AN INVESTIGATION INTO THE SEX DIFFERENCES BETWEEN OLDER ADULTS WITH OSTEOARTHRITIS IN KINETICS, KINEMATICS, AND MUSCLE ACTIVATION PATTERNS DURING SQUATTING

Olivia Zajdman

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies In partial fulfillment of the requirements For the M.Sc. degree in Human Kinetics School of Human Kinetics Faculty of Health Sciences University of Ottawa

© Olivia Zajdman, Ottawa, Canada, 2016
Table of Contents

List of Acronyms ................................................................................................. iv
List of Figures ........................................................................................................ v
List of Tables ......................................................................................................... vi
General Abstract.................................................................................................. 1
Introduction ........................................................................................................... 3
   Relevance .......................................................................................................... 3
   Purpose and Research Question ......................................................................... 6
Review of the Literature ..................................................................................... 7
   Age and Osteoarthritic-related Changes in Neuromuscular Function ............. 7
   Muscle Weakness and Proprioception ............................................................ 8
   Muscle Activation Magnitude, Co-activation, & Dynamic Knee Joint Stiffness.. 10
   Knee Adduction Moment and Malalignment ................................................... 12
   Squatting ........................................................................................................... 14
Methodology ......................................................................................................... 19
   Study Design .................................................................................................. 19
   Participants .................................................................................................... 19
      Participant Requirements ............................................................................ 20
   Data Collection and Equipment .................................................................... 20
      KOOS and Physical Activity Levels ............................................................... 20
      Muscle Activations ....................................................................................... 21
      Force Measurement ...................................................................................... 22
      Kinematic and Kinetic Measurements .......................................................... 22
      Biodex Multi-Joint System 4 Pro .................................................................. 22
   Protocol Setup ................................................................................................ 23
      Participant Setup ........................................................................................ 23
      Maximum Voluntary Isometric Contractions (MVICs) .................................. 24
      Squatting Protocol ....................................................................................... 25
   Data Analysis .................................................................................................. 25
      Maximum Voluntary Isometric Contractions ................................................. 25
      Muscle Activation Patterns .......................................................................... 26
      Co-activation Index ..................................................................................... 26
      Dynamic Knee Joint Stiffness ..................................................................... 27
      Kinematics and Kinetics ............................................................................. 28
   Statistical Analysis ......................................................................................... 29
ARTICLE 1: FEMALES WITH KNEE OSTEOARTHRITIS USE A DETRIMENTAL
   KNEE LOADING STRATEGY WHEN SQUATTING ...................................... 31
   Abstract ......................................................................................................... 32
   Introduction .................................................................................................... 33
   Methods ......................................................................................................... 34
   Results ............................................................................................................ 38
   Discussion ....................................................................................................... 44
   Conclusion ...................................................................................................... 49
   References ..................................................................................................... 50
ARTICLE 2: OSTEOARTHRITIS LEADS TO INCREASED MUSCLE CO-ACTIVATION AND KNEE STIFFNESS IN MALES AND FEMALES DURING SQUATTING

Abstract ................................................................................................................................................. 56
Introduction ............................................................................................................................................ 57
Methods .................................................................................................................................................. 59
Results .................................................................................................................................................... 63
Discussion .............................................................................................................................................. 69
Conclusion ............................................................................................................................................. 75
References .............................................................................................................................................. 76

General Discussion ............................................................................................................................... 80
Limitations ............................................................................................................................................. 85
Conclusion ............................................................................................................................................. 88
Acknowledgements ............................................................................................................................... 89
References ............................................................................................................................................. 90
Appendices ............................................................................................................................................ 103
## List of Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BF</td>
<td>Biceps femoris</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CI</td>
<td>Co-activation index</td>
</tr>
<tr>
<td>DKJS</td>
<td>Dynamic knee joint stiffness</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>FOA</td>
<td>Females with Osteoarthritis</td>
</tr>
<tr>
<td>FOC</td>
<td>Female Older Controls</td>
</tr>
<tr>
<td>GASTROCS</td>
<td>Summed gastrocnemius muscle activation</td>
</tr>
<tr>
<td>GRF</td>
<td>Ground Reaction Force</td>
</tr>
<tr>
<td>HAMS</td>
<td>Summed hamstrings muscle activation</td>
</tr>
<tr>
<td>KOOS</td>
<td>Knee Osteoarthritis and injury Outcome Score</td>
</tr>
<tr>
<td>LG</td>
<td>Lateral gastrocnemius</td>
</tr>
<tr>
<td>MG</td>
<td>Medial gastrocnemius</td>
</tr>
<tr>
<td>MOA</td>
<td>Males with Osteoarthritis</td>
</tr>
<tr>
<td>MOC</td>
<td>Male Older Controls</td>
</tr>
<tr>
<td>MVIC</td>
<td>Maximum voluntary isometric contraction</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>UOMAM</td>
<td>University of Ottawa motion analysis model</td>
</tr>
<tr>
<td>QUADS</td>
<td>Summed quadriceps muscle activation</td>
</tr>
<tr>
<td>RF</td>
<td>Rectus femoris</td>
</tr>
<tr>
<td>RPM</td>
<td>Repetitions per minute</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDAcc</td>
<td>Squat down acceleration</td>
</tr>
<tr>
<td>SDDec</td>
<td>Squat down deceleration</td>
</tr>
<tr>
<td>SENIAM</td>
<td>Surface Electromyography for Non-Invasive Assessment of Muscles</td>
</tr>
<tr>
<td>ST</td>
<td>Semitendinosus</td>
</tr>
<tr>
<td>SUAcc</td>
<td>Squat up acceleration</td>
</tr>
<tr>
<td>SUDec</td>
<td>Squat up deceleration</td>
</tr>
<tr>
<td>TFL</td>
<td>Tensor fascia lata</td>
</tr>
<tr>
<td>VL</td>
<td>Vastus lateralis</td>
</tr>
<tr>
<td>VM</td>
<td>Vastus medialis</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1: Group average and standard deviation of sagittal plane kinematics and kinetics for the hip and knee................................................................. 41

Figure 2: Group average and standard deviation of frontal plane kinematics and kinetics for the hip and knee and sagittal and frontal plane kinematics for the ankle.............42

Figure 3: Group average and standard deviation of muscle activation for rectus femoris (RF), tensor fascia latae (TFL), vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), semitendinosus (ST), lateral gastrocnemius (LG), and medial gastrocnemius (MG).................................................................43

Figure 4: Group average and standard deviation for co-activation indices during squat down acceleration (SDAcc) squat down deceleration (SDDec), squat up acceleration (SUAcc), and squat up deceleration (SUDec) for VM-ST, VL-BF, VM-MG, VL-LG, QUAD-HAM, and QUAD-GASTROC.................................................................67

Figure 5: Group average and standard deviation for dynamic knee joint stiffness (DKJS) indices during squat down acceleration (SDAcc) squat down deceleration (SDDec), squat up acceleration (SUAcc), and squat up deceleration (SUDec) for VM-ST, VL-BF, VM-MG, VL-LG, QUAD-HAM, and QUAD-GASTROC.................................................................68
List of Tables

Table 1: Group average and standard deviation of participant anthropometrics, KOOS scores, and time (seconds) spent during squat descent and ascent phases………………38

Table 2: Group average and standard deviation of participant anthropometrics, KOOS scores, and percent of squat movement spent in each phase ……………………………64

Table 3: Self-reported severity of females and males with knee osteoarthritis………………103

Table 4: Summary of the number of participants in each group with kinematic, kinetic, and electromyography data…………………………………………………………103

Table 5: Markers and description of their placement on the participant used to collect kinematic data in this study. The acronyms that begin with the letter “L” or “R” correspond to the left and right sides of the body, respectively………………109

Table 6: University of Ottawa Modified Plug-in-Gait model output of lower limb angles/moments in the local coordinate system and their positive and negative movement descriptors……………………………………………………………………………110

Table 7: Effect size and Cohen’s D values for peak knee flexion, knee flexion moment, hip flexion angle, knee power (ascent), hip abduction, and knee adduction angle…………112

Table 8: Pearson Correlation Coefficients for KOOS Pain sub-scores compared to peak knee flexion angle, knee extension moment, knee power (ascent), squat down deceleration stiffness (SDDec), and squat up acceleration stiffness (SUAcc). ………………………112

Table 9: Group average and standard deviation of absolute (N*m) and normalized torque (N*m/kg) …………………………………………………………………………………113
General Abstract

INTRODUCTION: Altered neuromuscular control and knee joint instability are commonly observed in populations with knee osteoarthritis (OA). Since knee OA is more prevalent in females, sex-related differences in muscle activation and movement strategies during activities of daily living (ADL) are theorized to be a contributing factor to the increased prevalence in females. PURPOSE: The aims of this thesis were: 1) identify sex differences in joint dynamics and muscle activation patterns in older adults with knee OA and healthy older adults; and 2) investigate whether differences in co-activation and dynamic knee joint stiffness exist between sexes in an OA and healthy populations. For both aims, squatting tasks were evaluated because it is a common and critical component in ADLs. METHODS: Thirty healthy individuals (15 females) and thirty individuals with knee OA (15 female) performed three two-legged squats at a self-selected pace on two force platforms. Hip, knee, and ankle sagittal and frontal plane joint angles, moments and powers were calculated and electromyography (EMG) of eight muscles crossing the knee joint was recorded for the test (OA affected or dominant) limb. Maximum voluntary isometric contractions were used to normalize the EMG data. Co-activation indices for six antagonist muscle pairings and dynamic knee joint stiffness (DKJS) were calculated for the acceleration and deceleration phases of squat descent and ascent. Two-way ANOVAs (Sex X OA status) were used to characterize differences in muscle activation patterns and movement strategies. RESULTS: For aim 1, decreased hip, knee and ankle sagittal plane range of motion was identified in the OA participants, with females showing the greater deficits compared to the males. Males with OA implemented a hip dominant strategy by increasing hip joint moments and decreasing
knee joint moments compared to the females. Indifferent of joint status, females performed the squat with more hip adduction compared to males. Females with OA demonstrated greater hip adduction and knee valgus angles throughout the squat, contributing to the decrease in the frontal plane range of motion. Additionally, hip joint power was lower in all female participants compared to males while knee joint power was lower in the OA participants. For aim 2, females with OA, and to lesser extent males with OA had greater DKJS around peak knee flexion compared to the healthy participants. Co-activation indices revealed sex differences in neuromuscular control: Females with knee osteoarthritis had higher muscle activation magnitude and co-activation of antagonistic muscles, whereas the males used a more selective increase in hamstring co-activation and more balanced quadriceps-hamstring recruitment.

CONCLUSION: Two-legged squats were able to detect sex and OA related functional deficits at the knee and adjacent hip and ankle joints. OA had a greater effect on the movement and neuromuscular control in females than males and the squat identified specific deficiencies that can be targeted for rehabilitation.
Introduction

Relevance

Osteoarthritis (OA) is a condition involving progressive degradation of articular cartilage, remodelling of subchondral bone and damage to surrounding soft tissue (ie. ligaments, menisci) (Felson et al. 2000/2006), most commonly found in the knee (Buckwalter et al., 2001). Affecting 13% of Canadian adults (Buckwalter et al., 2001), OA is a leading cause of long-term disability in populations over the age of 65 (Felson et al., 1990; Felson et al., 1995) and presents many limitations that threatens the autonomy of those affected. In older adults, the probability of mobility limitations accounted for by knee OA is equivalent to other chronic conditions like heart disease and stroke (Guccione et al., 1994). The burden on the Canadian economy associated with osteoarthritis in health care and other indirect costs was estimated to reach $1.8 billion in 2010 and is projected to reach $8.1 billion in 2031, with the increase in prevalence of people with OA (Sharif et al., 2012).

The precise etiology of knee OA is not well understood, although alterations in the local mechanical environment (Lohmander et al., 2007), changes in the tissues comprising the joint (Felson, 2006; Logerstedt et al., 2014), individual differences in joint mechanics (Segal et al., 2009), and the interaction between these risk factors are all speculated to have a great impact on the development of OA.

The progression of OA has been associated with both modifiable and non-modifiable risk factors. Obesity is a major modifiable risk factor for knee OA (Felson et al., 1997), linked to excessive knee joint loading (Arokoski et al., 2000) and mechanical degeneration of the joint (Felson et al., 1997). Other risk factors include occupational
bending and lifting (Cooper et al., 1994), ligament laxity (Loeser & Shakoor, 2003), ACL and/or meniscus injury (Lomander et al., 2004/2007), and previous knee surgery (Felson et al., 2004).

Sex and age are two of the most important non-modifiable risk factors. In 2010, nearly 48% of those newly diagnosed were over 60 years old (Arthritis Alliance of Canada, 2011). The elderly are most affected by this disease, with increased prevalence in females (Srikanth et al., 2005), who often have chronic disability (Guccione et al., 1994) affecting daily activities like squatting. Having difficulty holding a squatting position, which chiefly affects women (Jones & Reed, 2005), will negatively impact their ability to use a toilet, sit and rise from a chair, and use public transportation, thus reducing their functional independence. Considering that public restrooms are often inaccessible to those with disabilities (Jones & Reed, 2005), this physical limitation may further discourage elderly individuals with OA from leaving their homes. Moreover, those limited by arthritis have also reported using a vehicle, going out during the week, and traveling long distances less frequently than those who are not affected (Verbrugge et al., 2006). Inevitably, the inability to squat will negatively impact their standard of living, as inactivity and impaired mobility have predicted dependence and death in adults over 65 years of age (Hirvensalo et al., 2000).

Additionally, with healthy aging comes a decline in balance control (Hurley et al., 1998) and an increased risk of falling compared to younger healthy adults. The risk of falling increases from 30% in healthy older adults to 50% in an OA population (Takacs et al., 2013), and more often occurs during dynamic tasks in older adults (Niino, 2000) whilst moving laterally (Smeesters et al., 2001). This may impact women with OA more as
healthy females have a higher incidence of falls (Sattin, 1992). Moreover, having a fear of falling has previously been associated with being female and having arthritis (Liu et al., 2015).

Osteoarthritis results in mobility limitations, muscle atrophy, strength and increased pain (Glass et al., 2014; Segal et al., 2010) that worsen with disease progression. The disease is also linked to altered neuromuscular control and knee joint instability (Bigham, 2015) and it is hypothesised that muscles surrounding the joint change their activation patterns in an attempt to maintain stability, possibly resulting in joint degeneration (Piscoya et al., 2005). Differences in strength and muscle activation patterns are also present between sexes (Bigham, 2015). Males and females present different muscle activation strategies as a consequence of altered neuromuscular control with age, where by older females have shown less muscle specificity, having more general muscle activation to stabilize the knee and greater quadriceps activation compared to older males (Bigham, 2015). A ‘knee stiffening strategy’ is used in individuals with knee OA, by increasing muscle co-activation whilst reducing knee motion, which appears to be a safer short-term approach to movement (Rudolph et al., 2007). Furthermore, females with knee OA demonstrate quadriceps weakness compared to healthy older females (Pettersen et al., 2007) and this has been proposed as a risk factor (Brandt et al., 1999) and an early sign of OA. All these factors will affect the biomechanics of the lower limb and may cause kinematic and kinetic differences in activities of daily living (ADL) between males and females with OA.

Considering the above, investigating movement strategies and muscle activations during dynamic tasks, in particular how males and females with OA differ, seems
warranted. The disconnect that exists between OA severity and resultant disability (Harrison et al., 2004; McDonough et al., 2010) identifies the need to uncover factors influencing the progression of OA. Since squats are an everyday dynamic task, essential for using the toilet and other activities of daily living, this task may be a good indicator of functional ability. Squats are commonly used in clinical settings to assess functionality (Rossi et al, 2013; Hockings et al., 2013; Dwyer et al., 2010) yet there is little research on this task in older populations, particular those with knee OA. Having a concrete understanding of movement patterns in an OA population and how they differ from those uninhibited by OA may be critical for creating intervention strategies and rehabilitation protocols to reduce the burden of this disease.

A greater understanding of the kinematics, kinetics, and muscle activation during squatting will provide insight into deficits and compensations used by individuals with OA to accomplish activities of daily living. This can be used to develop targeted rehabilitation interventions in persons with OA and may also form the basis of clinically relevant measures to detect both sex and OA-related impairments.

**Purpose and Research Question**

The primary aims of this thesis are therefore to:

1) Identify differences in sagittal and frontal plane kinematics, kinetics, and muscle activation patterns among males and females with and without knee osteoarthritis.

2) Investigate whether differences in co-activation and dynamic knee joint stiffness exist between sexes and between older adults with and without knee osteoarthritis all during squatting.
Review of the Literature

Age and Osteoarthritic-related Changes in Neuromuscular Function

Knee joint stability is defined as the joint’s ability to remain in or quickly return to its initial homeostatic state upon disruption (Riemann & LePhart, 2002). The knee joint is stable when the tissues comprising the joint do not exceed their ability to dissipate forces and physiological threshold of deformation. Joint stability involves the combination of articular geometry (shape of condyles and menisci), body mass, as well as passive soft tissue restraints (ligaments), and active muscles. Knee stability is actively controlled by muscles (Flaxman et al., 2012). This requires the interaction of the musculoskeletal and nervous systems, which can change with age (Hortobagyi et al., 1995) and diseases such as OA (Lewek et al., 2005). Knee joint instability is a common problem in older adults, persons with OA, and females. Older adults have fewer fast twitch muscle fibres, resulting in a lower force generation capacity, and slower firing rates than younger adults. The combination of a slower motor neuron pool and a longer time to reach fused tetanus diminishes the ability to fine tune force output. This may limit the recruitment strategies available, as additional force cannot be produced while the motor unit (MU) is in tetanus or during its relaxation period (Connelly et al., 1999; Roos et al., 1997). This neuromuscular dysfunction seen with age is heightened with the presence of knee OA, as evidenced by their inability to recruit all motor units, resulting in muscle dysfunction and poor voluntary contraction of the quadriceps while performing an isometric and isokinetic strength test (Hurley & Newham, 1993). Inevitably, this may lead to a change in muscle activation patterns in attempt to maintain stability, altering joint loading, and resulting in joint degeneration (Piscoya et al., 2005). This is evident in the decreased mobility,
strength, and increased muscle atrophy and pain (Glass et al., 2014; Segal et al., 2010) seen with the progression of OA. Those suffering from these symptoms may continue to use an alternate muscle activation strategy creating a vicious cycle and further the development of OA.

**Muscle Weakness and Proprioception**

A loss of skeletal muscle and lower limb strength occurs with age (Stevens et al., 2001; Madhavan et al., 2009). Muscle atrophy, the reduction in fast twitch muscle fibres, and the total number of motor units in older adults (Yu et al., 2007) are partially responsible for the diminished force generation capacity, contractile speed, and resultant decline in strength. Lower MVC torque, MU firing rates, and longer twitch contractions have also been observed in the tibialis anterior of older adults compared to younger adults (Connelly et al., 1999). Discrepancies in muscle function observed in older women compared to men may be attributed to the sex differences in muscle contraction regulation, where older women have slower maximum velocities of the contractile unit of the muscle, which is not present in younger adults (Yu et al., 2007). This may be reflected in the differences in quadriceps and hamstring strength established between healthy male and female older adults (Huston & Wojtys, 1996), and exaggerated in women with knee OA (Petterson et al., 2007). Muscle weakness has been proposed as a risk factor for the initiation and progression of OA and an early sign of OA (Slemenda et al., 1997). Moreover, weaker quadriceps may be more susceptible to muscle fatigue and disuse (Youssef et al., 2009).
Reduced quadriceps strength will negatively impact the ability to perform daily activities (Hortobagyi et al., 2003; Fujita et al., 2011). Now consider that older females often use a quadriceps-dominant strategy (Youdas et al., 2007; Bigham, 2015). For example, Youdas et al., (2007) found that women activated their quadriceps 20% more than their hamstrings, while the males exhibited a more balanced hamstring-quadriceps activation when performing a single limb squat. Similarly, during a dynamic weight-bearing force-matching task, older males show greater hamstring activation to stabilise the knee joint, similar to healthy younger males (Bigham, 2015). Conversely, older females had reduced muscle specificity, having more general knee joint stabilisers (ie. symmetrical activation regardless of movement direction) and higher quadriceps activation (Bigham, 2015). This may be a compensation for reduced muscle strength and a greater quadriceps-hamstring imbalance seen in older females (Bigham, 2015; Hewett et al., 2002). Taken together, this would imply that females will suffer greater adverse effects on mobility from the development of OA, which is supported by the literature (Jones & Reed, 2005; Guccione et al., 1994; Kim et al., 2010).

In addition to muscle weakness, limitations in performing ADLs in those with osteoarthritis may also be affected by proprioceptive deficits (Bennell et al, 2003; Hurley et al., 1997; Pai et al., 1997). The sense of joint position in space comes from the integration of neural inputs from the joint, muscles, tendons, and ligaments (Sharma et al., 2003). Proprioceptive feedback from these receptors influences the amount of muscle activation needed to provide sufficient neuromuscular control and functional stability (ie. individuals perceived stability) while executing a movement (van der Esch et al., 2007). In persons with OA, altered sensory information from damaged articular mechanoreceptors
may contribute to the inability to completely activate their quadriceps voluntarily and exacerbate this weakness (Hurley et al., 1997; Hassan et al., 2001). Diminished proprioception seen in patients with knee OA (Bennell et al, 2003; Sharma et al., 1997) in conjunction with muscle weakness could negatively impact functional ability.

It is important to identify and implement prophylactic measures for these individuals as the higher prevalence of falls in the elderly, further elevated in the OA population (Takacs et al., 2013), have been associated with decreased mobility and lower limb disability (Whipple et al., 1997; Robbins et al., 1989).

Muscle Activation Magnitude, Co-activation, & Dynamic Knee Joint Stiffness

Activation of agonist and antagonist muscles occurs regularly during dynamic movements. The ability to effectively stabilise the knee and dissipate loads sustained through daily activities can be compromised as a result of altered neuromuscular function in older adults. The decline in muscle mass, fast twitch muscle fibres, and changes in regulation of muscle fibre recruitment in the elderly contribute to a slower contractile speed and reduced force generation compared to young adults (Yu et al., 2007). This may create a more desirable condition to continually elevate the coactivity of the agonist and antagonist muscles to reduce the demand on the neuromuscular system (Hasan, 1986). Co-activation may stem from the reduced reciprocal inhibition of the antagonist muscle via the 1a inhibitory interneuron and/or higher activation of the cortical centers that increase the activation of the antagonist motor neurons (Humphrey & Reed, 1983).

Elderly people have increased normalised muscle activity while performing downward steps (Hortobagyi & DeVita, 2000), and a single leg squat (Madhavan et al., 2009). Older adults also use increased antagonist coactivity as a strategy to maintain
balance in response to a perturbation, which is not present in young adults (Manchaster et al., 1989). Increased co-activation during activities of daily living (ADLs) was even greater in older adults with OA compared to healthy-age match controls and younger adults (Hortobagyi et al., 2005; Hubley-Kozey et al., 2008). As a result, a knee stiffening strategy is often implemented in those with OA to stabilise the knee, illustrated by increased co-contraction with reduced knee motion (Rudolph et al., 2007).

Dynamic knee joint stiffness characterizes the linear relationship between the knee joint moment (applied force) and knee angle (resultant displacement) over a given movement (Davis & DeLuca, 1996). Stiffness is an important component in stability, as it is a measure of the resistance to motion the joint provides, and depends on both passive and active joint structures. The ability to control and stabilize the knee is dependent upon the torque produced at a given angle. Higher knee joint moments and/or reduced range of motion at the knee will lead to increased dynamic joint stiffness. Joint stiffness may also be reflected in the change in agonist and antagonist muscle contributions, as muscles are the only active regulators of joint stability.

Dynamic knee joint stiffness has previously been used as an indicator of joint stability in conjunction with muscle co-activation during gait (Collins et al., 2014; Zeni & Higginson, 2009b). Increased joint stiffness during gait has been observed in patients with varying severities of OA (Zeni & Higginson, 2009; Dixon et al., 2010). Moreover, in response to an unexpected perturbation, individuals with medial knee OA have increased vastus medialis and medial hamstring co-activation to stabilize the knee joint compared to the control population (Lewek et al., 2005).
The increase in co-activation to stiffen the joint may be a result of the reduced ability to accurately scale muscle force (Hortobagyi et al., 2004; Williams et al., 2003). This strategy appears to be a safer short-term approach to movement (DeVita & Hortobagyi, 2000b). Increased antagonistic activity to increase stability affects dynamic joint stiffness, but increasing joint compression forces at the knee can promote degeneration over time (Piscoya et al., 2005; Griffin & Guilak, 2005). Quantifying the magnitude of co-activation in healthy and OA populations can provide information about the presence and severity of knee OA (Hubley-Kozey et al., 2006; Hubley-Kozey et al., 2008; Hubley-Kozey et al., 2009). In general, older adults who have quadriceps weakness but do not develop OA may retain a movement strategy and muscle activation pattern more similar to young adults (Hortobagyi et al., 2003). Using an altered muscle activation pattern to stabilize the knee joint, although seemingly beneficial, may in fact lead to altered cartilage loading that could compromise the long-term integrity of the joint (Rudolph et al., 2007). Examining whether greater dynamic knee joint stiffness is associated with increased co-activation of antagonist muscle activity may provide insight into this relationship.

**Knee Adduction Moment and Malalignment**

The external knee adduction moment (KAM) is defined as the combination of the ground reaction force, which passes medial to the centre of the knee joint, and the perpendicular distance of this force from the centre of the joint in the frontal plane. A higher KAM indicates greater load on the medial tibial plateau (Hurwitz et al., 1998). Previous studies have identified an increase in KAM in osteoarthritic populations during gait (Landry et al., 2007; Kumar et al., 2013; Hurwitz et al., 1998). An increase in KAM
of 1% has been shown to increase the risk of OA progression by 6.46 times (Miyazaki et al., 2002). Varus malalignment has been positively correlated with KAM (Wada et al., 2001). Reduced joint contact surfaces in the medial and/or lateral compartments of the knee will influence the contact stress, uniformity, laxity, and motion of the joint (Andriacchi, 1994). Moreover, with the increase in bone cyst formation and cartilage loss, the knee joint becomes malaligned (Felson et al., 1995). Changing the contact area to a region that is not accustomed to dissipating joint contact forces increases the likelihood of degeneration of the articular surface and joint cartilage (Wu, Herzog, & Epstein, 2000; Cicuttini et al., 2002).

Movement in the frontal plane is predominantly controlled by the hip abductor muscles (Chang et al., 2005). Employing a more valgus knee alignment and consequently increasing the knee joint load may be a result of decreased hip abductor activity (Chang et al., 2005; Claiborne et al., 2006). Previous works in our group has identified weaker hip abduction torque in older females with knee OA compared to healthy older females (Bigham, 2015), which may make females with OA more susceptible to abnormal knee joint loading. It is important that these individuals are identified in order to help slow disease progression, as the reduction in medial knee compartment OA progression has been directly linked to increased peak internal hip abduction moment (Chang et al., 2005).

A pilot study in older adults with early signs of knee OA (KL grade 1) found that peak KAM was reduced following a 8 week program using a single leg sit-to-stand exercise (Thorstensson et al., 2007). Reduced muscle function may be a modifiable risk factor contributing to greater knee joint loads that can be addressed with neuromuscular exercises. Interventions that are created to enhance neuromuscular control of everyday
activities, like squatting, may improve muscle activation and help control lateral knee movement, activating muscles that can generate an opposing moment to the KAM during everyday dynamic activities.

**Squatting**

Squatting is a complex, multi-joint movement that requires the coordination of individual limb segments. This is a fluid motion that begins with the hip and knees fully extended. It has been reported that decreased hamstring activity (Cheron et al., 1997; Hase et al., 2004) and increased activity of the tibialis anterior (Cheron et al., 1997; Dionisio et al., 2008; Hase et al., 2004) and gastrocnemius (Dionisio et al., 2008) are a preparatory response for squat initiation into the downward acceleration phase.

Once the initial upright position is unlocked, the centre of mass (COM) is lowered by flexing the trunk and lower limbs (Hase et al., 2004), and the quadriceps muscles increases activation as the individual decelerates to reach the target position (Cheron et al., 1997; Dionisio et al., 2008; Flanagan et al., 2003; Hase et al., 2004). Vastus medialis and vastus lateralis are 40-50% more active than rectus femoris (Escamilla, 2001; Isear et al., 1997), and may play a greater role in stabilizing the knee joint during this phase. When the trunk is erect during a squat, the rectus femoris may be more effective as a knee extensor in its’ lengthened position (Escamilla, 2001). The gastrocnemius also aids in facilitating this movement by eccentrically contracting to control ankle dorsiflexion towards the end of the deceleration phase of squat descent (Escamilla, 2001) to prevent the body from falling anteriorly.
Squat ascent is facilitated by activating the quadriceps to extend the knee, with the greatest activation seen in the vastus medialis and vastus lateralis, to help accelerate the body upward (Isear et al., 1997). The gastrocnemius are also activated to produce ankle plantar flexion during the acceleration into squat ascent (Escamilla, 2001). Previous studies have shown that hamstring activation is greatest during squat ascent to assist in hip extension (Escamilla et al., 1998; Isear et al., 1997). The hamstrings are bi-articular and do not significantly change length, as they shorten at the knee and lengthen at the hip during squat descent and vice versa during squat ascent (Jönhagen et al., 2009).

Throughout the squat movement a neuromuscular response is coordinated to maintain the COM within a person’s base of support to preserve dynamic stability. Aging and diseases like OA are known to affect neuromuscular control, resulting in decreased knee joint stability (Hurley & Newham, 1993). Furthermore, joint stability is only actively regulated through muscle recruitment (Flaxman et al., 2012) and older adults with OA have deficits in quadriceps strength (Lewek et al., 2004; Messier et al., 1992; Selmenda et al., 1997), further reduced in females (Petterson et al., 2007), altered muscle activation (Hurley et al., 1997), and reduced knee joint proprioception (Sharma et al., 1997; Hurley et al., 1997). Compounded together, for the female OA population we expect these changes to have a greater negative impact on their stability and ability to perform a squat.

In order to restore and preserve functional independence in squatting tasks it is important to have a concrete understanding of the squatting movement in order to prescribe proper movement techniques, correct injurious movements, and be able to identify differences in movement execution between sexes and as a result of disease.
The understanding of movement strategies of two-legged squats have focused on the analysis of lower limb kinematics (Lynn & Noffal, 2012; Dionisio et al., 2008; McKean et al., 2010; Hase et al., 2004; Pollard et al., 2011), kinetics (Almosnino et al., 2013; Lynn & Noffal, 2012; Dionisio et al., 2008; Pollard et al., 2011), and muscle activation (Lynn & Noffal, 2012; Dionisio et al., 2008; Gallagher et al., 2011) in healthy young adults, while limited research has been conducted in healthy older adults (Flanagan et al., 2003). Sex differences during squatting have been predominantly reported in young healthy populations. Females have higher quadriceps (Youdas et al., 2007; Dwyer et al., 2010; Zeller et al., 2003) and gluteus maximus (Dwyer et al., 2010) activation, increased hip extension and decreased knee flexion angles (Dwyer et al., 2010), while performing a single limb squat. Male adolescents performing a single leg squat demonstrate greater eccentric hip torque, trunk flexion, decreased hip adduction and knee abduction (Silva & Serrão, 2014; Graci et al., 2012).

Given that females regardless of OA, favour quadriceps dominant activation during single leg squats (Youdas et al., 2007) and demonstrate more general muscle activation during isometric tasks (Bigham, 2015), females are expected to have a greater overall magnitude of quadriceps activation during two-legged squats. General increases in activation are expected to be observed in the hamstrings, and gastrocnemius to control and direct throughout the squat descent and ascent in the females with OA. The lateral gastrocnemius has been classified as a general stabiliser in older females and individuals with osteoarthritis (Bigham, 2015) therefore it is expected to be activated to a greater extent and play a larger role in the knee stabilization strategy implemented in these individuals. Moreover, healthy male and female older adults performing two-legged squats
have achieved peak joint angles around 90° at the hip, 100° at the knee, and 30° at the ankle (Flanagan et al., 2003). Osteoarthritic participants, especially females, are expected to have reduced lower limb range of motion as a result of the previously described deficits in neuromuscular control and joint stability (Hurley & Newham, 1993).

Squats have been used to evaluate populations with patellofemoral pain syndrome (Nakagawa et al., 2012), Cerebral Palsy (Dan et al., 1999), ACL-deficiency (Roos et al., 2014; Miyaji et al., 2012) ACL-reconstruction (Roos et al., 2014, Neitzel et al., 2002), and total knee arthroplasty (Rossi et al., 2013). To our knowledge, there is no literature on OA sufferers, despite this being a population susceptible to functional disabilities during the squat. This represents a significant knowledge gap.

**KOOS and Self-reported Function**

The KOOS is a reliable and valid instrument for evaluating symptoms in populations with OA (Roos & Toksvig-Larsen, 2003). The KOOS questionnaire consists of 42 items in 5 subscales: symptoms, pain, function/daily living, function/sports and recreation, and quality of life, rated on a scale from 0 to 4. (Roos & Lohmander, 2003). The total score is then adapted to a scale from 0-100, zero meaning extreme knee problems, and 100 representing no symptoms (Roos et al., 1998). It was developed as a more detailed and extensive version of the WOMAC to increase the sensitivity of the questionnaire for knee related injury in the short and long term. The scores between the two questionnaires can be compared as all the WOMAC items were retained in the KOOS (Roos & Lomander, 2003).
Previous studies using the KOOS and WOMAC scales have reported significantly lower scores in the OA population compared to the healthy controls (Von Porat et al., 2004; Topp et al., 2000).

Self-reported and performance based measures have been used to assess functionality during everyday tasks in the knee OA population. A moderate negative correlation between KOOS scores and performance based measures has been established (Sabirli et al., 2013). Maximum knee flexion and extension torque, perception of joint pain and stiffness (WOMAC scale), have been shown to predict functional ability during stair ascent and descent and lowering and rising from the floor (Topp et al., 2000). Pain intensity in symptomatic knee OA patients has been associated with KOOS quality of life and functional scores, especially in persons with unilateral compared to bilateral pain (Riddle & Stratford, 2013). Harrison et al. (2004) found 42% of the variance associated with physical performance of function was attributable to functional self-efficacy (WOMAC) and balance. Lower WOMAC function and KOOS pain scores have been strongly associated with radiographic progression of osteoarthritis (Riddle & Jiranek, 2015) and can consequently be used as a representation of OA severity. Therefore, the KOOS questionnaire appears to be a valid measure to detect and differentiate healthy individuals from those who have OA.

In summary, squats are an everyday dynamic task that provide a good indication of functional ability. Given that squats require strength, coordination, and stability, they may also identify individuals with OA who are at increased risk of falling. Despite this, there is a significant lack of information regarding the biomechanics of a squat in an OA population. This thesis will address this knowledge gap and provide information regarding
the biomechanics and stabilisation strategies of older adults with OA during this important functional task.

**Methodology**

**Study Design**

This was a cross-sectional comparative study of the kinematics, kinetics, and muscle activation patterns between older males and females with OA compared to a control population. Lower limb joint angles, moments, powers and muscle activation patterns of eight muscles crossing the knee joint were recorded during a two-legged squat for the affected/dominant limb. This study design allowed for the investigation of the sex differences in movement strategies and muscle activation during squatting that may reveal deficits and compensations related to OA.

**Participants**

The participant recruitment, preparation, and data collection procedures were previously conducted by Bigham (2015), Flaxman et al., (2012), and Smith et al., (2012). Four groups including 15 females with knee OA (FOA) (63 ± 8.3 years), 15 males with OA (MOA) (64 ± 7.1 years), 16 healthy females (FOC), and 15 healthy males (61 ± 5.0 years) (MOC) (65 ± 7.7 years) participants (50+ years) were recruited for this study (See: Table 1). The participants had varying severities of OA (See: Appendix A), but were all able to complete the squatting task.

A post-hoc power analysis using G*Power (3.1.0) software was conducted on primary outcome data to determine the power of the results given the number of individuals that participated in the study (See: Appendix F).
The exclusion criteria for both groups included: previous lower limb sprain, muscle or tendon injury and fracture within 6 months prior to participating in the study; the presence of knee joint effusion; lower limb neurologic dysfunction; diabetes, or any other observed physical impairments that could affect the study’s results and/or BMI $\geq 35 \text{kg/m}^2$. Healthy participants were recruited using posters from Ottawa community centres and the knee osteoarthritis group was recruited from physiotherapy clinics throughout Ottawa, the Ottawa Hospital and community centres. All individuals with knee OA were diagnosed with radiographic and/or symptomatic knee osteoarthritis by a general practitioner. The University of Ottawa Research Ethics Board approved the study before commencing. All participants read and signed a consent form prior to data collection.

**Participant Requirements**

The data collection took place in the Human Movement Biomechanics Laboratory located at 200 Lees Ave., room E020 on one day for approximately four hours. Participants were reimbursed for parking fees or public transportation after proof of payment and were thanked for their contribution to the study. They were also notified that they would be given the results of the study after it was complete. The protocol did not present any safety or well-being concerns. Maximal isometric muscle contractions and squats may have caused temporary fatigue and discomfort. If the participant felt any discomfort throughout the session, they were encouraged to inform the researchers. Data was recorded under a participant code (ex. FOA01) for participant confidentiality.

**Data Collection and Equipment**

*KOOS and Physical Activity Levels*
All participants were asked to fill a Knee injury and Osteoarthritis Outcomes Score (KOOS) questionnaire (See: Appendix C) to qualitatively assess knee function and pain (Roos & Toksvig-Larsen, 2003). The KOOS is a reliable and valid instrument for evaluating symptoms in populations with OA (Roos & Toksvig-Larsen, 2003). The KOOS questionnaire consists of 5 subscales: symptoms, pain, function/daily living, function/sports and recreation, and quality of life, rated on a Likert scale from 0 to 4 (Roos & Lohmander, 2003). The totalled KOOS scores were calculated after the data collection. The frequency, intensity, and type of physical activity during an average week and if they were currently taking any medication were also recorded in the participant information form (See: Appendix G).

**Muscle Activations**

Each participant’s test limb (affected/dominant) had eight bipolar single differential silver surface electrodes (SP-E04, DE 2.1, Delsys Inc., MA) 10mm in length, 1mm in diameter, and spaced 10mm apart. A 16-channel system (DS-B04, Bagnoli-16, Delsys Inc., Boston, MA) was used to collect the EMG signals. Electrodes were connected to a portable input module (SP-N05, Bagnoli-8, Delsys Inc., Boston, MA) fastened to a custom harness worn by the participant. A grounding electrode (Dermatrode HE-R Farmado, BV, Nuland, Netherlands) was positioned on the right clavicular head. The input module was amplified using the main amplifier unit (SP-B08, Bagnoli-16, Delsys Inc., Boston, MA). Surface EMG was collected at 1000Hz, amplified by a gain of 1000 and band-pass filtered between 20-450Hz (Clancy et al., 2002, De Luca, 2010). Analog data were then converted to digital format with a 16-bit A/D conversion Board (Vicon MX control unit, Oxford Metrics, Oxford, UK).
**Force Measurement**

Ground reaction force (GRF) data were recorded from two force platforms (FP4060-08, Bertec Corporation, Columbus, OH, USA) at 1000Hz.

**Kinematic and Kinetic Measurements**

Trajectories of forty-five retroreflective markers (14mm diameter) were captured at 200 Hz with a ten-camera infrared Vicon motion analysis system. Lower limb kinematic and kinetic data were collected and processed using Nexus software (version 1.7, Oxford Metrics, Oxford, UK). Marker trajectories were reconstructed using the modified Plug in Gait model (See Appendix: D) (Beaulieu et al., 2010). Specifically, hip, knee and ankle joint angles, moments, and powers were collected for both the test (affected/dominant) and control (non-affected/non-dominant) limbs. In participants that had bilateral knee OA, the knee that was more adversely affected was taken as the ‘affected limb’. The dominant limb was defined as the leg used to kick a ball.

**Biodex Multi-Joint System 4 Pro**

An isokinetic dynamometer (850-000, Biodex, Shirley, NY) was used to collect maximum voluntary isometric contractions (MVIC). Position, velocity, and torque analog signals were exported in real time through a remote access port of the isokinetic dynamometer to an A/D conversion card (NI PCI 6229, National Instrument Corp., Austin, TX) and collected using a custom-made program (Labview 8.20; National Instruments Corp., Austin, TX) at 1000Hz.
Protocol Setup

**Participant Setup**

Upon arrival to the Human Movement Biomechanics Laboratory, participants were asked to fill out the study consent form and a KOOS questionnaire. Participants were asked to complete the questionnaire as truthfully and accurately as possible. Any medication being taken was recorded in the participant information form, in addition to the frequency, intensity, and type of physical activity performed during an average week. Participants were fitted in black spandex shorts and a t-shirt and anthropometric measurements including each participant’s height (cm), weight (kg), sex, age and lower limb anthropometrics (leg length, knee width, ankle width) were recorded and entered into Nexus software (v. 1.7, Oxford Metrics, Oxford, UK). Prior to electrode placement, an alcohol swab was used to clean the skin surface and hair was shaved with a disposable razor to maximise muscle activity conduction (DeLuca et al., 1997). Bipolar surface EMG electrodes were placed on the muscle bellies of eight lower limb muscles including: rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), long head of the biceps femoris (BF), semitendinosus (ST), tensor fascia latae (TFL), lateral gastrocnemius (LG), and medial gastrocnemius (MG) on the participant’s test leg. Electrodes were placed by palpation during manual resisted isometric contractions in accordance to SENIAM (Hermens et al., 2000). EMG signal to noise ratio was tested using various functional exercises (Hermens et al., 2000). Prior to data collection, participants performed a 5-minute warm up on a stationary bike with no resistance (Monarch AB, Sweden; 90 RPM). After completing the MVICs (See: Maximum Voluntary Isometric Contraction), forty-five reflective markers (14mm diameter) were placed on the body according to a modified
Plug-in-Gait model marker set (Beaulieu et al., 2010).

**Maximum Voluntary Isometric Contractions (MVICs)**

An isokinetic dynamometer (850-000, Biodex, Shirley, NY) was used to collect maximum voluntary isometric contractions (MVIC) and collect torque for the test leg (affected/dominant). For all isometric tests, participants were instructed to increase their perceived force from 0-100% effort gradually over a few seconds and maintain their maximum force for a minimum of three seconds and visual feedback of torque production and verbal encouragement from the researchers were provided. Joint torque and EMG were recorded and evaluated by custom-made software (Labview v.8.20; National Instruments Corp., Austin, TX). Position, velocity, and torque analog signals were exported in real time through a remote access port of the isokinetic dynamometer to an A/D conversion card (NI PCI 6229, National Instrument Corp., Austin, TX) and collected using a custom-made program (Labview 8.20; National Instruments Corp., Austin, TX) at 1000Hz. Three trials of knee flexion, extension and plantar flexion were measured on the participant’s test leg, while the participants were in a seated position with their hip, knee and ankle joints at 90° flexion, 30° flexion, and 10° plantar flexion, respectively. MVICs for the MG and LG were collected while participants were instructed to plantar flex against the dynamometer. Participants were then asked to perform knee extension and knee flexion trials to get the MVICs for the RF, VL, VM, ST and BF. Lastly, the TFL MVIC was obtained as the participants produced an abduction force against the thigh attachment while standing in a neutral hip and knee (0°) position (frontal plane). Participants were seated and secured in place with straps and attachments, to reduce the
chance of changing the joint angle and contribution of torque development from surrounding muscles.

**Squatting Protocol**

Following the completion of an isometric target matching protocol (see Flaxman et al. (2012) for details), three squat trials were collected. Participants performed three two-legged squats with each foot on a separate force platform (FP4060-08, Bertec Corporation, Columbus, OH, USA). The squat trial began when the participant deviated from maximum knee extension and ended upon return to full knee extension. They were instructed to have their arms straight out in front and squat down as low as possible, at a self-selected pace. Squat range was not standardised in order to capture the participants’ functional range of motion. Imposing a predetermined target peak knee flexion angle would prohibit the quantification of differences in functional range of motion between the groups. Nexus software (v. 1.7, Oxford Metrics, Oxford, UK) was used to collect EMG, GRF, and marker trajectories for successful trials.

**Data Analysis**

**Maximum Voluntary Isometric Contractions**

The EMG from the MVIC trials had the bias removed in order to have the mean signal equal to zero. This was done by calculating the mean of the signal and subtracting it from the whole signal. Raw EMG data from the MVIC was full wave rectified and smoothed with a 4\textsuperscript{th} order Butterworth dual low-pass 6 Hz filter by custom-made software (Labview 8.20; National Instruments Corp., Austin, TX, USA). Maximum muscle activity during the four MVIC movements for all eight muscles was calculated in Excel. An
average of 50ms about the absolute maximum EMG value from the three trials for each muscle was used as the MVIC value to normalize the EMG data from the squatting task, regardless of which MVIC movement it came from.

**Muscle Activation Patterns**

All EMG data were band-pass filtered, and a linear envelope (LE) was created using full wave rectification and filtering (4\textsuperscript{th} order Butterworth dual low-pass 6 Hz filter) in the custom-made Matlab application (2013a, The Mathworks, Natick, MA). The LE was then normalised as a %MVIC for each muscle. An ensemble average of the EMG signals from the three trials was calculated for each participant and for all four groups.

**Co-activation Index**

The co-activation index (CI) of the knee joint musculature is defined as the ratio between the antagonist’s and agonist’s summed activation during each phase of the task multiplied by the summed activity from both muscles (Rudolph et al., 2001; Lewek et al., 2005; Flaxman; 2012). The CI was calculated between vastus medialis and semitendinosus (VM-ST), vastus lateralis and biceps femoris (VL-BF), vastus medialis and medial gastrocnemius (VM-MG), vastus lateralis and lateral gastrocnemius (VL-LG), summed quadriceps and hamstring activity (QUAD-HAM) and quadriceps and gastrocnemius muscle activity (QUAD-GASTR). The QUAD group included the VM, VL, and RF, the HAM group included BF and ST, and the GASTR group included LG and MG. CI values were expected to be similar or higher, given the greater time spent squatting, to the results of Lewek et al. (2004) who evaluated co-activation during the loading phase of gait (initial
contact to peak adduction moment) and found CI values for VM-MG: 17.2 ± 7.3, VM-ST: 15.6 ± 6.6, VL-LG: 18.0 ± 7.8, VL-BF: 25.7 ± 10.8 in older adults with knee OA.

A CI was determined for the acceleration and deceleration phases of the squat descent and ascent, for a total of four CIs per muscle comparison. The time point where knee joint acceleration switched to deceleration was used to divide the squat ascent and descent phases, comparable to Dionisio et al. (2008) who characterized EMG patterns of downward squatting. A corresponding dynamic knee joint stiffness value (See: Dynamic Knee Joint Stiffness) was calculated for each CI value of the squat phases in order to evaluate knee joint stability between the four participant groups.

\[ CI = \frac{\text{lower EMG}}{\text{higher EMG}} \times (\text{lower EMG} + \text{higher EMG}) \quad (1) \]

Low co-activation values represent lower activation of both muscles or low level activation of one muscle and high activation of the other muscle in the pair, and indicate more selective muscle activation. Larger co-activation values denote higher activation of both agonist and antagonist muscles, representing a more general activation pattern. This CI provides a comprehensive description of the relative muscle activation (ratio) and the magnitude of activation (sum of muscle magnitudes).

**Dynamic Knee Joint Stiffness**

Dynamic knee joint stiffness (DKJS) was calculated by taking the slope of the linear regression line between knee joint moment and angle (Davis & DeLuca, 1996) using a custom made Matlab application (2013a, The Mathworks, Natick, MA). A DKJS value was determined for the acceleration and deceleration phases of the squat descent and ascent, matching the four CI values, previously calculated.
DKJS = \Delta M / \Delta \theta \quad (2)

M = Moment; \ \Theta = Degrees

**Kinematics and Kinetics**

A custom-made Matlab application (2013a, The Mathworks, Natick, MA) was used to extract GRF, kinematic and kinetic data for the squat task. A 4th order Butterworth dual low-pass (15Hz) filter was used for kinematic trajectories and GRFs using Nexus software (version 1.7, Oxford Metrics, Oxford, UK). This software calculated hip, knee, and ankle joint angles, moments, and powers in accordance with the modified Plug in Gait model (Beaulieu et al., 2010). An average of the squat trials was taken to derive the values for all dependent variables. All data were time normalised to 101 points for the squat descent and ascent phases. Squat trials began when the participant deviated from maximum knee extension and ended upon return to full knee extension. Faster squat velocities result in greater peak GRFs (Bentley, 2010), which could influence the comparison of power. Squats were therefore divided into ascent and descent, to account for the variability in time to peak knee between participants. Dividing the squat into phases was also used to determine if one phase was more difficult than the other based on the amount of joint torque and power required (ie. lowering body weight down or pushing up into extension).

Joint power is seldom reported in the literature, despite providing insight into the forces produced by the muscles as they relate to the speed of the contraction and task. Average joint power during squatting in healthy older adults indicates power absorption (negative) during descent and power generation during ascent (Flanagan et al., 2003).
Statistical Analysis

To characterize differences in squat descent and ascent strategies between males and females with and without knee OA the dependent variables (DV) of sagittal and frontal plane hip, knee, and ankle flexion angles, moments, and powers, normalised to body weight, were analysed for the affected/dominant leg using a two-way ANOVAs (Sex X OA Status; \(\alpha = .05\)). Bonferroni corrected post hoc tests were used to evaluate significant main effects and interactions to determine between group differences. Post-hoc analysis included four planned comparisons (FOA-FOC; FOA-MOA; MOA-MOC; FOC-MOC) using SPM independent t-tests. Each group was compared to two other groups, therefore significance was adjusted to the \(\frac{p=0.05}{2}=0.025\) for the number of comparisons to me made with each group.

ANOVA\'s and post hoc tests were carried out using Statistical Parametric Mapping (SPM), normalised to 100\% of the squat cycle. SPM is a valid tool that provides information about the entire curve (waveform) and how long significant differences occur over an entire movement cycle (Pataky, 2013). SPM includes all data within the waveform versus extracting a single finite value. The inherent problem of multiple comparisons is therefore taken into consideration, as it takes the entire waveform and covariance amongst the vector components into account.

Squat muscle activation patterns for the affected/dominant leg was analysed to compare stiffening strategies between OA participants and healthy controls using two-way ANOVAs (Sex X OA; \(\alpha = .05\)) implemented through SPM. Data were time normalised as a percentage of the squat cycle, as previously defined. SPM was able to indicate any differences in muscle activation magnitude and the timing of muscle activity over the
duration of squat phases. This allowed for the quantification of a muscle activation patterns for each of the four groups. Conducting an ANOVA through SPM in Matlab required the groups to have a balanced number of participants in each group. Due to technical errors, not all participants had EMG data; therefore the remaining participants were matched for age and body mass, resulting in the inclusion of 10 participants in each group.

Co-activation, dynamic joint stiffness were analysed for the affected/dominant leg between groups using two-way ANOVAs (Sex X OA status; α = .05) to analyse compensations and knee joint instability during squatting between sexes in the OA and control groups. Bonferroni corrected post hoc tests were used to evaluate significant main effects and interactions to determine between group differences. Data were time normalised as a percentage of the acceleration and deceleration phases of the squat descent and ascent, for a total of four comparisons. The Shaprio-Wilks test was used to determine normality of the data. Skewness and kurtosis (-2 < n > 2) was also analyzed to confirm the data was normally distributed. These analyses were performed using the statistical software package SPSS 21 for Windows (IBM Inc., Armonk, NY, USA).
ARTICLE 1: FEMALES WITH KNEE OSTEOARTHRITIS USE A DETRIMENTAL KNEE LOADING STRATEGY WHEN SQUATTING

Olivia R. Bayliss Zajdman¹, Heather J. Bigham¹, M.Sc.; Teresa E. Flaxman², M.Sc.;
Daniel L. Benoit¹,², Ph.D.

1. School of Human Kinetics, University of Ottawa, Canada
2. School of Rehabilitation Sciences, University of Ottawa, Canada

Corresponding Author: D. L. Benoit. Email address: dbenoit@uottawa.ca

Keywords: Knee Osteoarthritis, Sex differences, Squats, Kinematics, Kinetics, EMG
Abstract

Introduction: Older adults with knee osteoarthritis (OA) show altered muscle activation and females with OA demonstrate quadriceps weakness that will affect the biomechanics of the lower limb. Squatting is an important daily activity but the effects of these changes on this motion are limited. Objective: The purpose of this study was to identify sex differences in lower limb kinematics, kinetics, and muscle activation patterns between individuals with osteoarthritis and healthy controls during a two-legged squat. Methods: Thirty OA (15 females) and 30 healthy (15 females) participants performed three 2-legged squats. Sagittal and frontal plane hip, knee, and ankle kinematics and kinetics were calculated. Two-way ANOVAs (Sex X OA Status) were used to characterize differences in squatting strategies between sexes and between those with and without knee OA. Results: A greater decrease in sagittal hip, knee, and ankle range of motion and knee joint power was observed in the OA participants compared to the healthy controls. Females with OA had significantly reduced hip and knee adduction angles compared to the healthy females and males with OA. Females also had decreased hip power, flexion and adduction moments and knee adduction moments compared to their male counterparts, with the greatest deficits observed in the females with OA. Females with OA also had the highest magnitude of muscle activation for the quadriceps, hamstrings, and gastrocnemius throughout the squat, while males with OA showed increased activation of the vastus lateralis and medial gastrocnemius compared to the healthy males. Conclusion: OA significantly altered biomechanics and neuromuscular control during the squat, with males employing a hip-dominant strategy, allowing them to achieve a greater lower limb range of motion.
**Introduction**

Osteoarthritis (OA) occurs 2-3 times more frequently in females than in males (Badley, 1995) and is most commonly found in the knee (Buckwalter et al., 2001). The elderly are most affected by this disease (Bombardier et al., 2011) and often have chronic disability (Guccione et al., 1994) affecting daily activities. Older adults with OA have deficits in quadriceps strength (Lewek et al., 2004) and muscle activation (Hurley et al., 1997), and these deficits are exaggerated in women with knee OA (Petterson et al., 2007).

Squatting is one of the most common and arguably most important activities of daily living for personal hygiene and independence. It is a closed-kinetic chain movement that requires the hip, knee, and ankle joints to be coordinated. Difficulty holding a squatting position predominantly affects women (Jones & Reed, 2005) and will negatively impact their ability to use a toilet and sit and rise from a chair, thus reducing their functional independence.

To date, the biomechanics and neuromuscular control of the two-legged squat has primarily been studied in healthy young adults (Lynn & Noffal, 2012; Dionisio et al., 2008; Hase et al., 2004; Almosnino et al., 2013), with only limited research on healthy older adults (Flanagan et al., 2003) and to the author’s knowledge none with OA. Furthermore, sex differences have been predominantly reported in young healthy populations during a one-legged squat (Youdas et al., 2007; Zeller et al., 2003; Dwyer et al., 2010; Graci et al., 2012; Silva & Serrão, 2014). Two legged squats may be safer and a more relevant task to assess functional ability in older adults with OA, as they have shown deficits in quadriceps strength (Lewek et al., 2004), muscle activation (Hurley et al., 1997), and knee joint proprioception (Sharma et al., 1997; Hurley et al., 1997). These
factors may compound to have a greater negative impact on balance control and mobility (Hinman et al., 2002) putting them at an increased risk of falling while performing a single-leg squat.

Therefore the aim of this study is to characterize sex differences in lower limb kinematics, kinetics, and muscle activation patterns between males and females with knee OA and compared to a healthy control population during two-legged squatting. We hypothesized that the sex differences previously observed in younger healthy populations would be amplified with the presence of OA. Specifically, males with OA were expected to show movement patterns more similar to their healthy controls and to have increased sagittal plane range of motion (ROM), decreased hip adduction and knee abduction, and greater capacity to generate power compared to the females with OA. Females with OA were also expected to have greater magnitude of muscle activation than males.

**Methods**

**Participants**

Thirty participants with knee OA (15 females) and thirty healthy participants (15 females) were included in this study (Table 1). The exclusion criteria for both groups included: lower limb sprain, muscle or tendon injury and fracture within 6 months of participation, the presence of knee joint effusion, lower limb neurologic dysfunction, diabetes, or any other observed physical impairments that could alter movement characteristics. Healthy participants were recruited using posters in the Ottawa community centres and the knee osteoarthritis group was recruited from physiotherapy clinics throughout Ottawa, the Ottawa Hospital and community centres. All knee OA participants were diagnosed with radiographic and/or symptomatic knee osteoarthritis by a general
practitioner. This study was approved by the University of Ottawa Research Ethics Board (H02-08-07). All participants read and signed a consent form prior to data collection.

**Equipment**

Bipolar surface single differential silver surface electrodes (SP-E04, DE 2.1, Delsys Inc., MA) were placed on the rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), long head of the biceps femoris (BF), semitendinosus (ST), tensor fascia latae (TFL), lateral gastrocnemius (LG), and medial gastrocnemius (MG) muscle bellies of the test leg, in accordance to SENIAM (Hermens et al., 2000). Test leg of health controls was the dominant limb (defined as the leg used to kick a ball) while the affected limb was tested in the participants with OA. Surface electromyography (EMG) was sampled at 1000Hz, amplified by a gain of 1000, band-pass filtered between 20-450Hz and collected via a 16-bit A/D Board (Vicon MX control unit, Oxford Metrics, Oxford, UK).

Marker trajectories were captured at 200 Hz with a ten-camera infrared Vicon motion analysis system (Vicon MX-13, Oxford Metrics, Oxford, UK). Marker trajectories and ground reaction forces from two force plates (1000 Hz; FP4060-08, Bertec, Columbus, OH) were collected in Vicon Nexus software (version 1.8).

**Experimental Protocol**

**Healthy Outcome Questionnaires**

Upon arrival, participants were asked to fill out a consent form and a Knee injury and Osteoarthritis Outcomes Score (KOOS) questionnaire to qualitatively assess knee function and pain (Roos & Toksvig-Larsen, 2003). A score was calculated for the five subscales which include: Symptoms, Pain, Function, Daily Living (ADLs), Function, sports, and recreational activities (Sport/Rec), and Quality of Life (QOL). The frequency
and type of physical activity during an average week were also recorded in the participant information form.

Participants performed a 5-minute warm up on a stationary bike with no resistance (Monarch AB, Sweden; 90 RPM). Maximal voluntary isometric contraction (MVICs) during were performed using an isokinetic dynamometer (850-000, Biodex, Shirley, NY) and analysed with custom-made software (Labview 8.20; National Instruments Corp., Austin, TX, USA). Participants were instructed to increase their force from 0-100% effort gradually over a few seconds and maintain their maximum force for a minimum of three seconds, as researchers provided standardized verbal encouragement. Knee flexion, extension and plantar flexion torques were measured over three trials on the participant’s test leg while the participants were in a seated position with their hip, knee and ankle joints at 90° flexion, 30° flexion, and 10° plantar flexion, respectively. Hip abduction torque was measured in a standing position with the hip and knee at 0°.

Forty-five reflective markers (14mm diameter) were placed on the body according to a modified Plug-in-Gait model marker set (Beaulieu et al., 2010). Three two-legged squats performed at a self-selected pace were recorded Each foot was on a separate force platform and the squat trial began when the participant deviated from maximum knee extension and ended upon return to full knee extension.

Data Processing

Marker trajectories and GRFs were filtered with a 4th order low-pass zero-lag Butterworth filter at 15 Hz. Hip, knee, and ankle joint angles, moments, and powers were then calculated for the test limb using inverse dynamics. Internal net joint moments and powers were normalized to body weight (kg).
All EMG data were full wave rectified and smoothed using a 4th order dual low-pass Butterworth filter at 6 Hz. Maximum muscle activity (EMGmax) during MVIC trials for was calculated as the average of 50ms about the peak EMG value for each muscle across all trials. EMGmax was used to normalize experimental EMG data as percent (%) EMGmax.

All data were time normalized to 101 points for the descent phase (peak knee extension to peak knee flexion) and ascent phase (peak knee flexion to peak knee extension) for each trial. An ensemble average of the EMG signals from the three trials was calculated for each participant and for all four groups.

Statistical Analysis

Between group differences in the sagittal and frontal plane hip, knee, and ankle flexion angles, moments, powers, and EMG were tested using a two-way analysis of variance (ANOVA) with sex (female vs. male) and OA status (OA vs. healthy) as independent factors. ANOVAs and post hoc tests were carried out using Statistical Parametric Mapping (SPM) (Pataky et al., 2013). SPM is a valid tool that provides information about the entire curve (waveform) and how long significant differences occur over an entire movement cycle. Significant main effects and interactions were identified at the p=0.05 level. Post-hoc analysis included four planned comparisons (FOA-FOC; FOA-MOA; MOA-MOC; FOC-MOC) using SPM independent T-tests. Significance was adjusted to the p=0.05/2=0.025 for the number of comparisons to me made with each group.
Due to technical errors, not all participants had valid EMG data for all conditions. After ensuring that remaining participants were matched for age and body mass, a total of 10 participants in each group were included in the muscle activation analysis.

**Results**

*Anthropometrics and KOOS*

There were no differences between the groups (sex or OA status) for age or BMI. FOC and MOC had significantly higher KOOS scores than FOA and MOA (p < 0.001) (Table 1). No significant differences were found in the KOOS scores between the FOA and MOA groups (p > 0.05).

Table 1: Demographics, KOOS scores (Subscales: Symptoms, Pain, Function, Daily Living (ADLs), Function, sports, and recreational activities (Sport/Rec), and Quality of Life (QOL)), and time spent during each squat phase presented as mean with standard deviation for females with OA (FOA), males with OA (MOA), healthy older females (FOC), and healthy older males (MOC). Higher KOOS scores indicate better function.

<table>
<thead>
<tr>
<th></th>
<th>FOA</th>
<th>MOA</th>
<th>FOC</th>
<th>MOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.0 ± 8.3</td>
<td>64.0 ± 7.1</td>
<td>61.0 ± 5.0</td>
<td>65.4 ± 7.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 4.9</td>
<td>25.2 ± 4.0</td>
<td>23.3 ± 2.6</td>
<td>25.9 ± 2.9</td>
</tr>
<tr>
<td>Symptoms</td>
<td>68.8 ± 14.3*</td>
<td>69.6 ± 12.3#</td>
<td>93.6 ± 6.6*</td>
<td>94.0 ± 7.1#</td>
</tr>
<tr>
<td>Pain</td>
<td>71.4 ± 15.6*</td>
<td>72.6 ± 15.1#</td>
<td>97.2 ± 3.6*</td>
<td>96.9 ± 4.7#</td>
</tr>
<tr>
<td>ADLs</td>
<td>77.7 ± 13.4*</td>
<td>83.9 ± 13.2#</td>
<td>98.5 ± 3.3*</td>
<td>99.0 ± 2.1#</td>
</tr>
<tr>
<td>Sport/Rec</td>
<td>54.2 ± 24.0*</td>
<td>57.7 ± 25.0#</td>
<td>95.7 ± 8.3*</td>
<td>96.3 ± 8.3#</td>
</tr>
<tr>
<td>QOL</td>
<td>63.5 ± 12.6*</td>
<td>68.1 ± 13.1#</td>
<td>95.2 ± 5.5*</td>
<td>96.1 ± 4.7#</td>
</tr>
<tr>
<td>Squat Descent (s)</td>
<td>2.19 ± 0.6</td>
<td>3.10 ± 1.4</td>
<td>2.39 ± 1.0</td>
<td>2.58 ± 1.4</td>
</tr>
<tr>
<td>Squat Ascent (s)</td>
<td>1.63 ± 0.4</td>
<td>1.98 ± 0.7</td>
<td>1.91 ± 0.6</td>
<td>2.04 ± 1.2</td>
</tr>
</tbody>
</table>

Significant differences between ^FOC and MOC, *FOA and FOC, ~ FOA and MOA, and #MOA and MOC (p < 0.05).
Squat Kinematics

No significant main effects were found for time spent during squat descent or ascent between groups (p > 0.05; Table 1).

Significant main effect of sex was found for hip flexion angle (p = 0.045, 44-50%; p = 0.048, 51-61% squat cycle; Figure 1A), hip abduction angles (p = 0.027, 0-15%; p = 0.025, 83-100%; Figure 2A), knee adduction angle (p < 0.001; Figure 2B), and knee flexion angles (p < 0.001; Figure 1B), ankle dorsiflexion (p = 0.044), and ankle inversion (p = 0.032; Figure 2E,F).

Significant main effects of OA were found for ankle dorsiflexion (p = 0.041, 0-13%; p = 0.025, 24-35% squat cycle), ankle inversion (p < 0.001; Figure 2E,F), hip flexion angles (p = 0.024), knee flexion angles (p = 0.001, 19-50%; p < 0.002, 51-76% squat cycle), and hip adduction angles (p = 0.049; Figure 1A, B; Figure 2A).

Post-hoc analysis showed the FOA group had reduced hip abduction, knee adduction and knee flexion angles compared to the MOA (p = 0.007, p < 0.001, p < 0.001, respectively) and FOC (p = 0.007, p < 0.001, p < 0.001, respectively) groups. The MOA group had reduced ankle dorsiflexion compared to the MOC group (p = 0.024).

Squat Kinetics

Significant main effect of sex was found for hip extension moment (p < 0.001; Figure 1C), hip adduction moment (p < 0.001); Figure 2C), and knee adduction moment (p = 0.02; Figure 2D).

Significant effects of OA were found for knee extension moments (p = 0.047, 0-2%; p = 0.047, 22-24%; p = 0.048, 98-100% squat cycle), for knee joint power (p = 0.032,
0-2%; p < 0.001, 14-25%; p = 0.003, 43-49%; p < 0.001, 59-71% squat cycle) and hip joint power (p = 0.043, 49-50%; p < 0.001, 64-74% squat cycle; Figure 1D,F,E).

Post-hoc analysis showed the FOA group had reduced hip flexion moments and power generation compared to the MOA (p = 0.015, p = 0.019, respectively) and FOC (p = 0.015, p = 0.019, respectively) groups (Figure 1C,E).

**Muscle Activation Patterns**

A subset of ten participants for each group were included in the EMG analysis (FOA: n = 10; age = 62.7 ± 9.4 years; BMI = 24.5 ± 1.4; MOA: n = 10; age = 66.6 ± 6.0 years; BMI = 25.7 ± 4.2; FOC: n = 10; age = 59.8 ± 4.3 years; BMI = 24.0 ± 2.5; MOC: n = 10; age = 63.0 ± 7.0; BMI = 26.4 ± 3.1)

A significant main effect of sex was detected for muscle activation of the TFL (p = 0.045), RF (p = 0.046), VM (p = 0.046, 48-50%; p = 0.041, 51-53% squat cycle), VL (p < 0.001, 36-50%; p = 0.006, 51-57% squat cycle), ST (p < 0.001, 23-50%, p = 0.006, 51-68%; p = 0.049, 71-73% squat cycle), and LG (p = 0.038, 14-16%; p < 0.001 20-50%; p = 0.05, 51-61%; p = 0.02, 90-97%; p = 0.047, 99-100% squat cycle), and MG activity (p = 0.04, 2-4%; p < 0.001, 19-29%; p = 0.035, 65-68%; p < 0.001, 76-91%; p < 0.012, 94-100% squat cycle), whereby the female participants showed higher muscle activation (Figure 3).

Significant main effect for OA was found for VL (p = 0.037) and MG activity (p < 0.001, 17-28%; p < 0.001, 57-68%; 77-80% squat cycle; Figure 3C,H). A post hoc analysis revealed that the FOA group had a greater LG activation than MOA and FOC groups (p = 0.01, 29-32%; p = 0.01, 37-38%; and at p = 0.023, 43%; p = 0.025, 96% squat cycle).
Figure 1: Ensemble average and standard deviation of sagittal plane kinematics and kinetics for the hip and knee normalized to 100% of the squat cycle. A) Hip flexion angle (degrees); B) Knee flexion angle (degrees); C) Hip extension moment (N*m/kg); D) Knee extension moment (N*m/kg); E) Hip joint power (Watts/kg); F) Knee joint power (Watts/kg) for the FOA ( ), MOA ( ), FOC ( ), MOC ( ) groups. Significant differences are indicated for main effects of sex ( ) and OA status ( ) (p < 0.05).
Figure 2: Ensemble average and standard deviation of frontal plane kinematics and kinetics for the hip and knee and frontal and sagittal kinematics for the ankle normalized to 100% of the squat cycle. A) Hip abduction angle (degrees); B) Knee abduction angle (degrees); C) Hip adduction moment (N*m/kg); D) Knee adduction moment (N*m/kg); E) Ankle dorsiflexion angle (degrees); F) Ankle eversion angle (degrees) for the FOA ( ), MOA ( ), FOC ( ), MOC ( ) groups. Significant differences are indicated for main effects of sex ( ) and OA status ( ) (p < 0.05).
Figure 3: Ensemble average and standard deviation of muscle activation normalized to 100% of the squat cycle.  A) Rectus Femoris (RF) (degrees); B) Tensor Fascia Latae (TFL); C) Vastus Lateralis (VL); D) Vastus Medialis (VM); E) Biceps Femoris (BF); F) Semitendinosus (ST); G) Lateral Gastrocnemius (LG); H) Medial Gastrocnemius (MG) for the FOA ( ), MOA ( ● ● ● ● ), FOC ( ), MOC ( ● ● ● ● ) groups. Significant differences are indicated for main effects of sex ( ● ● ● ● ) and OA status ( ● ● ● ● ).
Discussion

The purpose of this study was to identify sex differences in kinematics, kinetics, and muscle activation patterns between an OA and healthy control population during two-legged squats. We compared four groups of age and BMI matched males and females with and without OA. Our hypothesis that the sex differences commonly observed in a young healthy population (Dwyer et al., 2010; Youdas et al., 2007; Zeller et al., 2003) would be amplified with the presence of OA was supported, as evidenced by the significant differences in functional ROM at the hip, knee and ankle joints, changes neuromuscular control patterns, and the decreased power absorption and generation compared to the healthy older adults.

Sagittal Plane Kinematics and Kinetics

The presence of OA was associated with altered sagittal plane biomechanics. Although both MOA and FOA groups demonstrated significantly different knee, ankle, and hip joint dynamics compared to their healthy counterparts, greater deficits in sagittal plane ROM at the hip and knee were observed in FOA compared to MOA group. Sex related differences in ROM have also been observed during single-limb squats (Dwyer et al., 2010). Since a sex-related increase in RF activation, accentuated with the presence of OA, was also observed, it is suggested that higher RF activation is required to control excessive knee flexion (Nene et al., 2004), but results in reduced knee flexion ROM (Piazza and Delp, 1996).

Our results also indicate that MOA implemented a hip dominant strategy, evidenced by decreased knee joint moments compared to MOC and increased hip joint moments compared to FOA. The gluteus maximus has a larger cross-sectional area (CSA)
than the four quadriceps muscles combined (Ito et al., 2003) and CSA is highly correlated to force generation capacity (Gans, 1982). Although gluteus maximus activity was not recorded in this study, it is possible that increasing its’ contribution to hip extension reduced the amount of hamstring co-activation and resultant quadriceps activation (knee extensor moment). Therefore, activating the hip extensors may decrease knee joint loading by reducing the required quadriceps activation (Bryanton et al., 2015), although this requires further investigation. The reduced vastii activation illustrated in the MOA group compared to the FOA group and the absence of a RF burst during ascent initiation further supports this theory.

In contrast, the quadriceps dominant neuromuscular control strategy employed in females during squatting (Youdas et al., 2007; Zeller et al., 2003; Dwyer et al., 2010), also illustrated in this study by the greater quadriceps activation, may reflect the strength and recruitment imbalance between quadriceps and hamstring muscles commonly observed in females (Hewett et al., 2002). Given that muscle forces modulate knee joint loads, this implies that the strategy used by females will alter the loading on the knee joint. Research using animal models (Wu, Herzog, & Epstein, 2000; Youssef et al., 2009; Herzog, Longino, & Clark, 2003) has clearly shown that small changes in the loading magnitude and location at the knee can trigger OA development. It may be that these altered neuromuscular strategies in females, leading to changes in loading, are thus linked to the increased incidence of OA in that population. The presence of OA in females further exacerbated this activation pattern and ROM deficit.

Greater hamstring activation has been observed in individuals with knee OA during knee extensor dominant tasks (Hortobagyi et al., 2005). Not surprisingly, our OA
groups also presented greater hamstring co-activation of ST compared to controls. However, FOA activated their ST to a greater extent than the MOA group. The change in muscle activation, possibly as an attempt to maintain stability, will alter knee joint loading and may expedite joint degeneration (Piscoya et al., 2005).

Both OA groups had reduced ankle ROM versus the controls. Individuals with knee OA have also displayed reduced ROM at the ankle during gait (Al-Zahrani et al., 2002) and while descending stairs (Hicks-Little et al., 2011). Alterations in ankle ROM as a result of OA may contribute to their increased fall rate (Takacs et al., 2013), as the combination of muscle weakness and decreased ankle flexibility has previously been proposed as a risk factor for falls in the elderly (Gehlesen & Whaley, 1990).

The role of the gastrocnemius is to help control the centre of mass from moving forward beyond the base of support (Hess & Woollacott, 2005), control dorsiflexion, and produce plantar flexion. However, our results suggest that gastrocnemii may be recruited differently between sexes and with the presence of OA, which may reflect differences in lower limb position during squatting. Altered activation of the gastrocnemius to compensate for knee instability, may lead to excessive knee joint loading in persons with OA, as increased activation of the gastrocnemius requires increased opposing muscle activity from the quadriceps to counter the knee flexor torque (Escamilla, 2001).

**Frontal Plane Kinematics and Kinetics**

Males and females displayed different frontal plane kinematics and kinetics at the hip and knee, regardless of the presence of OA. The MOA group executed the squat in a more adducted knee position than the females, possibly explaining the increased VL activation compared to the healthy males. Females executed and terminated the squat with
less hip abduction, with the FOA group maintaining a smaller abduction angle throughout the squat, contributing to the decrease in the frontal plane ROM and knee adduction. Higher TFL activation in FOA may have been required to help control frontal plane movement, as our FOA group also had the weakest normalized hip abduction torque compared to the other groups (See Appendix A). Given our strength and activation data, our results would thus indicate that hip muscle weakness plays a major role in the control of frontal plane knee joint motion.

Power

Hip joint power was significantly lower in both FOC and FOA groups during squat descent compared to their male counterparts. Since descent time was not significantly different between groups, the decline in hip power may be a reflection of the decreased ability to produce hip joint moments in the FOC, further reduced in the FOA group. Our results are supported by Resende et al. (2012) who found reduced hip joint power generation and absorption in women with knee OA during gait.

The presence of OA also impacted hip and knee joint power generation and is reflected by the reduction in sagittal hip flexion and knee extension moments in both OA groups compared to the healthy older adults. Decreased power absorption at the knee has previously been observed in osteoarthritic females during gait (Resende et al., 2012). The authors speculate that this strategy is used to reduce knee joint loading, but may also reflect the inability to properly deal with forces flowing through the knee in women with knee OA.

Older adults who have previously fallen have been shown to generate 24% less leg extensor power than non-fallers (Skelton et al., 1994), indicating the ability to generate
power swiftly is important in fall recovery. The negative influence of sex on the capacity to generate power at the hip combined with the effect of OA at both the hip and the knee may be a factor in the higher incidence of falls seen in females (Sattin, 1992) and in osteoarthritic individuals (Takacs et al., 2013). Falls occur more frequently during dynamic tasks (Niino et al., 2000) whilst moving laterally (Smeesters et al., 2001), and the females in this study had a reduced capacity to produce hip and knee joint moments and the FOA group demonstrated further mobility impairments in the frontal plane. Additionally, this will have a detrimental effect on the ability to squat down on to a toilet and push back up to a standing position independently, especially in the absence of an external aid (ie. handle/bar). As a result, functional independence and overall quality of life will be negatively affected.

It is important to note that participants were instructed to squat to their lowest comfortable depth. As such, squat depth was not normalized since we wished to evaluate self-perceived functional ability. This may have contributed to the increased variability seen in the frontal plane kinematics and kinetics, as the only instructions given to execute the squat were to keep their arms straight out in front of them. In addition, individuals with OA commonly display reduced knee extensor strength (Lewek et al., 2004) and deficits in quadriceps muscle function (Hurley & Newham, 1993). Since our EMG was normalized to a maximum value obtained during MVIC exercise, our EMG amplitudes may be overestimated and the observed increased in RF activity may not be a neuromuscular adaption in individuals with OA but rather a limitation in signal normalization.
Conclusion

Our study shows that two-legged squats are effective in detecting both sex and OA related impairments in movement patterns and muscle activation. As evidenced by the smaller ROM and higher muscle activation, males and females with osteoarthritis had more difficulty performing a two-legged squat compared to the healthy participants, with the negative influence being more prominent in the females with OA. For example, the males with OA were able to implement a hip-dominant strategy, decreasing knee joint loading (Lynn et al., 2012) and allowing them to achieve greater lower limb ROM. Differences in VL and MG activation in the OA participants, and ST activation in the females with OA around peak knee flexion, played a role in the OA participant’s decreased sagittal ROM, as the quadriceps, hamstrings, and gastrocnemii help control movement in the sagittal plane.

Our results indicate that interventions should aim to improve hip abductor and knee extensor strength, in particular in females with OA, in order to increase functional ROM and improve lower limb joint power. This may allow females to use a hip dominant squat strategy thereby correcting injurious movements, reducing knee joint loading, and slowing OA progression (Chang et al., 2005).
References


ARTICLE 2: OSTEARTHRITIS LEADS TO INCREASED MUSCLE CO-ACTIVATION AND KNEE STIFFNESS IN MALES AND FEMALES DURING SQUATTING

Olivia R. Bayliss Zajdman¹, Heather J. Bigham¹, M.Sc.; Teresa E. Flaxman², M.Sc.;
Daniel L. Benoit¹,², Ph.D.

1. School of Human Kinetics, University of Ottawa, Canada
2. School of Rehabilitation Sciences, University of Ottawa, Canada

Corresponding Author: D. L. Benoit. Email address: dbenoit@uottawa.ca

Keywords: Knee osteoarthritis, Dynamic joint stiffness, Co-activation, Squat, Sex differences
Abstract

Introduction: Knee osteoarthritis (OA) occurs 2-3 times more in females than males. It has been speculated that increased antagonistic muscle co-activation seen with knee osteoarthritis (OA) is a compensatory strategy to increase dynamic knee joint stability. 

Objective: The aim of this study was to identify sex differences between older adults with knee OA compared to healthy controls for dynamic knee joint stiffness and co-activation of muscles surrounding the knee. Methods: Thirty OA (15 FOA/30 MOA) and 30 healthy (15 FOC/MOC) participants each performed three 2-legged squats. Motion capture and electromyography of eight major muscles crossing the knee were recorded. Six co-activation indices and dynamic knee joint stiffness were calculated for squat descent and ascent phases. Two-way ANOVAs (Sex X OA Status) were used to characterize differences in squatting strategies between sexes with OA and healthy subjects. FOA had greater co-activation in 2 indices compared to FOC and MOA during the squat descent/deceleration phase. Results: OA participants had greater co-activation during the squat ascent/acceleration phase. The FOA group had higher co-activation in all QUAD-GASTROCN indices compared to the MOA and FOC groups during squat termination. Females also showed greater overall QUAD-HAM activity during the final phase. The OA participants had greater dynamic knee joint stiffness during squat descent deceleration and squat ascent acceleration compared to the healthy participants, with the FOA showing the greatest stiffness. Conclusion: Females with OA implemented a non-specific increase in antagonist activity, while the males used a more selective increase in co-activation, contributing to their greater stiffness. Females with OA use a different muscle co-
activation strategy compared to males and healthy females, which may put them at an increased risk of OA progression.

**Introduction**

Osteoarthritis is most commonly found in the knee (Buckwalter et al., 2001), and occurs 2-3 times more frequently in women than in men (Badley, 1995), resulting in decreased mobility, muscle atrophy, strength, and increased pain (Glass et al., 2014; Segal et al., 2010) with OA progression. Age is a risk factor for OA (Arthritis Alliance of Canada, 2011), and older adults have increased normalised muscle activity while performing a downward step (Hortobagyi & DeVita, 2000) and a single leg squat (Madhavan et al., 2009). Moreover, older females demonstrate a more general activation of the muscles surrounding the knee and increased quadriceps activation while performing a weight-bearing force-matching task (Bigham, 2015). This may be a compensation for reduced muscle strength and greater quadriceps-hamstring imbalance seen in older females (Bigham, 2015; Hewett et al., 2002). Furthermore, individuals with OA have greater normalised lower limb EMG activity while performing dynamic tasks (Schmitt & Rudolph, 2007). These alterations in neuromuscular function lead to an increase in co-activation during activities of daily living (ADLs), such as gait and stair ascent and descent, in older adults with OA compared to healthy-age match controls and younger adults (Hortobagyi et al., 2005).

Increasing co-activation may be a joint stiffening strategy in response to instability (Lewek et al., 2004), proprioceptive impairments (Hurley et al., 1997; Pai et al., 1997), or muscle dysfunction in OA individuals (Hurley & Newham, 1993). This co-activation strategy appears to be a safer short-term strategy during movement (DeVita & Hortobagyi,
Knee joint stability involves the combination of articular geometry (shape of condyles and menisci), body mass, passive soft tissue restraints and active muscles. Dynamic joint stiffness is an important component in stability, as it is a measure of the resistance to motion the joint provides, and depends on both passive and active joint structures. Joint stiffness may be reflected in the change in agonist and antagonist muscle contributions, as muscles are the only active regulators of joint stability (Flaxman et al., 2012). Dynamic knee joint stiffness (DKJS) has been used as an indicator of joint stability in conjunction with muscle co-activation during gait (Collins et al., 2014; Zeni & Higginson, 2009b). The combination of decreased range of motion at the knee (Messier, 1992) and altered muscle activation patterns (Hurley & Newham, 1993) commonly observed in the OA population could impact DKJS. To our knowledge, the DKJS has only been evaluated during gait in participants with knee OA, but have not evaluated sex differences despite the significant differences between male and females OA sufferers in terms of incidence, biomechanics, and neuromuscular control.

Two-legged squats are an everyday dynamic task, essential for personal hygiene and thus independence. It requires strength, coordination, and stability, and our research indicates that females with OA perform the task with significant biomechanical and neuromuscular adaptations (Zajdman et al., unpublished, 2016). Understanding these adaptations as they relate to stabilizing the knee and controlling joint loads is critical joint for developing intervention strategies to improve mobility and independence in the OA
population. Examining the relationship between DKJS and muscle co-activation during the squat may provide this insight. Therefore, the purpose of this study was to identify differences in co-activation and DKJS between sexes and between adults with and without knee OA during squatting. It was hypothesised that there would be a significant increase in DKJS and muscular co-activation with the presence of OA and that females would be most affected by these changes.

Methods

Participants

Thirty healthy older adults (15 male) and thirty individuals with knee OA (15 males participated in this study (Table 2). Individuals with previous lower limb sprain, muscle or tendon injury, and/or fracture within 6 months prior to participating in the study; the presence of knee joint swelling; lower limb neurologic dysfunction; diabetes were excluded from the study.

Individuals with knee osteoarthritis were recruited from the Ottawa Hospital, physiotherapy clinics throughout Ottawa, and both OA and healthy older adults were recruited from local Ottawa community centres. A general practitioner diagnosed all OA participants with radiographic and/or symptomatic knee osteoarthritis. The University of Ottawa Research Ethics Board approved this study. Prior to data collection, all participants read and signed a consent form.

Healthy Outcome Questionnaires

Upon arrival, participants were asked to fill out a consent form and a Knee injury and Osteoarthritis Outcomes Score (KOOS) questionnaire to qualitatively assess knee
function and pain (Roos & Toksvig-Larsen, 2003). The frequency and type of physical activity during an average week were also recorded in the participant information form.

**Participant preparation**

Participants were then fitted in black spandex shorts and a t-shirt and anthropometric measurements were recorded. Bipolar single differential silver surface electrodes with a 10mm separation (SP-E04, DE 2.1, Delsys Inc., MA) were placed on the muscle bellies of the dominant or OA affected limb rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), long head of the biceps femoris (BF), semitendinosus (ST), tensor fascia latae (TFL), lateral gastrocnemius (LG), and medial gastrocnemius (MG) following the SENIAM guidelines (Hermens et al., 2000). The dominant limb was defined as the leg the participant would use to kick a ball. Following the maximum voluntary isometric contractions (MVIC) trials (see below) forty-five reflective markers (14mm diameter) were placed on the whole body according to a modified Plug-in-Gait model marker set (Beaulieu et al., 2010).

**Maximum Isometric Voluntary Contractions**

A 5-minute warm up on a stationary bike with no resistance was completed before the data collection (Monarch AB, Sweden; 90 RPM). An isokinetic dynamometer (850-000, Biodex, Shirley, NY) was used to collect MVICs, and EMG and torque were evaluated using custom-made software (Labview 8.20; National Instruments Corp., Austin, TX). Participants were given visual and verbal encouragement and instructed to progressively increase their force from 0-100% over a few seconds and were required to maintain maximum force for at least three seconds. The participant’s test leg was used to test knee extension, flexion, and plantar flexion, while the participants were in a seated
position with their hip, knee, and ankle joints at 90° flexion, 30° flexion, and 10° plantar flexion, respectively. Participants stood with their hip and knee at 0° to measure hip abduction.

Participants completed three consecutive squats, using two force platforms (1000 Hz; FP4060-08, Bertec, Columbus, OH), and were instructed to have their arms straight out in front and squat down as low as possible, at a self-selected pace. Squat trials began at maximum knee extension and ended when the subject returned back to full knee extension. A ten-camera infrared Vicon motion analysis system was used to capture marker trajectories to establish squat initiation and termination (200 Hz; Vicon MX-13, Oxford Metrics, Oxford, UK).

Kinematics and Kinetics

Knee kinematic and kinetic data were collected using a ten-camera Vicon motion capture system and Nexus 1.8 software (Oxford Metrics, UK). Data were filtered with a 4th order low-pass zero-lag Butterworth filter at 15 Hz and exported to Matlab (2013a, The Mathworks, Natick, MA) for further data reduction. Knee joint angles and moments were calculated for the affected/dominant limb. Joint moments were normalized to body weight (kg) and expressed as internal net joint moments.

Electromyography

EMG was recorded at 1000Hz, amplified by a gain of 1000 and band-pass filtered between 20-450Hz (SP-B08, Bagnoli-16, Delsys Inc., Boston, MA). Analog data were then converted to digital format with a 16-bit A/D conversion Board (Vicon MX control unit, Oxford Metrics, Oxford, UK). The data were full waved rectified and filtered with a 4th order Butterworth dual low-pass 6 Hz filter using a custom Matlab (Matlab version
13a; The Mathworks, Natick USA) or Labview script (Labview 8.20; National Instruments Corp., Austin, TX, USA) for the squat and MVIC trials, respectively. Maximum muscle activity values were calculated in Excel for each MVIC task for each muscle from the average of 50ms about the absolute peak EMG value, regardless of the MVIC task it originated from. The data were then normalised as a %MVIC for each muscle and an average of the three squat trials was calculated for each subject and for all four groups. Squat events were determined from knee marker trajectories and squat descent and ascent phases were normalized to 101 points. Co-activation indices (CI) were determined for the acceleration and deceleration phases of the squat descent and ascent, for a total of four CIs per muscle comparison. The frame where knee joint acceleration switched to deceleration was used to divide the squat ascent and descent phases, comparable to Dionisio (2008) who characterized muscle roles during downward squatting. Not all subjects had EMG data due to technical errors, resulting in the inclusion of 10 participants per group for the co-activation analysis.

Co-activation was assessed using a CI, defined as the ratio between the antagonist’s and agonist’s summed activation during each squat phase multiplied by the summed activity from both muscles (Rudolph et al., 2000). The CI was calculated between (VM-ST), (VL-BF), (VM-MG), (VL-LG), (QUAD-HAM) and quadriceps and gastrocnemius muscle activity (QUAD-GASTR). The QUAD group included the VM, VL, and RF, the HAM group included BF and ST, and the GASTR group included LG and MG.

\[ CI = \frac{\text{lower EMG}}{\text{higher EMG}} \times (\text{lower EMG} + \text{higher EMG}) \]  

(1)
DKJS was calculated using a custom made Matlab application (2013a, The Mathworks, Natick, MA) by computing the slope of the linear regression line between knee joint moment and angle (Davis & DeLuca, 1996). A DKJS value was determined for the acceleration and deceleration phases of the squat descent and ascent, to match the four CI values.

\[ \text{DKJS} = \frac{\Delta M}{\Delta \theta} \]  

(2)

Statistical Analysis

The dependent variables of co-activation and DKJS for the affected/dominant leg were used to compare muscle activation and stiffening strategies between females with OA (FOA), males with OA (MOA), healthy older females (FOC), and healthy older males (MOC) using two-way ANOVAs (Sex X OA status; \( \alpha = .05 \)). Statistical significance was set at \( p < .05 \). Four planned comparisons were included in the post hoc analysis (FOA-FOC; FOA-MOA; MOA-MOC; FOC-MOC). A Bonferroni corrected post hoc test was used to correct for multiple comparisons and evaluate significant main effects and interactions to determine between group differences. Statistical analyses were conducted using SPSS 21 for Windows (IBM Inc., Armonk, NY, USA).

Results

Anthropometrics and KOOS

There were no main effects found for sex or OA status for age or BMI. Healthy males and females had significantly higher KOOS scores than females and males with OA (p < 0.001; Table 2). No significant differences were found in the KOOS scores between the FOA and MOA groups (p > 0.05). No significant differences were found between groups for the time spent during each squat phase (p > 0.05; Table 2).
Table 2: Participant characteristics, KOOS scores (Subscales: Symptoms, Pain, Function, Daily Living (ADLs), Function, sports, and recreational activities (Sport/Rec)), and Quality of Life (QOL), and percent of squat movement spent during each phase presented as mean with standard deviation. Higher KOOS scores indicate better function.

<table>
<thead>
<tr>
<th></th>
<th>FOA</th>
<th>MOA</th>
<th>FOC</th>
<th>MOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.0 ± 8.3</td>
<td>64.0 ± 7.1</td>
<td>61.0 ± 5.0</td>
<td>65.4 ± 7.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 4.9</td>
<td>25.2 ± 4.0</td>
<td>23.3 ± 2.6</td>
<td>25.9 ± 2.9</td>
</tr>
<tr>
<td>KOOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>68.8 ± 14.3*</td>
<td>69.6 ± 12.3#</td>
<td>93.6 ± 6.6*</td>
<td>94.0 ± 7.1#</td>
</tr>
<tr>
<td>Pain</td>
<td>71.4 ± 15.6*</td>
<td>72.6 ± 15.1#</td>
<td>97.2 ± 3.6*</td>
<td>96.9 ± 4.7#</td>
</tr>
<tr>
<td>ADLs</td>
<td>77.7 ± 13.4*</td>
<td>83.9 ± 13.2#</td>
<td>98.5 ± 3.3*</td>
<td>99.0 ± 2.1#</td>
</tr>
<tr>
<td>Sport/Rec</td>
<td>54.2 ± 24.0*</td>
<td>57.7 ± 25.0#</td>
<td>95.7 ± 8.3*</td>
<td>96.3 ± 8.3#</td>
</tr>
<tr>
<td>QOL</td>
<td>63.5 ± 12.6*</td>
<td>68.1 ± 13.1#</td>
<td>95.2 ± 5.5*</td>
<td>96.1 ± 4.7#</td>
</tr>
<tr>
<td>Squat Descent (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acceleration</td>
<td>33.5% 66.5%</td>
<td>33.7% 66.3%</td>
<td>30.3% 70.0%</td>
<td>28.7% 71.3%</td>
</tr>
<tr>
<td>Deceleration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squat Ascent (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acceleration</td>
<td>58.7% 41.3%</td>
<td>65.8% 34.2%</td>
<td>61.8% 38.2%</td>
<td>61.0% 39.0%</td>
</tr>
<tr>
<td>Deceleration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant differences between ^FOC and MOC, *FOA and FOC, ~FOA and MOA, and #MOA and MOC (p < 0.05).

Muscle Co-activation Indices

Squat Down Acceleration

Significant main effect differences for sex (p < 0.019) were revealed for VM-ST, where the FOA group had increased co-activation compared to the MOA (p < 0.018) and FOC groups (p < 0.05) (Figure 4A).

A significant main effect of OA was found (p < 0.002) for VL-BF. The post-hoc analysis revealed that the FOA and MOA groups had greater co-activation than FOC (p < 0.015) and MOC (p < 0.035) groups, respectively (Figure 4B).
Significant differences were revealed for sex (p < 0.003; p < 0.04) and OA (p < 0.014; p < 0.004) for VM-MG and VL-LG, respectively. The post-hoc analysis showed that the FOA group had greater VM-MG (p < 0.045) and VL-LG (p < 0.018) co-activation than the FOC group, and greater VM-MG (p < 0.017) activation that the MOA group (Figure 4C,D).

A significant main effect of sex (p < 0.007) was detected for the co-activation of the QUAD-HAM, while both OA (p < 0.013) and sex (p < 0.017) were significant for QUAD-GASTROC activation. The FOA group increased QUAD-HAM co-activation compared to the healthy females FOC (p < 0.025). The FOA group also had greater QUAD-GASTROC co-activation in comparison to the MOA (p < 0.017) and FOC (p < 0.014) groups (Figure 4E,F).

Squat Down Deceleration

A significant interaction effect of OA and sex was found for VM-ST antagonist muscle activation (p < 0.028). The FOA group had increased co-activation in comparison to the FOC (p < 0.032) and MOA (p < 0.002) groups (Figure 4).

The females had significantly greater VL-LG (p < 0.012) co-activation, in addition to the FOA group having significantly greater VL-LG co-activation than the MOA group (p < 0.037).

No significant differences were detected for VL-BF, VM-MG, QUAD-HAM, or QUAD-GASTROC.

Squat Up Acceleration

The ANOVA revealed a significant effect of OA (p < 0.022), as the MOA group had increased VL-BF co-activation versus the MOC group (p < 0.035; Figure 4B). A
significant main effect of OA (p < 0.004) was also determined for VM-MG, where by the MOA group showed greater co-activation than MOC (p < 0.014) (Figure 4C). Females had significantly higher co-activation of VL-LG during the squat up acceleration phase than the males (p < 0.047; Figure 4D).

Differences in the co-activation of the QUAD-HAM (p < 0.04) and QUAD-GASTROC (p < 0.025) were significantly affected by OA. The post-hoc revealed that the MOA (p < 0.023) and FOC (p < 0.033) groups had increased co-activation of QUAD-GASTROC muscles compared to the healthy males.

No significant differences were detected for VM-ST co-activation.

**Squat Up Deceleration**

The MOA group displayed an alternate muscle activation pattern seen in the decreased co-activation in all muscle pairings in the final squat phase compared to the other groups (Figure 4). The MOA group had significantly less VM-ST co-activation compared to the FOA (p < 0.03) and MOC (p < 0.01) groups.

A significant sex effect was detected for VM-MG (p < 0.0001) and VL-LG (p < 0.047). The post hoc revealed that the FOA group had higher VL-LG co-activation than MOA group (p < 0.011). The post-hoc also showed that the FOA greater VM-MG co-activity than the FOC group (p < 0.001), who had increased co-activation than the MOC group (p < 0.024).

Differences in co-activation of the QUAD-HAM (p < 0.046) and QUAD-GASTROC (p < 0.001) were significantly affected by sex. The FOA and FOC group had greater hamstring antagonist activity than the MOA and MOC groups, respectively. The FOA group also had significantly increased co-activation of the QUAD-GASTROCS
compared to the MOA group (p < 0.001) (Figure 4E,F). No significant differences were detected for VL-BF co-activation.

Figure 4: Co-activation indices (with SD) during squat down acceleration (SDAcc) squat down deceleration (SDDec), squat up acceleration (SUAcc), and squat up deceleration (SUDec) for A) VM-ST; B) VL-BF; C) VM-MG; D) VL-LG; E) QUAD-HAM; F) QUAD-GASTROC for the FOA ( ), MOA ( ), FOC ( ), MOC ( ) groups. Significant differences between ^FOC and MOC, *FOA and FOC, ~FOA and MOA, and #MOA and MOC (p < 0.05).
Dynamic Knee Joint Stiffness

Dynamic knee joint stiffness was calculated over four distinct phases of the squat cycle. There were no differences between groups with respect to the percent squat-time spent by each group in each phase (Table 2). An interaction effect of OA x sex was detected (p < 0.037) for DKJS during the squat down deceleration phase. The post-hoc analysis revealed that the FOA group had increased stiffness compared to the FOC group (p < 0.002). An interaction effect of OA x sex was also detected (p < 0.048) for DKJS during the squat up acceleration. The post-hoc analysis showed that the FOA group had increased stiffness compared to the FOC group (p < 0.001) (Figure 5).

Figure 5: Dynamic knee joint stiffness (DKJS) indices (with SD) during squat down acceleration (SDAcc) squat down deceleration (SDDec), squat up acceleration (SUAcc), and squat up deceleration (SUDec) for A) VM-ST; B) VL-BF; C) VM-MG; D) VL-LG; E) QUAD-HAM; F) QUAD-GASTROC for the
FOA (□), MOA (■), FOC (▲), MOC (▼) groups. Significant differences between ^FOC and MOC, *FOA and FOC, –FOA and MOA, and #MOA and MOC (p < 0.05).

No significant main effects for sex or OA status were found for DKJS during the squat down acceleration or squat up deceleration phases. Additionally, no significant differences were detected for DKJS between the FOC and MOC groups in any phase.

Discussion

The aim of this study was to analyse the muscular co-activation and DKJS between males and females with knee OA compared to healthy older adults. We found that the presence of knee OA alters the neuromuscular control strategy to stabilize the knee during squatting. Our results also indicate that females with knee OA had greater DKJS during the squat descent deceleration and squat ascent acceleration phases, whereas the males with OA had slightly higher DKJS during the squat up acceleration phase. The females with OA also presented the greatest co-activation throughout the squat movement, whereas the males with OA only used select increases in muscular co-activation. Our hypothesis were therefore partially supported by our results since increased DKJS and muscular co-activation is greater with the presence of OA, and heightened in females.

Squat Descent Muscle Co-activation

During the squat down acceleration phase, females with knee OA had greater VM-ST co-activation and all OA participants had greater VL-BF co-activation than healthy participants, derived from increased activation of both agonist and antagonist muscles. The increased co-activity in the females with knee OA continued as the participants transitioned into the squat descent deceleration phase, whereas the males showed a similar magnitude of QUAD-HAM co-activation to the healthy males. The increase in QUAD-
HAM co-activation in the OA participants, particularly in the females with OA, may be impacted by their lower knee extensor torque compared to the healthy participants (See: Appendix J). Previous studies have also shown that older adults use greater hamstring and quadriceps activation compared to younger adults during the knee flexion phase of a single leg squat (Madhavan et al., 2009) and as a strategy to maintain balance in response to a perturbation (Manchester et al., 1989). Older adults with knee OA present further increases in co-activation during activities of daily living (ADLs) compared to healthy-age match controls and younger adults (Hortobagyi et al., 2005).

The vasti muscles are considered general joint stabilizers in healthy older adults, but a general increase in quadriceps activation may be a sex related muscle recruitment pattern to compensate for muscle weakness (Huston & Wojtys, 1996). Older females have previously shown reduced specificity of activation of muscles surrounding the knee and increased quadriceps activation compared to males (Bigham, 2015). This may be a compensation for reduced knee extension and hip abduction torque females in this study demonstrated (See: Appendix J) and a greater quadriceps-hamstring imbalance seen in older females (Bigham, 2015; Hewett et al., 2002). The pre-disposition for females to implement a more general muscle activation to stabilize the knee joint may have been exaggerated in females with OA as a result of quadriceps weakness (Lewek et al., 2004; Slemenda et al., 1997) and proprioceptive deficits (Hurley et al., 1997; Pai et al., 1997), and may have contributed to the overall increased QUAD-HAM co-activation.

Furthermore, although the males with OA in this study have less absolute peak knee extensor torque than the healthy males (Bigham, 2015), the heightened VL antagonist activity seen in the MOA group may also be a reflection of the greater knee
adduction employed by the MOA group (Zajdman et al., unpublished, 2016) compared to the females, to help control the outward movement of the knees and decrease loading on the medial knee compartment (Lewek et al., 2004).

As the participants moved into the squat down deceleration phase, the gastrocnemius was activated to help control dorsiflexion and prevent the individual from falling forward. The medial and lateral gastrocnemii may be recruited differently between sexes and with the presence of OA, as greater VL-LG co-activation was seen in all females. Older females have previously used the LG as a general stabilizer whereas the males only activated it when moving in a specific direction, while performing a dynamic weight-bearing force-matching task (Bigham, 2015).

The greater QUAD-GASTROC co-activation observed in the FOA group in comparison to the MOA and FOC groups may also be a response to frontal plane instability and thus part of their knee stabilization strategy. A similar strategy was seen in patients with medial compartment knee OA, whereby they increased medial muscle co-activation in response to a lateral platform perturbation (Lewek et al., 2005). Moreover, Rudolph et al. (2007) observed that healthy older adults and knee osteoarthritic individuals used greater MG activity during gait than young and middle-aged individuals. The increased co-activation of the thigh muscles in the FOA group may be a protective mechanism used to prevent knee valgus during squat descent (Zajdman et al, 2016; manuscript 1).

Overall, the groups showed the greatest amount of co-activation during the squat down deceleration phase. The increase in eccentric activation of the quadriceps and
gastrocnemius may be a mechanism to help stabilize the knee and required more antagonistic muscle activity to control the joint motion.

_Squat Ascent Muscle Co-activation_

The quadriceps are activated to extend the knee (Isear et al., 1997), while hamstring activation peaks during squat ascent to assist in hip extension (Escamilla et al., 1998; Isear et al., 1997). Our OA participants had greater VL-BF and overall QUAD-HAM co-activation than the healthy participants. Our healthy participants may have been more efficient in selectively recruiting their hamstrings to help with hip extension, while the OA individuals maintained constant activation, which is both inefficient and increases knee joint loading.

The FOA group had increased QUAD-HAM co-activation around peak knee flexion compared to the healthy females. Conversely, the MOA group maintained similar VM-ST co-activation, while increasing VL-BF co-activation compared to the healthy males while accelerating upward. Greater lateral versus medial co-activation may help control the external adduction moment during the stance phase of gait (Schipplein & Andriacchi, 1991) and redistribute the load to the lateral knee compartment (Lewek et al., 2004). The ability for the males with knee OA to emulate this increased lateral quadriceps-hamstring co-activation may be a factor in the reduced incidence and severity of OA seen in males, while increased quadriceps activation during a dynamic task may be an important factor in understanding the greater prevalence of OA in females (Srikanth et al., 2005).

The increase in BF antagonistic activity observed in the MOA group may have been a strategy implemented to achieve greater knee range of motion (Zajdman et al,
2016; manuscript 1) compared to the females with OA. Youdas et al. (2007) has also shown that healthy females use a more quadriceps dominant strategy, while males are more hamstring dominant during the knee extension phase of a single leg squat. The MOA strategy may therefore allow the males to execute the squat more efficiently and generate more power (Zajdman et al, 2016; manuscript 1) to help push themselves back into full extension.

The medial and lateral gastrocnemii appear to be affected by OA in older females, requiring increased activation throughout the squat ascent, evidenced by greater VM-MG and VL-LG co-activation used by the females with OA during the squat up acceleration phase. Greater co-activation may be a result of the lower plantar flexor torque observed in the females (See: Appendix J). The MG in osteoarthritic males may also be affected by OA, demonstrated by the greater VM-MG co-activation in the males with OA. Altered activation of the gastrocnemius may be a compensatory mechanism in attempt to stabilize the joint, but may lead to greater joint degeneration in persons with OA by increasing the joint compression forces (Piscoya et al., 2005). Altered proprioception seen with knee OA may also play a role in the muscle activation differences seen, as the neural inputs from the soft tissue restraints of the joint and muscles impact the commands sent from the central nervous system (Hewett et al., 2002). As a result, the neuromuscular system may be ill equipped to activate the muscles efficiently, leading to an increase in co-activation.

**Dynamic Knee Joint Stiffness**

One method of analyzing knee joint stability is through DKJS, as decreased range of motion at the knee and increased co-activation of agonist and antagonist muscle activity will increase joint stiffness and impact the stabilization strategy implemented. The healthy
older adults significantly reduced their DKJS while decelerating into peak knee flexion and during the acceleration back to full knee extension. This coincided with their lower levels of co-activation throughout the squat, thus demonstrating the greatest efficiency and optimal dynamic joint stabilization strategy. The overall increased QUAD-HAM and QUAD-GASTROC co-activation during the squat ascent acceleration seen in the OA participants appears to contribute to the greater DKJS.

The females with OA had the greatest stiffness during these phases. The greater magnitude of antagonistic muscle activity also observed may highlight the role of muscles acting as active regulators of joint stability, which contributed to the increased stiffness and the least efficient movement strategy. Increasing DKJS and co-activation may be required to maintain their joint stability but the excessive muscle activation could negatively impact knee joint loading. The ability of the healthy older adults to maintain greater knee joint motion and largely reduce excessive antagonistic muscle activation may help explain their higher KOOS scores. It could also be hypothesized that their strategy may have helped prevent them from developing OA, however this requires further investigation.

Greater activation of the quadriceps could be contributing to the greater co-activation results in OA participants, in particular in females. Arthrogenous muscle inhibition with OA, in particular females with OA (Hurley & Newham, 1993), could result in greater muscle activation but not necessarily with the same relative increase in muscle force. As such, the increased quadriceps activation we observed may not contribute to increased compression forces acting across the knee joint. However, the increase in DKJS
seen in the OA population, particularly the females, would seem to confirm that this increased activation leads to the stiffening and stabilization strategy.

**Conclusion**

Older females and OA sufferers displayed a joint stabilization strategy driven by an increase in co-activation and contributing to increased DKJS which coincides with the reduced muscle specificity during weight-bearing force controls tasks identified in these populations (Bigham, 2015). Females with OA, and to a lesser extent males with OA, maintained greater DKJS around peak knee flexion, however the corresponding co-activation indices revealed differences in neuromuscular control in antagonistic muscle activation between sexes. The osteoarthritic females had a less specific increase in quadriceps and gastrocnemius antagonist activity during the squat descent and antagonistic hamstring activation during the squat ascent, while the males with OA employed a more selective increase in hamstring co-activation during squat ascent. Additionally, neuromuscular deficits in muscles that control frontal plane movement may put females at a predisposed risk of developing OA at a higher rate than males.

Our results indicate that future studies should aim to improve hip abductor, knee extensor strength, and hamstring-quadriceps coordination in females with OA to determine if targeted neuromuscular training leads to a more efficient stabilization strategy that reduces knee joint loading, inevitable slowing disease progression.
References


General Discussion

The two major goals of this thesis were to: 1) Identify differences in muscle activation patterns, kinematics, and kinetics between older males and females with and without OA; 2) Investigate whether differences in co-activation and dynamic knee joint stiffness exist between sexes and between older adults with and without knee osteoarthritis during squatting. We did this using the two-legged squat as it is a critically important ADL and important task for independent living. Sex differences in sagittal and frontal plane kinematics and kinetics, muscle activation patterns, and dynamic knee joint stiffness were analysed for the affected/dominant limb in the participants while performing three 2-legged squats. Males and females with OA had more difficulty squatting compared to the healthy participants, illustrated by the decreased functional range of motion, smaller power absorption and generation, and neuromuscular control patterns; the negative influence of the disease was more prominent in the females.

Squatting identified a greater decrease in sagittal plane range of motion at the hip, with the OA participants maintaining a more extended joint position throughout the squat movement. Furthermore, the females with OA had a smaller hip and knee range of motion than the males with OA and showed a greater deficit compared to the healthy females than the males did compared to their control group.

The osteoarthritic males employed a hip dominant strategy, whereby they decreased their knee joint moments and increased hip joint moments. This decreases knee loading (Lynn et al., 2012) and may have allowed males to increase peak knee flexion by using a more efficient squatting technique. Activating the hip extensors, in particular gluteus maximus, may decrease knee joint loading by reducing the required quadriceps
activation (Powers et al., 2010). This appears to be the strategy implemented by the MOA group who illustrated reduced magnitude of vasti activation compared to the FOA group, in addition to the absence of a RF burst during initial squat ascent observed in all other groups. The increased RF activation seen in the females, elevated with the presence of OA, may have limited their knee flexion, as previously demonstrated during a model gait simulation (Piazza and Delp, 1996).

Moreover, the decreased knee joint moments around peak knee flexion in the female participants may have reflected the increased co-activation of antagonist muscles commonly observed in the OA population (Hortobagyi et al., 2005). This is reflected in the increased QUAD-HAM co-activation seen around peak knee flexion in the FOA group compared to the healthy females, whereas the males with OA maintained similar co-activation compared to the healthy males. Lower hip joint moments in the healthy females, even lower in the FOA group, was also observed. Reduced eccentric hip torque, required to control anterior displacement of the centre of mass during squatting, is present in healthy females (Powers, 2010). Reduced capacity to generate hip torque and the strength deficit present in the females (Huston & Wojtys, 1996), and accentuated with OA (Petterson et al., 2007; Bigham 2015), may have prevented them from reaching maximal knee flexion.

Female participants also executed and terminated the squat with more hip adduction, with the FOA group maintaining a greater adduction angle over the entire squat movement, contributing to the decrease in the frontal plane range of motion and greater knee valgus position compared to the males, which was also observed in healthy females performing a single limb squat (Silva & Serrão, 2014; Graci et al., 2012). Increased TFL
activation in the females with OA may have been required to help control frontal plane movement, as our FOA group also had the weakest hip abduction torque compared to the other groups (Bigham, 2015). The FOA group also demonstrated ST activation with greater knee flexion while other groups decreased activation. The constant elevation of muscle activity in the females with OA could be a mechanism to stabilize the knee against valgus collapse in combination with their reduced torque generation capacity. Healthy younger male adults have previously demonstrated greater hip abductor, knee flexor, and knee extensor torque compared to females, which has been related to decreased valgus motion during a single leg squat (Claiborne et al., 2006). It is evident that hip muscle weakness and decreased neuromuscular control play major roles in the control of frontal plane knee joint motion. Our results indicate that females, irrespective of OA, may be predisposed to executing a squat using a less efficient strategy than males, putting them at a greater risk of overloading the knee joint.

The medial and lateral gastrocnemii were recruited differently between sexes and with the presence of OA. The females incrementally increased LG activity during the squat descent and maintained this higher activation during the ascent phase, while males with OA maintained a constant LG activation similar to the healthy males. Only the MG appeared to be affected by OA in the osteoarthritic males during squatting as demonstrated by the greater activation during the initial acceleration upward compared to healthy males. Increased muscle activation of both gastrocnemii and decreased plantar flexor torque in FOA compared to the MOA group (See: Appendix J) may have negatively impacted their functional range of motion at the ankle during squats.
Increased VM-MG co-activation seen in the males with OA and the even greater VM-MG and VL-LG co-activation seen in the females with OA during the squat down deceleration and squat up acceleration phases may be a compensatory mechanism used to counter knee joint instability or pain but contributed to greater joint stiffness and reduced mobility. Furthermore, older females have previously shown less specificity of activation of muscles surrounding the knee and higher quadriceps activation compared to males (Bigham, 2015). The pre-disposition for females to implement more generalized muscle activation to stabilize the knee joint may have been exaggerated in females with OA as a result of quadriceps weakness (See: Appendix A; Lewek et al., 2004; Slemenda et al., 1997) and proprioceptive deficits (Hurley et al., 1997; Pai et al., 1997), contributing to the overall increased QUAD-HAM co-activation.

The overall increased muscular co-activation during the squat down deceleration and the acceleration into squat ascent in the OA participants contributed to their greater DKJS. The females with OA had the greatest stiffness values during these phases, reflective of the greater magnitude of antagonistic muscle activity observed in these individuals. Increasing co-activation and using a ‘stiffening’ strategy may help counteract the increased knee instability commonly reported in OA sufferers, but limiting the range of motion at the knee and excessive muscle activation could increase joint compression forces and promote degeneration over time (Piscoya et al., 2005; Griffin & Guilak, 2005).

The different muscle activation strategies implemented between the sexes was illustrated in differences in co-activation indices, which resulted in similar increases seen in DKJS around peak knee flexion. The osteoarthritic females had an overall increase in quadriceps and gastrocnemius antagonist activity during the squat descent and antagonistic
hamstring activation during the squat ascent, while the males with OA employed a more selective increase in hamstring co-activation. Preferential activation of the quadriceps over the hamstrings to stabilize the knee may heighten the strength and recruitment imbalance between these muscles (Hewett et al., 2002). Females used a quadriceps dominant strategy to stabilize the knee during squatting but this can cause an unequal distribution of forces acting across the joint, increasing the likelihood of degeneration of the articular surface and joint cartilage by changing the contact area to a region not accustomed to dissipating joint contact forces (Wu, Herzog, & Epstein, 2000; Cicuttini et al., 2002).

Furthermore, the healthy older adults significantly reduced their DKJS while decelerating into peak knee flexion and during the acceleration back to full knee extension compared to the OA participants. This may be an indication of greater dynamic joint stability (since less co-activation was needed to support the joint during the task) and the decreased resistance to movement resulting from coordinated antagonist muscle activation. The ability for the healthy older adults to maintain greater knee joint motion and largely reduce excessive antagonistic muscle activation may provide insight into why they have not yet developed OA and should be further explored.

Finally, the influence of sex and OA on the reduced capacity to generate power at the hip and knee may be a factor in the higher incidence of falls seen in females (Sattin, 1992) and in osteoarthritic individuals (Takacs et al., 2013). Hip joint power was significantly reduced in both healthy and OA females during squat descent, mirrored in the decreased ability to produce hip joint moments in the FOC that was further reduced in the FOA group. The reduction in knee flexion moments in both OA groups during the descent phase may have contributed to their decreased knee joint power. Squat ascent revealed a
greater discrepancy in hip power generation between the OA and healthy subjects.

Supporting our results, a study analysing joint power in women with knee OA during gait found that power generation and absorption at the hip and knee were also decreased compared the asymptomatic group (Resende et al., 2012). The authors speculated that this strategy was used to reduce knee joint loading, but may also reflect the inability to properly deal with forces flowing through the knee in women with knee OA. The decline in muscle mass and proportion of fast twitch muscle fibres seen in older adults (Connelly et al., 1999) may also affect their ability to generate sufficient power. The capacity to generate power quickly is essential for fall recovery, and older adults who have previously fallen have been shown to generate 24% less leg extensor power than non-fallers (Skelton et al., 1994). Falls occur more frequently during dynamic tasks (Niino et al., 2000) whilst moving laterally (Smeesters et al., 2001), and the females in this study had a reduced capacity to produce hip and knee joint moments, with the female osteoarthritic group demonstrating greater mobility impairments in the frontal plane.

Limitations

One limitation of this study was that participants were only instructed to squat down as low as possible with their arms straight out in front of them, but not given any instruction as to how to execute the squat, which may be reflective of the increased variability seen in the frontal plane kinematics and kinetics. Moreover, squat range of motion was not standardised in order to capture the participants’ functional range of motion. Imposing a predetermined target peak knee flexion angle would prohibit the quantification of differences in functional range of motion between the groups. Interpreting the muscle activation at the same degree of flexion would also have been
compromised as the muscles take on different roles during acceleration into squat descent and deceleration towards peak flexion. For example, females with OA had a smaller range of motion than then healthy females and would reach the deceleration phase earlier; therefore the increased magnitude of muscle activation would not be an accurate interpretation of their neuromuscular control as the comparison would be made between two different phases.

Another limitation inherent in the methodology used to analyze muscle activation was that SPM could not analyse data that does not have an even number of values in each group. Although one subject from the FOC group had to be removed in order to have equal numbers in each group, information given about the entire squat and how long significant differences occurred versus using single peak values outweighs this drawback.

The participants in this study included unilateral and bilateral knee OA sufferers. Although the unaffected/less affected limb was not analyzed in this study, it is possible that participants used this limb to compensate for any deficits in their (more) affected limb and this level of compensation was not accounted for. Future studies should aim to limit the sample of OA participants to either those with unilateral OA, or assess asymmetry between the two OA-affected limbs as a confounding factor.

Furthermore, individuals who took part in this study were only required to be physically active twice a week, but the type and frequency were not controlled for. The variability observed in muscle activations may have been influenced by the differences in physical activity between participants, as muscle activation has been shown to vary as a function of physical activity level (da Fonseca et al., 2006). Future studies should aim to include participants who engage in similar levels of physical activity.
Elevated magnitude of quadriceps activation may be partly attributable to the quadriceps weakness commonly found in people with knee osteoarthritis (Lewek et al., 2004; Slemenda et al., 1997) and could be contributing to the greater co-activation and the stiffening strategy seen in the OA population. Caution should be taken when interpreting the elevated magnitude of quadriceps muscle activation in the osteoarthritic participants, as studies have found arthrogenous muscle inhibition, resulting in reduced voluntary quadriceps activation while performing isometric and isokinetic strength tests in females with OA (Hurley and Newham, 1993). Therefore the knee extensor maximum voluntary contractions in the OA participants may not be an accurate representation of their true maximum.

The muscle activity of the gluteus maximus, gluteus medius and tibialis anterior were not recorded, but should be included in future studies. The gluteus maximus is a primary hip extensor and understanding how its activation may change between sexes and with OA may provide a better understanding of the factor that limit functional range of motion during a squat. Analysing the activation patterns of the gluteus medius could provide greater insight into the neuromuscular control of frontal plane kinematics and kinetics in males and females with OA, as it plays a principal role in hip abduction. Although the tibialis anterior (TA) does not contribute to compressive forces at the knee, its role is to provide ankle dorsiflexion. Alterations in neuromuscular control of the TA in the presence of OA may help explain the decreased range of motion.

Finally, individuals with knee OA often report that pain affects their ability to perform everyday tasks (Jordan et al., 1997). Pain was monitored throughout the data collection, and participants were asked to report any changes in pain. Although none of the
participants indicated any change in pain level while completing the squatting task, the KOOS pain subscale was correlated to some of the main outcome variables, including peak knee flexion (See: Appendix I). Since pain did not change during testing, we conclude that any influence of pain on our results, in particular knee flexion range of motion, are a reflection of participant compensations used in their everyday activities.

**Conclusion**

In conclusion, our results indicate that females with OA use a different muscle activation strategy to execute two-legged squats compared to males with OA and healthy females. This includes a knee stiffening strategy that could put them at a greater risk of OA development and progression and may be indicative of a native instability at their knee joint requiring compensation. Neuromuscular deficits in muscles that control frontal plane movement may also put females at a predisposed risk of developing OA at a higher rate than males.

Osteoarthritis had a negative impact on the movement strategy executed, which was influenced by the combination of knee joint instability, lower limb muscle weakness, and quadriceps dysfunction, resulting in a decreased functional range of motion in the males and females with osteoarthritis compared to the healthy participants, although the females with OA were affected the most. It is evident that increased DKJS around peak knee flexion in the females with OA, and to a lesser extent males with OA, limited their range of motion, however the corresponding co-activation indices revealed differences in neuromuscular control in antagonistic muscle activation between sexes.
Our study shows that two-legged squats are able to detect sex and OA related movement impairments, as well as altered biomechanics at adjacent lower limb joints, and can therefore be used as a clinically relevant measure.

Interventions should aim to improve hip and knee extensor strength in order to increase functional range of motion and improve lower limb joint power. This may allow females to use a hip dominant squat strategy thereby correcting injurious movements, reduce abnormal knee joint loading and slow OA progression. Increased mobility from a more targeted intervention will help improve functional limitations, prevent further disability due to OA, increase quality of life, and reduce the burden on the Canadian health care system and subsequently society.

**Acknowledgements**

This research was supported by the Natural Sciences and Engineering Research Council, the Canadian Foundations for Innovation and the University of Ottawa, Faculty of Health Sciences. I would also recognize Teresa Flaxman, Heather Bigham, and Andrew Smith for their contribution to the data collection and Luis Licon for his assistance with the data processing in Matlab.

I would like to thank my committee members Dr. Linda McLean and Dr. Martin Bilodeau for their involvement during the development of this project. I would also like to thank my family and friends for their endless support and encouragement throughout the duration of my master’s thesis. Finally, I would like to thank Dr. Daniel Benoit for supervising my master’s thesis project. This has been an invaluable learning experience and I would not have been able to complete this project without his guidance and patience for my infinite number of “quick” questions.
References


Hirvensalo, M., Rantanen, T., & Heikkinen, E. (2000). Mobility Difficulties and Physical Activity as Predictors of Mortality and Loss of Independence in the Community-


Appendices

Appendix A: Reported Severity

**Table 3:** Reported number of female (FOA) and male (MOA) participants with bilateral/unilateral knee osteoarthritis (OA) and their self-reported severity.

<table>
<thead>
<tr>
<th>OA Severity</th>
<th>FOA</th>
<th>MOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mild-Moderate</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Unilateral</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Bilateral</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Appendix B: Variable Summary

**Table 4:** Summary of the number of females with OA (FOA), males with OA (MOA), healthy older females (FOC), and healthy older males (MOC) in each group with kinematic, kinetic, and electromyography (EMG) data.

<table>
<thead>
<tr>
<th>Squat</th>
<th>Kinematics/Kinetics</th>
<th>EMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOA: 15 FOA: 10</td>
<td>FOC: 16 FOC: 11</td>
<td></td>
</tr>
<tr>
<td>MOA: 15 MOA: 10</td>
<td>MOC: 15 MOC: 10</td>
<td></td>
</tr>
</tbody>
</table>
Appendix C: KOOS (Knee Osteoarthritis and injury Outcome Score)

KOOS KNEE SURVEY

Today's date: ____/____/______ Date of birth: ____/____/____

Name: ________________________________

INSTRUCTIONS: This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to perform your usual activities. Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Symptoms
These questions should be answered thinking of your knee symptoms during the last week.

S1. Do you have swelling in your knee?

Never Rarely Sometimes Often Always
☐ ☐ ☐ ☐ ☐

S2. Do you feel grinding, hear clicking or any other type of noise when your knee moves?

Never Rarely Sometimes Often Always
☐ ☐ ☐ ☐ ☐

S3. Does your knee catch or hang up when moving?

Never Rarely Sometimes Often Always
☐ ☐ ☐ ☐ ☐

S4. Can you straighten your knee fully?

Always Often Sometimes Rarely Never
☐ ☐ ☐ ☐ ☐

S5. Can you bend your knee fully?

Always Often Sometimes Rarely Never
☐ ☐ ☐ ☐ ☐

Stiffness
The following questions concern the amount of joint stiffness you have experienced during the last week in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

S6. How severe is your knee joint stiffness after first wakening in the morning?

None Mild Moderate Severe Extreme
☐ ☐ ☐ ☐ ☐

S7. How severe is your knee stiffness after sitting, lying or resting later in the day?

None Mild Moderate Severe Extreme
☐ ☐ ☐ ☐ ☐
### Pain

**P1. How often do you experience knee pain?**

- **Never**
- **Monthly**
- **Weekly**
- **Daily**
- **Always**

What amount of knee pain have you experienced the **last week** during the following activities?

**P2. Twisting/pivoting on your knee**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**P3. Straightening knee fully**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**P4. Bending knee fully**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**P5. Walking on flat surface**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**P6. Going up or down stairs**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**P7. At night while in bed**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**P8. Sitting or lying**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**P9. Standing upright**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

### Function, daily living

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

**A1. Descending stairs**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>

**A2. Ascending stairs**

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
</table>
For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A3. Rising from sitting

None | Mild | Moderate | Severe | Extreme

A4. Standing

None | Mild | Moderate | Severe | Extreme

A5. Bending to floor/pick up an object

None | Mild | Moderate | Severe | Extreme

A6. Walking on flat surface

None | Mild | Moderate | Severe | Extreme

A7. Getting in/out of car

None | Mild | Moderate | Severe | Extreme

A8. Going shopping

None | Mild | Moderate | Severe | Extreme

A9. Putting on socks/stockings

None | Mild | Moderate | Severe | Extreme

A10. Rising from bed

None | Mild | Moderate | Severe | Extreme

A11. Taking off socks/stockings

None | Mild | Moderate | Severe | Extreme

A12. Lying in bed (turning over, maintaining knee position)

None | Mild | Moderate | Severe | Extreme

A13. Getting in/out of bath

None | Mild | Moderate | Severe | Extreme

A14. Sitting

None | Mild | Moderate | Severe | Extreme

A15. Getting on/off toilet

None | Mild | Moderate | Severe | Extreme
For each of the following activities please indicate the degree of difficulty you have experienced in the \textit{last week} due to your knee.

A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc)

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A17. Light domestic duties (cooking, dusting, etc)

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textbf{Function, sports and recreational activities}

The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the \textit{last week} due to your knee.

SP1. Squatting

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SP2. Running

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SP3. Jumping

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SP4. Twisting/pivoting on your injured knee

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SP5. Kneeling

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textbf{Quality of Life}

Q1. How often are you aware of your knee problem?

<table>
<thead>
<tr>
<th>Never</th>
<th>Monthly</th>
<th>Weekly</th>
<th>Daily</th>
<th>Constantly</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q2. Have you modified your lifestyle to avoid potentially damaging activities to your knee?

<table>
<thead>
<tr>
<th>Not at all</th>
<th>Mildly</th>
<th>Moderately</th>
<th>Severely</th>
<th>Totally</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q3. How much are you troubled with lack of confidence in your knee?

<table>
<thead>
<tr>
<th>Not at all</th>
<th>Mildly</th>
<th>Moderately</th>
<th>Severely</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q4. In general, how much difficulty do you have with your knee?

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textit{Thank you very much for completing all the questions in this questionnaire.}
Appendix D: University of Ottawa Modified Plug-in Gait Marker Set
Table 5: The markers and description of their placement on the participant used to collect kinematic data in this study. The acronyms that begin with the letter “L” or “R” correspond to the left and right sides of the body, respectively.

<table>
<thead>
<tr>
<th>Head</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFHD &amp; RFHD</td>
<td>Left temple</td>
</tr>
<tr>
<td>LBHD &amp; RBHD</td>
<td>Left back of head</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Torso</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C7</td>
<td>7th cervical vertebrae</td>
</tr>
<tr>
<td>T10</td>
<td>10th thoracic vertebrae</td>
</tr>
<tr>
<td>CLAV</td>
<td>Jugular notch</td>
</tr>
<tr>
<td>STRN</td>
<td>Xiphoid process</td>
</tr>
<tr>
<td>RBAK</td>
<td>Middle of right scapula</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSHO &amp; RSHO</td>
<td>Acromio-clavicular joint</td>
</tr>
<tr>
<td>LUPA &amp; RUPA</td>
<td>Upper arm</td>
</tr>
<tr>
<td>LELB &amp; RELB</td>
<td>Lateral epicondyle</td>
</tr>
<tr>
<td>LFRA &amp; RFRA</td>
<td>Forearm</td>
</tr>
<tr>
<td>LWRA &amp; RWRA</td>
<td>Wrist bar thumb side</td>
</tr>
<tr>
<td>LWRB &amp; RWRB</td>
<td>Wrist bar pinkie side</td>
</tr>
<tr>
<td>LFIN &amp; RFIN</td>
<td>Dorsum of the hand head of 2nd metacarpal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pelvis</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LASI &amp; RASI</td>
<td>Anterior superior iliac crest</td>
</tr>
<tr>
<td>LPSI &amp; RPSI</td>
<td>Posterior superior iliac spine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Legs</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTHI &amp; RTHI</td>
<td>Lateral thigh</td>
</tr>
<tr>
<td>LMKN &amp; RMKN</td>
<td>Medial epicondyle of the knee</td>
</tr>
<tr>
<td>LKNE &amp; RKNE</td>
<td>Lateral epicondyle of the knee</td>
</tr>
<tr>
<td>LTIB &amp; RTIB</td>
<td>Lateral shank</td>
</tr>
<tr>
<td>LANK &amp; RANK</td>
<td>Lateral malleolus</td>
</tr>
<tr>
<td>LMAN &amp; RMAN</td>
<td>Medial malleolus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feet</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTOE &amp; RTOE</td>
<td>2nd metatarsal head of foot</td>
</tr>
<tr>
<td>LHEE &amp; RHEE</td>
<td>Posterior calcaneus</td>
</tr>
</tbody>
</table>
Appendix E: Joint Angle and Moment orientation

Table 6: University of Ottawa Modified Plug-in-Gait model output of lower limb angles/moments in the local coordinate system and their positive and negative movement descriptors.

<table>
<thead>
<tr>
<th>Description</th>
<th>Segment Axes</th>
<th>Movement</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle X</td>
<td>Tibia</td>
<td>Dorsiflexion/Plantarflexion</td>
<td>Dorsiflexion</td>
<td>Plantarflexion</td>
</tr>
<tr>
<td>Ankle Y</td>
<td>Tibia</td>
<td>Abduction (inv)/Adduction (ever)</td>
<td>Adduction</td>
<td>Abduction</td>
</tr>
<tr>
<td>Knee X</td>
<td>Thigh</td>
<td>Flexion/Extension</td>
<td>Flexion</td>
<td>Extension</td>
</tr>
<tr>
<td>Knee Y</td>
<td>Thigh</td>
<td>Varus (add)/Valgus (abd)</td>
<td>Varus (add)</td>
<td>Valgus (abd)</td>
</tr>
<tr>
<td>Hip X</td>
<td>Pelvis</td>
<td>Flexion/Extension</td>
<td>Flexion</td>
<td>Extension</td>
</tr>
<tr>
<td>Hip Y</td>
<td>Pelvis</td>
<td>Abduction/Adduction</td>
<td>Adduction</td>
<td>Abduction</td>
</tr>
</tbody>
</table>

Appendix F: G*Power (3.1.0) Post-hoc power analysis

<table>
<thead>
<tr>
<th>Primary Outcome Data</th>
<th>Sample Size</th>
<th>Alpha</th>
<th>Effect Size</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Flexion Angle</td>
<td>15</td>
<td>.05</td>
<td>0.4</td>
<td>0.861</td>
</tr>
<tr>
<td>Raw Quadriceps EMG</td>
<td>10</td>
<td>.05</td>
<td>0.4</td>
<td>0.692</td>
</tr>
</tbody>
</table>
Appendix G: Participant Information form

Knee Muscle Activation Study
Participant Information

Date: ______________________  Subject Code: __________________

Name: __________________________________________________________

Phone: ______________  Email: ______________________

Birth date: __________________  Age: ________  Sex: ________

Leg Dominance: __________ (What leg would you use to kick a soccer ball as far as possible?)

Check all that apply to you below:

☐ Previous traumatic knee injury (i.e. ligament rupture or meniscal tear)

☐ Previous lower extremity motor nerve lesion

☐ Diabetes

☐ Ankle or knee sprain within the past 6 months

☐ Lower limb muscle or tendon injury within the past 6 months

☐ Lower limb bone fracture within the past 6 months

☐ Recent pain and/or swelling in the knee or ankle joint (within the last 2 weeks)

Physical activities/sports (please specify intensity levels – light/moderate/high, duration per session and number of sessions per week):
__________________________________________________________
__________________________________________________________
__________________________________________________________
__________________________________________________________
__________________________________________________________

Further notes: ______________________________________________
__________________________________________________________
__________________________________________________________
Appendix H:

Table 7: Effect size and Cohen’s D for peak knee flexion angle, knee flexion moment, hip flexion angle, knee power (ascent), hip abduction angle, and knee adduction angle for females with OA (FOA), males with OA (MOA), healthy older females (FOC), and healthy older males (MOC).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comparison</th>
<th>Cohen’s D</th>
<th>r</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Flexion Angle</td>
<td>FOA-MOA</td>
<td>1.944</td>
<td>0.697</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td>FOA-FOC</td>
<td>2.197</td>
<td>0.739</td>
<td>0.546</td>
</tr>
<tr>
<td></td>
<td>MOA-MOC</td>
<td>0.917</td>
<td>0.417</td>
<td>0.174</td>
</tr>
<tr>
<td>Knee Extension Moment</td>
<td>MOA-MOC</td>
<td>0.955</td>
<td>0.431</td>
<td>0.186</td>
</tr>
<tr>
<td>Hip Flexion Angle</td>
<td>FOA-MOA</td>
<td>0.859</td>
<td>0.395</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>FOA-FOC</td>
<td>1.411</td>
<td>0.577</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td>MOA-MOC</td>
<td>0.734</td>
<td>0.345</td>
<td>0.119</td>
</tr>
<tr>
<td>Knee Power (Squat up)</td>
<td>FOA-MOA</td>
<td>0.588</td>
<td>0.282</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>FOA-FOC</td>
<td>1.051</td>
<td>0.465</td>
<td>0.216</td>
</tr>
<tr>
<td></td>
<td>MOA-MOC</td>
<td>0.830</td>
<td>0.383</td>
<td>0.147</td>
</tr>
<tr>
<td>Hip Abduction Angle</td>
<td>FOA-MOA</td>
<td>0.931</td>
<td>0.422</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td>FOA-FOC</td>
<td>1.181</td>
<td>0.509</td>
<td>0.259</td>
</tr>
<tr>
<td>Knee adduction Angle</td>
<td>FOA-MOA</td>
<td>1.144</td>
<td>0.496</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td>FOC-MOC</td>
<td>1.031</td>
<td>0.458</td>
<td>0.210</td>
</tr>
</tbody>
</table>

Appendix I:

Table 8: Pearson Correlation Coefficients for KOOS Pain sub-scores compared to peak knee flexion angle, knee extension moment, knee power (ascent), squat down deceleration stiffness (SDDec), and squat up acceleration stiffness (SUAcc) for all participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pearson Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Flexion Angle</td>
<td>.523*</td>
</tr>
<tr>
<td>Knee Extension Moment</td>
<td>.310*</td>
</tr>
<tr>
<td>Knee Power (ascent)</td>
<td>.444*</td>
</tr>
<tr>
<td>SDDec Stiffness</td>
<td>.209*</td>
</tr>
<tr>
<td>SUAcc Stiffness</td>
<td>.329*</td>
</tr>
</tbody>
</table>

Significant differences indicated by * at α = .01 (2 tailed)
### Appendix J:

**Table 9:** Absolute (N*m) and normalized torque (N*m/kg) presented as mean with standard deviation for females with OA (FOA), males with OA (MOA), healthy older females (FOC), and healthy older males (MOC).

<table>
<thead>
<tr>
<th>Variable</th>
<th>FOA</th>
<th>MOA</th>
<th>FOC</th>
<th>MOC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Peak Torque (N*m)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee Extension</td>
<td>78.21 ± 27.0</td>
<td>96.95 ± 20.6</td>
<td>78.92 ± 19.0</td>
<td>115.76 ± 30.0</td>
<td>* p = 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>^ p = 0.001</td>
</tr>
<tr>
<td>Knee Flexion</td>
<td>50.35 ± 20.7</td>
<td>63.36 ± 19.3</td>
<td>47.37 ± 11.5</td>
<td>62.33 ± 16.6</td>
<td>* p = 0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>^ p = 0.036</td>
</tr>
<tr>
<td>Ankle Plantar Flexion</td>
<td>55.99 ± 20.0</td>
<td>71.71 ± 16.1</td>
<td>48.54 ± 20.5</td>
<td>70.78 ± 19.9</td>
<td>* p = 0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>^ p = 0.006</td>
</tr>
<tr>
<td>Hip Abduction</td>
<td>49.79 ± 16.5</td>
<td>63.18 ± 14.9</td>
<td>46.60 ± 12.5</td>
<td>63.53 ± 14.7</td>
<td>* p = 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>~ p = 0.035</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>^ p = 0.009</td>
</tr>
<tr>
<td>Normalized Peak Torque (N*m/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee Extension</td>
<td>1.08 ± 0.32</td>
<td>1.34 ± 0.33</td>
<td>1.32 ± 0.30</td>
<td>1.48 ± 0.38</td>
<td>* p = 0.001</td>
</tr>
<tr>
<td>Knee Flexion</td>
<td>0.68 ± 0.24</td>
<td>0.87 ± 0.25</td>
<td>0.79 ± 0.15</td>
<td>0.79 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>Ankle Plantar Flexion</td>
<td>0.79 ± 0.25</td>
<td>1.01 ± 0.28</td>
<td>0.83 ± 0.38</td>
<td>0.89 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Hip Abduction</td>
<td>0.69 ± 0.18</td>
<td>0.88 ± 0.26</td>
<td>0.78 ± 0.19</td>
<td>0.80 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

Significant main effects of sex (*) and OA status (#) differences between FOC and MOC (^), and FOA and MOA (~) calculated from a two-way between group ANOVA (p < 0.05).