = 0.000$, partial $\eta^2 = 0.549$, power = 1.000) VL & BF ($F(1, 29) = 9.510$, $p = 0.005$, partial $\eta^2 = 0.268$, power = 0.843), VM & ST ($F(1, 29) = 6.643$, $p = 0.016$, partial $\eta^2 = 0.192$, power = 0.701). 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 11: Statistical significance of main effects of gender and target direction on CCI. A two way within-between factorial ANOVA was used to determine the reported p values.

	Main Effect of Direction	Direction *Age	Main Effect of Age
QUADS & HAMS	0.000*	0.126	0.000*
QUADS & GASTROCS	0.000*	0.010*	0.000*
VL & BF	0.000*	0.093	0.005*
VM & ST	0.000*	0.759	0.016*
VL & LG	0.003*	0.236	0.062
VM & MG	0.000*	0.126	0.000*

Abbreviations: Rectus femoris (RF); vastuslateralis (VL); vastusmedialis (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 $p < 0.025$ level (Bonferonni adjustment for repeated measures: $\alpha = 0.05/2 = 0.025$) (Table 12). Older adults had significantly greater Quads & Hams, Quads & Gastrocs, VL & BF, and VM & ST, CI values at the target locations of 0° ($M=0.11$, $SD=0.04$ vs. $M=0.04$, $SD=0.03$, $t(24) = -5.061$, $p < 0.005$), 30° ($M=0.11$, $SD=0.05$ vs. $M=0.05$, $SD=0.03$, $t(17.751) = -3.880$, $p = 0.001$), 90° ($M=0.14$, $SD=0.07$ vs. $M=0.06$, $SD=0.04$, $t(24) = -3.585$, $p = 0.001$), 150° ($M=0.16$, $SD=0.08$ vs. $M=0.08$, $SD=0.04$, $t(24) = -3.136$, $p = 0.004$), 210° ($M=0.13$, $SD=0.07$ vs. $M=0.05$, $SD=0.03$, $t(14.912) = -3.595$, $p = 0.003$), and 330° ($M=0.15$, $SD=0.08$ vs. $M=0.06$, $SD=0.04$, $t(24) = -3.341$, $p = 0.003$). Post hoc independent T test examinations yielded significant differences at specific target locations between males and females at the $p < 0.025$ level (Bonferonni adjustment for repeated measures: $\alpha = 0.05/2 = 0.025$) (Table 12). Older adults had significantly greater Quads & Hams, Quads & Gastrocs, VL & BF, and VM & ST, CI values at the target locations of: Quads & Hams 0° ($M=0.271$, $SD=0.146$ vs $M=0.11$, $SD=0.048$, $t(16.863) = -4.087$, $p < 0.001$), 30° ($M=0.161$, $SD=0.076$ vs $M=0.067$, $SD=0.038$, $t(20.405) = -4.346$, $p < 0.000$), 60° ($M=0.207$, $SD=0.108$ vs $M=0.092$, $SD=0.057$, $t(29) = -3.736$, $p < 0.001$), 90° ($M=0.295$, $SD=0.139$ vs $M=0.138$, $SD=0.073$, $t(20.992) = -3.916$, $p < 0.001$), 120° ($M=0.34$, $SD=0.176$ vs $M=0.155$, $SD=0.078$, $t(19.058) = -3.745$, $p < 0.001$), 150° ($M=0.313$, $SD=0.176$ vs $M=0.152$, $SD=0.075$, $t(18.706) = -3.278$, $p < 0.004$), 180° ($M=0.286$, $SD=0.142$ vs $M=0.148$, $SD=0.069$, $t(19.936) = -3.413$, $p < 0.003$), 210° ($M=0.294$, $SD=0.124$ vs $M=0.171$, $SD=0.106$, $t(29) = -2.965$, $p < 0.006$), 240° ($M=0.295$, $SD=0.127$ vs $M=0.17$, $SD=0.104$, $t(29) = -3.018$, $p < 0.005$), 270° ($M=0.28$, $SD=0.112$ vs $M=0.187$, $SD=0.132$,

$t(29)=-2.114$, $p<0.043$), and 300° ($M=0.322$, $SD=0.151$ vs $M=0.179$, $SD=0.101$, $t(29)=-$
 3.136 , $p<0.004$), Quads & Gastrocs 0° ($M=0.31$, $SD=0.159$ vs $M=0.11$, $SD=0.036$,
 $t(15.328)=-4.767$, $p<0.00$), 30° ($M=0.135$, $SD=0.053$ vs $M=0.082$, $SD=0.03$, $t(29)=-$
 3.432 , $p<0.002$), 60° ($M=0.237$, $SD=0.071$ vs $M=0.111$, $SD=0.046$, $t(29)=-5.866$,
 $p<0.000$), 90° ($M=0.376$, $SD=0.173$ vs $M=0.16$, $SD=0.082$, $t(29)=-4.5$, $p<0.000$), 120°
($M=0.389$, $SD=0.221$ vs $M=0.169$, $SD=0.085$, $t(17.871)=-3.623$, $p<0.002$), 150°
($M=0.365$, $SD=0.217$ vs $M=0.162$, $SD=0.081$, $t(17.613)=-3.409$, $p<0.003$), 180°
($M=0.29$, $SD=0.127$ vs $M=0.138$, $SD=0.069$, $t(21.341)=-4.109$, $p<0.000$), 210°
($M=0.328$, $SD=0.127$ vs $M=0.13$, $SD=0.06$, $t(19.729)=-5.494$, $p<0.000$), 240° ($M=0.265$,
 $SD=0.073$ vs $M=0.134$, $SD=0.074$, $t(29)=-4.984$, $p<0.000$), 270° ($M=0.274$, $SD=0.097$ vs
 $M=0.137$, $SD=0.078$, $t(29)=-4.344$, $p<0.000$), 300° ($M=0.405$, $SD=0.163$ vs $M=0.144$,
 $SD=0.063$, $t(17.854)=-5.796$, $p<0.000$), and 330° ($M=0.378$, $SD=0.144$ vs $M=0.146$,
 $SD=0.06$, $t(18.472)=-5.784$, $p<0$), VL & BF 0° ($M=0.251$, $SD=0.186$ vs $M=0.088$,
 $SD=0.047$, $t(12.078)=-2.973$, $p<0.012$), 30° ($M=0.139$, $SD=0.1$ vs $M=0.068$, $SD=0.038$,
 $t(13.364)=-2.344$, $p<0.035$), 60° ($M=0.22$, $SD=0.124$ vs $M=0.124$, $SD=0.091$, $t(26)=-$
 2.392 , $p<0.024$), 180° ($M=0.335$, $SD=0.215$ vs $M=0.186$, $SD=0.106$, $t(26)=-2.425$,
 $p<0.023$), 270° ($M=0.31$, $SD=0.142$ vs $M=0.183$, $SD=0.136$, $t(26)=-2.394$, $p<0.024$),
 300° ($M=0.359$, $SD=0.256$ vs $M=0.137$, $SD=0.075$, $t(12.444)=-2.908$, $p<0.013$), and 330°
($M=0.337$, $SD=0.223$ vs $M=0.105$, $SD=0.055$, $t(13.185)=-3.659$, $p<0.003$).

Table 12: Post hoc evaluation of coactivation differences at each target location of males and females. Independent T tests were used to determine significance.

Direction	Sex	Quads & Hams			Quads & Gas			VM & ST			VL & BF		
		Mean	SD	pValue	Mean	SD	pValue	Mean	SD	pValue	Mean	SD	pValue
0°	Young	0.117	0.071	0.001*	0.088	0.047	0.000*	0.117	0.071	0.024*	0.088	0.047	0.012*
	Older	0.263	0.208		0.251	0.186		0.263	0.208		0.251	0.186	
30°	Young	0.055	0.03	0.000*	0.068	0.038	0.002*	0.055	0.03	0.012*	0.068	0.038	0.035
	Older	0.163	0.138		0.139	0.1		0.163	0.138		0.139	0.1	
60°	Young	0.047	0.024	0.001*	0.124	0.091	0.000*	0.047	0.024	0.031	0.124	0.091	0.024*
	Older	0.161	0.175		0.22	0.124		0.161	0.175		0.22	0.124	
90°	Young	0.073	0.05	0.001*	0.183	0.131	0.000*	0.073	0.05	0.046	0.183	0.131	0.033
	Older	0.191	0.197		0.327	0.208		0.191	0.197		0.327	0.208	
120°	Young	0.083	0.047	0.001*	0.185	0.114	0.002*	0.083	0.047	0.032	0.185	0.114	0.041
	Older	0.232	0.229		0.363	0.256		0.232	0.229		0.363	0.256	
150°	Young	0.097	0.06	0.004*	0.196	0.125	0.003*	0.097	0.06	0.032	0.196	0.125	0.043
	Older	0.253	0.24		0.378	0.263		0.253	0.24		0.378	0.263	
180°	Young	0.114	0.073	0.003*	0.186	0.106	0.000*	0.114	0.073	0.013*	0.186	0.106	0.023*
	Older	0.264	0.213		0.335	0.215		0.264	0.213		0.335	0.215	
210°	Young	0.15	0.111	0.006*	0.204	0.143	0.000*	0.15	0.111	0.028	0.204	0.143	0.077
	Older	0.277	0.185		0.302	0.134		0.277	0.185		0.302	0.134	
240°	Young	0.167	0.119	0.005*	0.191	0.133	0.000*	0.167	0.119	0.057	0.191	0.133	0.032
	Older	0.281	0.191		0.327	0.183		0.281	0.191		0.327	0.183	
270°	Young	0.179	0.139	0.043	0.183	0.136	0.000*	0.179	0.139	0.092	0.183	0.136	0.024*
	Older	0.288	0.198		0.31	0.142		0.288	0.198		0.31	0.142	
300°	Young	0.18	0.127	0.004*	0.137	0.075	0.000*	0.18	0.127	0.05	0.137	0.075	0.013*
	Older	0.313	0.221		0.359	0.256		0.313	0.221		0.359	0.256	
330°	Young	0.172	0.123	0.001*	0.105	0.055	0.000*	0.172	0.123	0.054	0.105	0.055	0.003*
	Older	0.306	0.231		0.337	0.223		0.306	0.231		0.337	0.223	

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).

Appendix E – Average Joint Moments

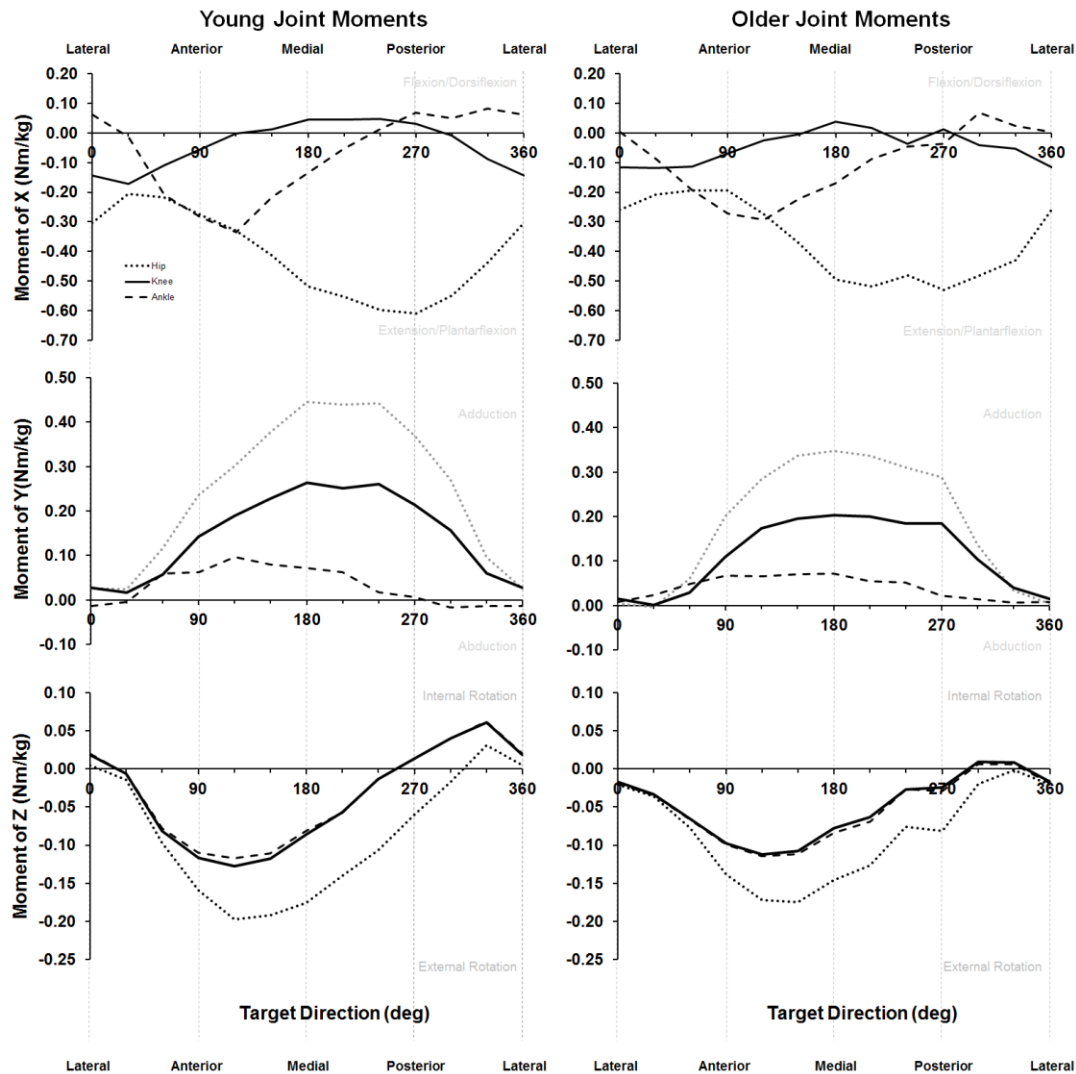


Figure 14: Hip, Knee and ankle moments of healthy young and older adult participants dominant leg. Values along the x axis represent target locations (angle in degrees). Values along the y axis represent joint moments, normalised to body weight (Nm/kg). Positive values indicate flexion/dorsiflexion, adduction, and internal rotation and negative values indicate extension/plantarflexion, abduction, and external rotation in moments of X, Y, and Z respectively.

Appendix F – Statistical Results of Gender Analysis

Table 13: Descriptive statistics of the muscle activation characteristics for each muscle of older men and women participants. Significant differences between sexes was determined with independent T tests and indicated by *p* values.

		XEMG						SI					
		Young			Older			Young			Older		
Muscle	Sex	Mean	SD	<i>p</i> Value	Mean	SD	<i>p</i> Value	Mean	SD	<i>p</i> Value	Mean	SD	<i>p</i> Value
RF	Men	0.069	0.058	0.243	0.117	0.047	0.544	0.110	0.091	0.395	0.199	0.101	0.037*
	Women	0.097	0.034		0.134	0.055		0.154	0.112		0.098	0.035	
VL	Men	0.116	0.063	0.061	0.328	0.107	0.048	0.059	0.059	0.078	0.188	0.118	0.188
	Women	0.184	0.074		0.218	0.095		0.127	0.085		0.117	0.087	
VM	Men	0.098	0.051	0.140	0.205	0.082	0.31	0.097	0.047	0.293	0.191	0.122	0.447
	Women	0.150	0.080		0.266	0.131		0.131	0.078		0.138	0.14	
BF	Men	0.128	0.064	0.259	0.426	0.145	0.149	0.361	0.114	0.223	0.427	0.2	0.13
	Women	0.178	0.104		0.309	0.149		0.271	0.168		0.284	0.118	
ST	Men	0.139	0.063	0.477	0.215	0.127	0.555	0.393	0.132	0.211	0.439	0.103	0.079
	Women	0.115	0.071		0.265	0.183		0.325	0.078		0.335	0.108	
LG	Men	0.087	0.056	0.198	0.162	0.078	0.938	0.215	0.133	0.957	0.178	0.146	0.469
	Women	0.145	0.102		0.166	0.108		0.219	0.154		0.134	0.086	
MG	Men	0.133	0.035	0.578	0.321	0.113	0.598	0.449	0.098	0.026*	0.342	0.157	0.879
	Women	0.147	0.060		0.286	0.142		0.273	0.181		0.354	0.161	
TFL	Men	0.030	0.030	0.327	0.059	0.038	0.437	0.230	0.208	0.711	0.276	0.165	0.065
	Women	0.044	0.025		0.083	0.07		0.266	0.193		0.119	0.148	

Abbreviations: Rectus femoris (RF); vastuslateralis (VL); vastusmedialis (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}); specificity index (SI). Asterisks (*) identifies significant differences at the $p < 0.05$ level.

Table 14: Statistical significance of main effects of gender and target direction on EMG amplitude of young and older adults. A two way within-between factorial ANOVA was used to determine the reported *p* values.

Younger						
Muscle	Main Effect of Direction	Power	Interaction	Power	Main Effect of Gender	Power
RF	0.149	1.000	0.803	0.551	0.243	0.089
VL	0.001*	0.998	0.006*	0.471	0.061	0.522
VM	0.033*	0.571	0.331	0.365	0.140	0.176
BF	0.000*	1.000	0.394	0.614	0.259	0.296
ST	0.000*	0.998	0.741	0.123	0.477	0.087
LG	0.092	0.531	0.848	0.199	0.198	0.051
MG	0.000*	1.000	0.668	0.289	0.578	0.079
TFL	0.002*	0.375	0.770	0.296	0.327	0.115
Older						
Muscle	Main Effect of Direction	Power	Interaction	Power	Main Effect of Age	Power
RF	0.000*	1.000	0.205	0.551	0.544	0.089
VL	0.000*	0.998	0.256	0.471	0.048*	0.522
VM	0.082	0.571	0.249	0.365	0.289	0.176
BF	0.000*	1.000	0.082	0.614	0.149	0.296
ST	0.000*	0.998	0.675	0.123	0.555	0.087
LG	0.064	0.531	0.375	0.199	0.938	0.051
MG	0.000*	1.000	0.338	0.289	0.598	0.079
TFL	0.174	0.375	0.261	0.296	0.437	0.115

Abbreviations: Rectus femoris (RF); vastuslateralis (VL); vastusmedialis (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 15: Statistical significance of main effects of gender and target direction on CCI. A two way within-between factorial ANOVA was used to determine the reported *p* values.

Younger						
Muscle Pairs	Main Effect of Direction	Power	Interaction	Power	Main Effect of Gender	Power
QUADS & HAMS	0.000*	1.000	0.390	0.292	0.589	0.081
QUADS & GASTROCS	0.000*	0.987	0.984	0.080	0.037*	0.573
VL & BF	0.000*	0.990	0.707	0.130	0.775	0.059
VM & ST	0.000*	0.979	0.560	0.133	0.708	0.065
VL & LG	0.030*	0.726	0.837	0.110	0.057	0.489
VM & MG	0.101	0.443	0.334	0.216	0.021*	0.673
Older						
Muscle	Main Effect of Direction	Power	Interaction	Power	Main Effect of Age	Power
QUADS & HAMS	0.000*	1.000	0.110	0.659	0.621	0.088
QUADS & GASTROCS	0.001*	0.971	0.984	0.055	0.549	0.442
VL & BF	0.000*	0.985	0.104	0.606	0.072	0.083
VM & ST	0.001*	0.973	0.289	0.390	0.577	0.080
VL & LG	0.064	0.594	0.533	0.191	0.592	0.681
VM & MG	0.000*	0.994	0.023*	0.763	0.021*	0.076

Abbreviations: Rectus femoris (RF); vastuslateralis (VL); vastusmedialis (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.