SEX DIFFERENCES IN KNEE OSTEOARTHRITIS PROCESSES: THE ROLE OF MUSCLE STRENGTH IN EXPLAINING ACUTE PAIN INTENSITY, PAIN SENSITIZATION, KNEE JOINT MOMENT AND MUSCLE ACTIVATION RESPONSES TO A STANDARD CONTINUOUS WALK

by

Bernadette A. McCann

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ABSTRACT

Walking is prescribed for knee osteoarthritis management but evidence to support specific walking prescriptions is lacking. Given that osteoarthritis manifests itself differently between sexes, are sex-specific walking prescriptions needed? This study determined differences between sexes in pain, moment and muscle activation responses to a 30-minute walk in individuals with radiographic knee osteoarthritis, and whether muscle strength explained variability in responses. Forty-five (23 females) participants with radiographic medial knee osteoarthritis were included. Independent t-tests determined males had higher strength, knee flexion moment-knee extension moment difference, and lower pain sensitization and muscle activity than females ($p<0.05$). Two-way mixed ANOVAs found significant sex by time interactions ($p<0.1$) where males, but not females, increased their pain sensitization and knee adduction moment (KAM) features post-walk. Linear regression models indicated strength explained 11% of the variance in KAM 1st peak response. Different magnitudes and directions in responses between sexes support the need for sex-specific walking prescriptions.

LIST OF ABBREVIATIONS USED

3D: Three-dimensional

ANOVA: Analysis of variance

BE: Beta-endorphins

BMI: Body mass index

CNS: Central nervous system

COMP: Cartilage oligomeric matrix protein

DOHM: Dynamics of Human Motion

ECRL: Extensor carpi radialis longus

EMG: Electromyography

JSN: Joint space narrowing

IRED: Infrared emitting diode

KE: Knee extensor

KF: Knee flexor

KFA: Knee flexion angle

KAM: Knee adduction moment

KFM: Knee flexion moment

KEM: Knee extension moment

KL: Kellgren-Lawrence

KOOS: Knee injury and osteoarthritis outcome score

LH: Lateral hamstring

MH: Medial hamstring

MRI: Magnetic resonance imaging

MVIC: Maximum voluntary isometric contraction

mPD-Q: Modified painDETECT questionnaire

NPRS: Numeric Pain Rating Scale

NSAIDs: Non-steroidal anti-inflammatory drugs

OA: Osteoarthritis

OG1: Over-ground walk 1 (pre-intervention)

OG2: Over-ground walk 2 (post-intervention)

OKS: Oxford Knee Score

PC: Principal component

PCA: Principal component analysis

PCS: Pain catastrophizing scale

PPT: Pressure pain threshold

QST: Quantitative sensory testing

RF: Rectus femoris

RMS: Root mean square

TKA: Total knee arthroplasty

US: Ultrasound

VL: Vastus lateralis

VM: Vastus medialis

WOMAC: Western Ontario and McMaster Universities Index

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CHAPTER 1: INTRODUCTION

The overall goal of this thesis was to improve our understanding of whether muscle strength can explain the differences between males and females with radiographic knee osteoarthritis (OA) in acute responses to continuous walking in pain intensity and sensitization, knee joint moment and muscle activation features during walking that have been previously associated with knee OA progression. Chapter 1 provides a brief overview of the background and rationale for this thesis, followed by the specific objectives and hypotheses, and the thesis outline.

1.1 BACKGROUND AND RATIONALE

Osteoarthritis (OA) is a serious disease with a large personal, healthcare, and economic burden[1]. OA affects over 500 million people worldwide[2] and over 4 million Canadians[3] and these numbers are expected to rise (e.g., 9 million Canadians in 2040)[3]. Canadian statistics show that OA is more prevalent in females (23%) than males (17%)[3], and this is consistent with the global literature[4]. There is literature to support that females with OA self-report worse pain and physical function[5–9], lower physical activity levels[10] than males with OA, and that specific differences exist in joint structure[11–15], pain mechanisms[9,16–19], gait biomechanics[20–23] and muscle activation[21,23] during walking, biochemical biomarkers[24–26] and muscle strength[16,23,27–31]. Together, differences in these factors may help explain the increased OA prevalence and burden in females and influence how we manage OA as current OA therapies are generalized and not sex-specific.

There is currently no cure for OA, and the most common therapeutic interventions include pharmaceuticals aimed primarily at managing pain (e,g., non-steroidal anti-inflammatory drugs (NSAIDs), opioids)[32] and end-stage treatments involving surgery (i.e., tibial osteotomies and joint replacements)[33]. However, many of these therapies are not sustainable due to serious and negative side effects (e.g., addiction and various long-term health problems)[32,34,35] and the difficulty meeting current surgical demand[36]. There has been a shift towards movement-based interventions such as exercise and in particular walking, based on evidence from intervention studies showing improved pain and general health[37]. Yet current uptake by healthcare providers and patients for these interventions is poor[38–41] and can in part be explained by vague guidelines based on general population guidelines (e.g., 2018 Physical Activity Guidelines for Americans[42]) which do not provide specific parameters (e.g., duration, frequency, intensity) for those with OA[37]. Furthermore, there remains a gap in direct evidence on precisely how walking impacts joint health (e.g., cartilage degradation) and other OA processes (e.g., pain, inflammation, joint structure, gait mechanics and muscle function) to support specific parameters. Given the discordance between the disease (i.e., structure) and illness (i.e., symptoms)[43–45], both structure and symptom outcomes must be examined.

One model that can provide direct evidence on the impacts of loading on various OA processes is to examine acute or immediate responses to a specific walking condition. Studies report that 40 to 50% of participants with knee OA increase their pain intensity immediately after 6 to 30 minutes of continuous walking[46–48], and these pain responses differ between interval and continuous walking for the same overall time period with interval walking resulting in no pain increases[49]. Pain increases after walking have also

been associated with specific changes in knee joint mechanics and muscle function measures[46–49]. These studies provide emerging evidence that individuals with knee OA do not have consistent pain responses to a continuous walking condition. Important to the objectives of this thesis, past walking studies reported only one dimension of pain (i.e., pain intensity), despite the multidimensional nature of pain which includes psychological and physiological components[50]. Furthermore, they did not separate individuals by sex in their analysis despite the multiple differences between sexes as indicated above. Given that females typically have lower muscle strength than males[16,23,27–31], it is plausible that their lower body muscles may fatigue more quickly while walking. This may result in changes in knee joint moments similar to changes that occur after a knee extensor (KE) muscle fatigue protocol including increases in the knee adduction moment (KAM) and decreases in the early stance knee flexion moment (KFM) to late-stance knee extension moment (KEM) difference measure (i.e., KFM-KEM difference)[51].

Pertinent to walking interventions is the growing evidence that knee joint biomechanics[52–59] and muscle activation patterns[54,60–62] during walking are predictive of OA progression and these are modifiable risk factors than can be addressed through neuromuscular exercises and gait re-training[63–66]. Since there is no cure for OA, understanding how these patterns can be modified to slow progression is key to ensuring patients obtain the best possible outcomes. Outcomes for OA progression can include measures of structural and/or symptom worsening with the clinical end-point treatment for severe OA being joint replacement surgery such as total knee arthroplasty (TKA)[33,67]. For this thesis, OA progression studies on both structure and/or symptom progression outcomes were examined to identify important knee joint moment and muscle activation

features associated with progression since both structural changes and symptoms are evaluated in clinical decision-making. The two most common features examined related to joint loading and knee OA progression[57] are the frontal plane external KAM which provides a measure of the ratio between medial-to-lateral joint loading[68] and the sagittal plane external KFM which can provide an estimate of overall joint loading[69]. Several KAM features have been linked primarily to structural progression metrics[57,52,53] but unique to clinical progression that includes both structural and symptom worsening is the KFM-KEM difference[58] indicative of a stiff-knee gait.

There are limitations in relying on joint moments only to estimate joint contact loads as muscle forces account for a large part of internal joint contact loads[70,71]. KE and knee flexor (KF) muscle strength, and KE and medial hamstring (MH) prolonged muscle activation patterns are also highly correlated with the KFM-KEM difference measure linked to OA progression[72]. Muscle strength is also independently linked to OA progression[73,74], although when sex-specific analyses are conducted, this relationship is stronger or only present in females[31,75,76]. The few studies that have looked at muscle activation measures linked to knee OA progression provide evidence that greater magnitude and duration of co-activation[60,62] and higher and more prolonged muscle activity, consistent with a stiff-knee gait pattern[54,60,61] are linked to OA progression. A thorough review of these features will be provided in Chapter 2.

Of particular interest is the evidence that knee joint moments and muscle activation features linked to knee OA progression, also differ between males and females. This includes evidence of females having lower KAM magnitudes[59,77] and KFM-KEM differences[20,59], and higher muscle co-activation[23] and overall muscle activity[59] than

males. Since higher KAM features are primarily associated with structural progression[57,52,53] and the KFM-KEM difference measure with clinical progression[58] it appears that females may be at greater risk of clinical progression given that they have a stiffer-knee gait pattern and higher muscle activation patterns, whereas males may be at greater risk of structural progression given that they have higher KAM features. It is unclear whether these sex differences in gait patterns could be explained by the lower muscle strength[20,23,29,30] and/or higher pain reported in females[5–9] since lower KF and KE muscle strength has been correlated with a smaller KFM-KEM difference[72] and pain has shown a relationship with reduced gait speed[78,79]. Individuals with knee pain have shown different knee joint moments such as higher KAM peaks and impulse[80–83] and mid-stance KFM[83] but lower peak KFMs[82] and higher KF and KE muscle activation[82]. Experimental pain relief has been shown to increase peak KAM and KEM[84] and overall compressive knee joint forces[85]. There is also an association between higher pain and lower muscle strength[86–92]. Most of the above studies investigating between sex differences examined each variable individually, and this study focused on examining how these variables interact with one another, specifically the interactions among sex, muscle strength, pain, knee joint moments, and muscle activation and if they differ following a continuous bout of walking. Furthermore, since females typically have lower muscle strength than males, and muscle strength is associated with pain, knee joint moments and muscle activation features, it is plausible that some sex differences in these measures are due to differences in muscle strength.

Therefore, the overall goal of this thesis was to improve our understanding of whether muscle strength can explain the differences between males and females with

radiographic knee OA in acute responses to a continuous walk in pain intensity and sensitization, knee joint moment and muscle activation features during walking that have been previously associated with knee OA progression. Four specific objectives aimed to address the overall goal.

To address the overall goal of this thesis as described above, four specific objectives were included. Objectives 1 and 2 compared muscle strength, multiple dimensions of pain, knee joint biomechanics, and muscle activation patterns between sexes. Objective 3 examined whether there were differences between sexes in pain, knee joint biomechanics, and muscle activation pattern responses to a continuous walking protocol to determine whether sex-specific walking parameters are needed. Objective 4 determined how much variance muscle strength explained in these responses, to better understand the role of muscle strength in these responses to walking.

1.2 THESIS OBJECTIVES AND HYPOTHESES

Objective 1: To determine if there are differences between males and females with radiographic medial compartment knee OA in KE and KF muscle strength, demographic (i.e., age, mass etc.) and clinical characteristics (i.e., self-reported measures of pain catastrophizing, OA-specific pain, physical function, symptoms, and physical activity levels).

Hypothesis 1: Females will have significantly lower KF and KE muscle strength, higher pain catastrophizing, and worse OA-specific pain, physical function, symptoms, and physical activity levels than males.

Objective 2: To determine if there are differences between males and females with radiographic medial compartment knee OA in pain intensity, pain sensitization, and knee joint moments and muscle activation patterns during walking previously linked to OA progression.

Hypothesis 2: Females will have higher pain intensity and pain sensitization, a lower KAM magnitude, a smaller KFM-KEM difference, and higher and more prolonged muscle activation magnitude than males.

Objective 3: To determine if there are differences between males and females with radiographic medial compartment knee OA in responses to a standard 30-minute self-selected speed walk in pain intensity, pain sensitization, knee joint moments and muscle activation patterns during walking previously linked to OA progression.

Hypothesis 3: There will be significant sex (male/female) by time (pre-post-walk) interactions where females will have significantly greater increases in pain intensity, pain sensitization, KAM magnitude, muscle activity magnitude, prolonged muscle activity responses, and decreases in the KFM-KEM difference measure than males.

Objective 4: To determine how much variance in pre-post-walk response scores following a standard 30-minute self-selected speed walk (Objective 3) is explained by muscle strength (Objective 1) in individuals with radiographic medial compartment knee OA.

Hypothesis 4: Muscle strength will explain significant variance in pain intensity, pain sensitization, the KFM-KEM difference measure, KE and KF muscles overall activity magnitude and prolonged activity pre-post-walk response scores.

For clarity throughout this thesis, variables that were measured before and after the 30-minute walking intervention are defined as:

i) Pre-walk variables: Variables measured before the 30-minute walk.

ii) Post-walk variables: Variables measured after the 30-minute walk.

iii) Pre-post-walk response score: Difference between the post-walk value and pre-walk value.

1.3 THESIS OUTLINE

This Master's thesis consists of six chapters. Chapter 2 provides a detailed overview of the relevant background literature on this topic. Chapter 3 provides a detailed description of the study methodology. Chapters 4 and 5 provide the results and discussion for Objectives 1 and 2, and Objectives 3 and 4 respectively. A summary and discussion of key findings, implications and a conclusion are presented in Chapter 6.

CHAPTER 2: REVIEW OF RELEVANT LITERATURE

This chapter contains an overview of the literature on the burden of knee OA and on the evidence supporting walking as a therapy for OA management. Next, a synthesis of the literature on key outcome variables assessed in this study including pain, muscle strength, knee joint moments, and muscle activation is provided. Finally, a review of studies investigating acute responses to walking in the current OA literature is included, followed by a chapter summary.

2.1 KNEE OSTEOARTHRITIS

Osteoarthritis (OA) is a prevalent (>500 million people worldwide[2]) and serious disease with a large personal, healthcare, and economic burden[1]. The knee is the most commonly affected joint, accounting for 83% of OA cases[93]. Walking, an aerobic exercise, is a recommended intervention for knee OA management in non-pharmacological/non-surgical guidelines[37,38,94–96], but uptake by health providers and people with OA is poor[97]. While a recent systematic review indicates that walking interventions have shown long-term improvements in pain in those with knee OA[98], compliance with walking programs is low[38–41], and this may in part be explained by acute pain intensity increases found in response to walking[99]. Furthermore, walking intervention trials in knee OA have high dropout rates[100], are prone to bias and placebo effects[101] , and most focus on only one dimension of pain which limits our understanding of how other factors can contribute to pain intensity increases such as pain type (e.g. nociceptive[40,102–105], neuropathic[102,106–110], constant[111–113], intermittent[111–113], cognitive[114–119]), walking mechanics[78,82,120], muscle strength[16,121–123] or biochemical biomarkers[25,113,124,125].

Guidelines are vague with respect to walking prescriptions; they do not include recommendations on the frequency, duration, and intensity of walking specific for those with knee OA as there is minimal direct evidence to support dose parameters[37]. Only a few fundamental studies have assessed acute responses to loading during walking for standard durations and they have reported changes in pain intensity, gait mechanics and muscle activation, biomarkers, and joint structure in response to walking[46,47,49,126–130]. There is emerging evidence of a large subgroup of 40 to 50% of individuals with knee OA who experience immediate increases in pain intensity following a continuous walk of 30-minutes or less at a self-selected speed[46–48]. These acute response studies also showed differences between the pain and no pain increase groups in baseline and post-walk changes in gait biomechanics and muscle activation[46–48] and suggest a poorer gait pattern and higher cartilage oligomeric matrix protein (COMP) levels in the pain increase group. To date, these studies have only looked at pain intensity, despite the multidimensional nature of pain[50] and they did not include sex in their analyses, despite differences between sexes reported in pain and other OA-specific measures. While acute walking studies provide a model to understand the effects of joint loading on OA processes (e.g., pain, inflammation, structural joint damage, gait mechanics and muscle function), there is a gap in evidence on the interactions of different OA processes, and how sex and muscle strength influence gait knee joint moments and muscle activation responses to walking. This gap will be addressed in this study.

2.2 PAIN IN KNEE OSTEOARTHRITIS

Pain is a main symptom of knee OA[124,131] and some recognize its presence and severity as risk factors for disability and radiographic progression[132]. Pain is multifactorial, with biological and psychosocial components contributing to the experience of pain[50]. It is therefore important to look at different components of pain because the type of pain an individual experiences can alter the type of pain relief prescribed[133].

There are different types of pain mechanisms including nociceptive and neuropathic mechanisms. Nociceptive pain in OA is thought to arise when inflammation caused by joint tissue damage causes chemical mediators to be released into the joint[134]. These chemical mediators result in an increased sensitization of the primary afferent nerves so that previously innocuous movements (e.g., walking) are now painful[134]. After an extended period, this increased peripheral neuronal activity causes plastic changes in the peripheral nerves, leading to neuropathic pain and increased pain sensitivity. If the pain stimulus continues, plastic changes at the central nervous system (CNS) may lead to increased general or whole-body pain sensitization, i.e., central sensitization[134]. Other psychosocial factors (e.g., pain catastrophizing, depression) also influence pain perception in those with OA[134]. Thus, OA pain may result from nociceptors in the knee joint tissue becoming sensitized during inflammation (acute peripheral sensitization) or pathological and chronic neural signals causing CNS changes (chronic central sensitization) or a combination of both[113].

Important biological components of pain perception include endogenous analgesic mediators such as endocannabinoids[135,136] and beta-endorphins (BE)[137], whose release enables acute pain relief[138] . Exercise typically results in an increase in circulating endocannabinoid and BE levels, and this is thought to contribute to the improvement in

pain with exercise[139,140]. What has not been well studied is that individuals with chronic pain have shown dysfunctional pain responses with exercise[141], and dysfunctional endogenous anti-nociceptive ligands have been identified in OA[102]. A recent study found that higher resting BE levels were associated with increased mechanical pain sensitivity in participants with knee OA[142]. They also showed that females had higher pain sensitivity[142], and this is consistent with previous findings of higher pain sensitivity in females with symptomatic OA[19]. Thus, it is plausible that higher resting BE levels contribute to a dysfunctional BE response to exercise and to increases in pain with walking in some individuals with knee OA.

Pain catastrophizing, a measure of pain cognition characterized by the tendency to ruminate, magnify, and feel helpless in the presence of pain[116,143], has been associated with knee pain and lower physical activity levels in individuals with knee OA[114,115,117,118,144,145]. Higher pain catastrophizing levels have been shown to be predictive of worse post-TKA pain, disability, and functional outcomes[115,117,119,145] and worse stair climbing abilities in individuals with knee OA[118]. Furthermore, pain catastrophizing was found to moderate the association of day-to-day increases in physical activity levels and increases in pain intensity, with those with high levels of pain catastrophizing reporting higher pain intensity increases (98% increase) on high, relative to low, physical activity days, compared to lower pain intensity increases (24% increase) in those with low pain catastrophizing[114]. Pain catastrophizing appears to have a significant effect on knee joint pain and how it influences physical activity, and evidence is needed to examine whether it influences acute walking responses in OA processes. Evidence on whether this differs between sexes is also needed, given the mixed reports

on pain catastrophizing where some report higher levels in females[7,146], with others reporting no differences[147].

Central sensitization is a pain hypersensitivity thought often to result from chronic neuropathic pain[148]. There is evidence of more central sensitization in individuals with OA, suggesting an important role of central components in the pain perception in knee OA[148] and a possible contributor to the discordance between cartilage structure and pain[149]. This structure and symptom discordance was examined in how participants with congruent versus discordant clinical pain and radiographic severity differed in measures of quantitative sensory testing (QST) which provide measures of pain sensitization[144]. Participants were separated into four groups based on high/low clinical pain and radiographic severity. Two groups were classified as congruent (high pain/high radiographic severity, low pain/low radiographic severity) and two as discordant (low pain/high radiographic severity, high pain/low radiographic severity). The results indicated that the discordant high pain/low radiographic severity group demonstrated the highest levels of central sensitization suggesting that sensitization may play a key role in clinical pain perception and in the structure and symptom discordance[144].

Pain pressure threshold (PPT) testing is one method of QST that measures sensitivity to a mechanical stimulus[106] typically measured in kgf/cm^2. A higher PPT suggests a greater tolerance for pain and lower pain sensitivity. Performing PPT testing at multiple sites can aid in determining the primary mechanism of pain as lower PPTs at the affected joint site are thought to be associated with peripheral sensitization whereas lower PPTs at a remote site suggest a combination of peripheral and central sensitization[150]. A systematic review[106] found that individuals with OA were more sensitive to painful

stimuli than healthy controls. This review included several PPT studies that reported individuals with OA having lower PPT (i.e., higher sensitization) at both affected and remote sites, with larger differences found at the affected site[106].

Two studies comparing PPTs differences between sexes in knee OA found higher pain sensitization in females at both the local (i.e., knee joint) and at remote sites[9,19]. Tonelli et al. (2011)[9] examined differences between sexes in both pressure and heat pain sensitivity at the affected and contralateral knee in a severe knee OA group and Bartley et al. (2016)[19] examined pain sensitivity to various stimuli (mechanical pressure, heat, cold) at multiple local and remote testing sites in a knee OA group with mild to moderate symptoms. Both studies found that females had lower pain thresholds (i.e., a greater sensitivity) at the local (i.e., knee joint) and remote sites compared to males[9,19] and Bartley et al. (2016)[19] found that females reported more widespread pain (i.e., greater number of pain sites) which is another measure of central sensitization. These findings suggest that both peripheral and central sensitization can contribute to the pain experience in knee OA and may influence differences between sexes in pain perception.

A recent meta-analysis[151] examined how exercise affects pain sensitization in individuals with OA. The results indicated very low-quality evidence that PPTs at the local, but not remote site, increase in response to exercise, indicating a decrease in local pain sensitivity. However, the studies included in this review[151] did not separate their participants by sex, and given the above-mentioned sex differences in PPTs, the changes in PPTs following exercise may differ between males and females.

Evidence supports a subset of up to one third of individuals with OA who express neuropathic pain[107,109,110,152]. This neuropathic pain group has been characterized as

significantly younger, and trending towards more females, a longer duration of OA and higher pain intensity and Western Ontario and McMaster Universities Index (WOMAC) pain scores[110]. The higher neuropathic pain prevalence in females with knee OA is consistent with higher neuropathic pain prevalence in females with chronic pain[153] and with findings of higher central sensitization in females with OA[9,19]. Greater neuropathic pain is a plausible contributor to the greater self-reported pain and worse physical function in females with OA[5–9]. Current clinical tools such as the modified painDETECT questionnaire (mPD-Q) have shown high face and content validity[109] and high reliability with QST signs of central sensitization[108]. The mPD-Q and similar questionnaires have the potential to become practical and accessible tools to identify the neuropathic component of pain in individuals with knee OA, so that proper pain relief therapies can be provided, given that these differ for nociceptive and neuropathic pain[133].

Similarly, knee joint specific pain assessments include self-reported pain, symptoms, and physical function measures (e.g., WOMAC, Knee Injury and Osteoarthritis Outcome Score (KOOS), Oxford Knee Score (OKS)) which are widely used in knee OA research[154–156]. These assessments provide a comprehensive picture of everyday pain, symptoms, and physical function, and their impact on daily living. They can have important clinical value due to their accessibility and ease of completion, and they can be used as screening tools to determine whether gait assessments or other clinical tests are needed. The Numeric Pain Rating Scale (NPRS)[157] provides a quick and simple method to assess pain intensity at a specific moment in time, by asking participants to rate their current pain as a whole number between 0 (no pain) and 10 (worst pain imaginable). Although the pain experience is highly subjective, self-reported

pain measures can provide a general estimate of pain for an individual or group. They are also useful to compare changes in pain score over an acute period (NPRS) or a longer duration or intervention (WOMAC, KOOS, OKS) within participants, and this within-subject design reduces the potential of between-subject errors due to pain subjectivity. Some studies using self-reported pain and function measures in OA populations report no differences between sexes[18,158] but more often, higher pain is reported in females than males [5–9,17]. However, the magnitude of the difference between sexes in these studies is typically small and less than clinically meaningful differences[159,160].

Given that pain studies in the OA literature are typically limited to only one pain measure (i.e., pain intensity or self-reported knee joint specific pain assessments), there remains a gap in evidence comparing the different dimensions of pain between sexes, and specifically acute pain responses to walking. It is also unclear if or how muscle strength may affect these pain responses but given the association between pain and muscle strength[86] there is reason to believe a relationship exists. Intervention studies report lower strength after an induced pain stimulus[87] and greater strength after induced pain relief[88,89]. Further, individuals with knee pain compared to asymptomatic individuals[91,92], and painful knees compared to asymptomatic knees within individuals who experience knee pain[90,92] have lower muscle strength than asymptomatic individuals or knees. This relationship between higher pain and lower muscle strength is true for multiple dimensions of pain including patient-reported knee OA pain from WOMAC and KOOS questionnaires[122,161], pain catastrophizing[162], pain intensity[163] and local pain sensitivity from PPTs[164]. There are numerous potential reasons for this relationship. Chronic pain, in particular, may prevent regular activity resulting in disuse and muscle atrophy and this is

supported by physical inactivity being associated with lower muscle strength[86]. Conversely, lower strength may result in insufficient joint stabilization and subsequent joint damage and pain. Thus, lower muscle strength may be both a cause and a consequence of knee OA.

2.3 MUSCLE STRENGTH IN KNEE OSTEOARTHRITIS

Muscle strength, in particular KE muscle strength, has been linked to the risk of developing knee OA[165–167] as well as to progression outcomes[31]. There is evidence that individuals with knee OA have lower KE[168–170] and KF[170] muscle strength, but the literature is less clear on whether participants with moderate severity knee OA have lower muscle strength. No differences in KE or KF muscle strength between moderate OA and asymptomatic groups have been reported[171] whereas others found a significant difference in KE strength between their OA and healthy control groups[168,169]. However, the studies that found significant differences between groups did not specify the OA severity of participants, but since they were all scheduled for an opening wedge high tibial osteotomy[168,169], it is likely that participants had greater clinical severity than those that did find significant strength differences[171]. This suggests a relationship between muscle strength and clinical OA severity. Studies have reported a significant correlation between incident symptomatic OA or symptom progression and lower KE[112,148,153,154] and KF[173] muscle strength. In contrast, other studies have found no significant relationships between muscle strength and radiographic OA[173] or risk of structural progression at follow-up[121,132,172]. These findings suggest that muscle strength has a stronger relationship with symptoms than joint structure in knee OA, and given its relationship

with symptom progression, suggest that low muscle strength has causal role in symptom progression, rather than symptoms resulting in lower muscle strength. However, these studies did not control for sex, and in studies where sex was considered, lower KE baseline muscle strength was found to be a risk factor for radiographic OA[28,174] and its progression[66,67] in females but not males. Thus, the lack of a relationship between strength and radiographic severity in the above mentioned studies[121,132,172,173] may be due to a potential sex effect.

Many studies report that females with knee OA have lower KE[20,23,29,30,175] and KF[20,175] strength than males with knee OA and this relationship was consistent across severity levels[176]. Furthermore, KE muscle strength was predictive of future knee replacement at 2-2.5 and 7 years in females but only at the 2-2.5 year timeframe in males[31]. KE and KF muscle strength was predictive of future knee replacement $\leq$ 2 years later in females but not males[177]. Since knee replacement decisions are based on symptoms and structural severities[33], these findings are consistent with previous studies highlighting sex as a factor influencing the relationship between strength and radiographic severity[28,174] and progression[66,67]. These findings[31] are also consistent with lower KE[16,73] and KF[73] strength being a risk factor for worsening knee pain in females but not males[16], or to a lesser degree in males[64]. KE strength has been identified as a risk factor for symptomatic knee OA in females and to a lesser degree in males[27]. Together this evidence suggests that strength shows a stronger relationship with symptomatic and radiographic OA in females than males.

Studies have suggested that the stronger relationship between muscle strength and knee OA in females may be due to an absolute muscle strength threshold needed to

protect the knee joint and females being less likely to meet this threshold[16,27–30]. Evidence supporting this theory includes males showing no associations between baseline KE muscle strength and physical function after five years whereas females with higher baseline strength had better physical function compared to females with lower baseline strength[29]. Interestingly, females in the lowest baseline muscle strength group who showed increased strength over five years did not improve their physical function, and it is plausible that these females did not gain sufficient strength to cross the necessary KE strength threshold estimated to be between 30 and 60 Nm dependent on the functional task (e.g., sit-to-stand and walking)[30]. This contrasts with males who showed increases in strength, as they showed no improvements in physical function, likely because they were already above this threshold. It is important to understand whether muscle strength interventions can improve OA symptoms and progression, and if so, for which subset of individuals. Furthermore, understanding how strength interacts with other risk factors for OA progression (e.g., gait biomechanics and muscle activation, pain, biochemical biomarkers) will aid in developing optimal interventions.

Adequate muscle strength is important, as muscles are key contributor to knee joint stability[70] and loading[178,179]. When instability occurs during gait, abnormal gait patterns may arise leading to increased structural damage[180], reduced shock absorption[181], and increased contact stress[71]. In an attempt to increase joint stability, a less dynamic loading pattern may be adopted, and this may lead to greater cartilage degradation as static loading has been linked to catabolic cartilage changes in cartilage explant[124] and animal models[182,183]. Furthermore, weaker muscles fatigue more quickly, leading to poor neuromuscular control and abnormal gait patterns[184,185]. Most studies have focused on KE

muscle strength, and although the KEs play a crucial role in gait mechanics, the KF muscles must also be examined given their significant role in joint stability and joint contact forces during gait[70,186].

Of clinical importance is the evidence that lower KF and KE muscle strength and less dynamic knee joint moment patterns during walking are directly correlated in individuals with knee OA[72]. Since these specific gait mechanics are linked to OA severity and progression, muscle strength may be a suitable intervention to improve gait patterns.

2.4 GAIT MECHANICS AND MUSCLE ACTIVATION IN KNEE OSTEOARTHRITIS PRESENCE AND SEVERITY

Gait is a common model used to study the local joint biomechanical environment associated with OA, and how altered biomechanics, specifically the KAM and KFM, and muscle function can affect and be affected by knee OA[187]. Studies have used both discrete metrics and principal component analysis (PCA) to characterize these mechanical and muscle function features. Discrete metrics provide information typically at a specific moment or over a specific interval of time whereas PCA is pattern recognition a technique used to reduce large sets of data into a number of principal patterns or principal components (PCs), which are quantitative and interpretable patterns of data[188,189]. PCA has gained popularity in the OA literature and has shown reliability in OA populations[189]. The benefits of PCA include that it does not require a priori selection of discrete features and that it can detect patterns of relevant information that discrete features may not capture in waveform data[190]. However, the interpretation of PCs can be subjective, and the lack of clinically meaningful units also presents a limitation[190].

Studies have reported that individuals with knee OA have higher KAM peaks[191,192], overall magnitude and impulse[192,193], where the KAM provides a measure of medial-to-lateral knee compartment loading[71] and impulse accounts for the loading magnitude and duration[191,192]. Higher mid-stance KAM, and higher and more sustained overall KAM magnitudes have been correlated with greater radiographic[78,191], symptomatic[82,192], and clinical[116,120,121] severity. Although there are some inconsistencies with which KAM measures are most important at varying severity levels, these overall findings demonstrate greater medial-to-lateral loading being linked to knee OA.

Lower early-stance KFM magnitudes, where the KFM provides a measure of overall joint loading[69], have been associated with mild to severe radiographic[194], symptomatic[82], and clinical OA[193,195,196]. In addition, a less dynamic KFM-KEM difference and knee flexion angle (KFA), i.e., a stiffer-knee gait pattern, was found in individuals with radiographic[78,197] and clinical OA[195,196], with progressive increases in stiffness with increasing OA severity[78,195,196]. These patterns suggest that the KFM-KEM difference may play a role in OA incidence and severity, but more evidence is needed to determine whether less dynamic overall joint loading contributes to or is a by-product of OA progression. Key to interpreting external knee joint moments, is including measures of lower-limb muscle function as muscles are the key contributors to joint contact loading magnitudes and patterns[70,198,199] . For example, without muscle function measures, a lower net external KFM may be misinterpreted as KE, or quadricep weakness, when in reality the lower KFM is a result of co-activation of the KF muscles during the KE contraction[200].

Specific muscle activation patterns have been correlated with specific knee joint moment patterns (e.g., KF and KE prolonged activity correlated with the KAM and KFM dynamic loading patterns)[72], but they have also been independently linked to OA presence and severity in several studies. Higher overall magnitude and duration of KE and KF muscle activity, and more co-activation in individuals with knee OA were found during walking, and were influenced by OA severity levels[78,82,170,171,196,201–204]. Muscle activation of the rectus femoris (RF) and lateral muscles (vastus lateralis (VL) and lateral hamstring (LH)) demonstrate progressive increases in activity with increasing clinical severity whereas medial muscles (vastus medialis (VM) and MH) appear to have higher activity in severe OA only[171,196,201]. These findings remain consistent when using a radiographic definition of OA[204]. In participants with severe clinical OA, MH and LH waveforms differ in shape and amplitude, whereas VL, VM, and LH waveforms demonstrate similar patterns and amplitudes[170] and this supports the differing MH and LH activity patterns across OA severities.

Similarly, when comparing a symptomatic and asymptomatic group both of moderate radiographic severity (Kellgren-Lawrence (KL) grade = 2), higher activity was found in all KE muscles and the LH but not in the MH in the symptomatic group[82]. Taken together, these studies suggest that changes in the activity of medial muscles, and particularly the MH, may only occur in severe OA, when greater overall joint stability is needed. Increasing lateral muscle activity may be used as a first adaptation to increase lateral forces, unload the medial compartment and increase joint stiffness, although it is not yet clear whether this is a response to pain or structural changes. Interestingly, pain but not radiographic severity, was correlated with MH activity during stance in a mild to

moderate OA cohort[78] which suggests that the MH activity may be most associated with pain and symptoms.

Internal muscle activation measures allow researchers to better interpret biomechanical data. The lower KFM magnitude and less dynamic KFM-KEM range reported in OA and across OA severities has been thought to be directly related to internal KE muscle moments. This assumption would only hold true if there was no antagonistic muscle activity, but studies have found higher muscle co-activation in those with OA[78,82,170,171,196,201–204], and these findings do not support the KE avoidance hypothesis[205]. This emphasizes the limitations of interpreting external moments as these do not directly relate to internal joint contact forces and highlights the importance of including electromyography (EMG) results to provide a measure of muscle activity during interpretation.

Important from a clinical intervention perspective is the growing evidence that gait biomechanics and muscle activation patterns can be predictive of OA progression. This is especially important given that there is no cure for OA, and that gait biomechanics and muscle function are modifiable risk factors. Structural and clinical progression endpoints have been well defined where the endpoint for structural progression is based on imaging such as a KL grade of four whereas TKA provides a clinical endpoint as decisions for surgery are based on both structural and symptom severity[33,58].

2.5 GAIT MECHANICS AND MUSCLE ACTIVATION IN KNEE OSTEOARTHRITIS PROGRESSION

Evidence is growing that knee joint moment features are linked to knee OA progression including higher KAM peaks and overall magnitudes linked to radiographic[52–56], symptomatic[120], and clinical[58] OA progression and lower KAM early- to mid-stance difference measure linked to clinical OA progression[58]. Studies have reported divergent findings on the relationship between peak KFM and radiographic OA progression with reports of higher peak KFM being associated with radiographic progression[56] and others finding no associations between peak KFM and radiographic progression[53,54,61]. The reason for these discrepancies is unclear given that the samples appear to be of similar clinical severity (similar walking speeds and KL grades). However, most of these studies (except Chang (2015)[53]) had small sample sizes, and further research is needed to confirm these findings. Lower KFM peaks have been identified in individuals with mild to severe radiographic[194], symptomatic[82], and clinical OA[193,195,196], but there is not sufficient evidence to support whether lower peak KFM in individuals is a cause or a results of OA progression. A less dynamic KFM-KEM pattern has been linked to clinical OA progression[58], and this stiff-knee gait pattern is consistent with the less dynamic KFM-KEM patterns associated with increasing radiographic and clinical OA severity[78,195,196].

Specific muscle activation patterns linked to knee OA progression include higher magnitudes and more prolonged muscle activation[54,60–62] linked to radiographic[54,61,62], symptomatic[61], and clinical[60] OA progression although some evidence suggests joint moments to be more predictive of radiographic progression than muscle activation patterns[61]. There are only a few studies on muscle activation patterns related to radiographic knee OA progression, and results include longer duration of medial muscle

(VM and MH) co-activation in a one-year follow-up study of medial tibial cartilage volume assessed using MRI[62] but also higher and prolonged LH activity in a three-year follow up study of increases in joint space narrowing (JSN) via radiographs[54]. In both studies, only structural measures were assessed and in a recent study when structural and clinical progression to TKA were examined after 7 years, muscle activation patterns were found to be predictive of progression to TKA but not structural changes[61]. Comparing the results of these studies is difficult given the use of different definitions of radiographic progression and the varying lengths of follow-up but together these findings suggest different muscle patterns may be important in different phases of radiographic OA progression. Two studies looked at clinical progression to TKA after 5-8 years[60] and 7 years[61] and found that progression was linked to higher lateral muscle co-coactivation[60], higher KF muscles activity magnitude[60,61] and more prolonged activation in the KF[60] and KE muscles[60,61]. This prolonged KF and KE muscle activity is significantly correlated with the less dynamic KAM and KFM-KEM unloading patterns predictive of OA progression[72] and this relationship may be due to prolonged muscle activity causing muscles to fatigue more quickly and consequently being less likely to produce large KFMs[51]. This leads to less dynamic unloading, which is problematic increased static loading on the joint is shown to elicit a catabolic response in cartilage tissue, compared to dynamic loading which elicits an anabolic response in explant cartilage studies[124]. Higher and prolonged muscle activity resulting in increased static loading has also been associated with increased cartilage cell death in animal models[182,183].

Lower KF and KE muscle strength is also correlated with a less dynamic KFM-KEM pattern[72]. This lower strength may also cause the muscles to fatigue more quickly

reducing peak KFMs. There were no significant correlations found between KE or KF muscle activation or muscle strength and the overall magnitudes for the KAM and KFM, and this suggests that alternative interventions to improve these features are needed. Interestingly, a yoga based KE strengthening and neuromuscular training program did not improve KAM magnitude but did improve the KFM-KEM pattern by moving towards a more dynamic KFM-KEM loading pattern[206]. This finding suggests that interventions focusing on neuromuscular training and muscle strengthening have the potential to improve the stiff-knee gait patterns linked to OA progression. Nonetheless, there is limited evidence on whether sex influences gait mechanics and muscle activation patterns predictive of OA progression, and future research must examine if and how sex plays a role in the relationship between gait mechanics, muscle activation, pain, and muscle strength.

2.6 SEX DIFFERENCES IN GAIT MECHANICS AND MUSCLE ACTIVATION IN KNEE OSTEOARTHRITIS

Differences between sexes in walking biomechanics have been identified in a small number of knee OA studies. Males with severe knee OA have shown higher KAM peaks and magnitude than females[59,77], whereas no KAM magnitude differences between sexes have been found in a moderate OA sample[20]. Since higher KAM peaks and magnitudes have been associated with radiographic OA progression[52–56], these higher KAM measures may suggest a more mechanical disease mechanism in males. Females with moderate and severe OA have shown a lower KFM-KEM difference indicative of a stiffer-knee gait pattern[20,59] than males which is a feature predictive of clinical OA progression[58]. Differences between sexes with respect to KFM magnitudes have been less clear, with

some studies reporting lower KFM amplitude (magnitude and difference measures)[20] and others reporting higher KFM magnitude[153] and KEMs in females[59,207]. These mixed KFM findings are consistent with the divergent findings on the relationship between KFM peaks and magnitude, and OA progression. However, the higher KFM magnitude in females was found in a more severe OA group which suggests that KFM magnitudes may play a greater role in later stages of the disease.

Previous studies identified higher muscle activation in different KE and KF muscles in females[23,59,176,208]. Bigham et al. (2018)[208] found that females with knee OA had higher muscle activation in the LH but not in other KF and KE muscles during a specific standing ground reaction force marching protocol. Sisante et al. (2020)[23] found higher KF muscle co-activation in females during isokinetic quadriceps strength testing. Astephen Wilson et al. (2015)[59] looked at muscle activation during self-selected speed over-ground walking and found that females with severe knee OA had higher overall muscle activation in the KE but not KF muscles. Interestingly, males had more prolonged KE muscle activity than females, which is not consistent with the stiff-knee gait pattern that was found in the females' knee joint moments (i.e., lower early to mid-stance difference). Hubley-Kozey et al. (2022)[176] found higher overall activation in the VL, VM, and MH during over-ground walking in females at all severity levels (asymptomatic, moderate OA, severe OA), whereas the LH did not significantly differ between sexes. Females with severe OA also had more prolonged muscle activity in the VL and VM[176] than males. Overall, these results suggest that females have greater overall KE and to a lesser extent KF muscle activity during gait[59,176] patterns associated with OA progression[60] and higher OA severity levels[78,82,170,171,196,201–204].

The differences between sexes in gait mechanics and muscle activation measures suggest that mechanisms of OA progression may differ between sexes and may in part explain the greater prevalence and symptom severity, and lower physical activity levels in females. Walking as an intervention may also affect OA progression in males and females differently. There is only a small number of studies looking at how joint mechanics and muscle activation change in response to continuous walking[46,47,49], and they focused on pain intensity responses with little attention to other pain, structural, functional or biochemical responses associated with OA, while also not examining sex as a factor. Despite these limitations, evidence from these studies aid us in better understanding walking as an OA intervention.

2.7 ACUTE WALKING RESPONSES IN KNEE OSTEOARTHRITIS

To better understand walking as an intervention for knee OA, we must first understand how joint loading during walking affects OA processes (pain, inflammation, structural joint damage, joint and muscle function) and how these processes in turn influence walking patterns. Walking imposes an additional load onto the joint, and higher loads have been found to elicit a catabolic response in diseased tissues[209,210]. If individuals with knee OA show significant tissue damage, loading may accelerate disease progression. However, immobilization has also been found to cause tissue degradation[211,212] so it appears that there is an optimal load to promote overall health benefits. Walking is also a cyclical pattern, and dynamic and cyclical loading have demonstrated positive joint outcomes compared to static loading[209,213,214]. Thus, optimal loading conditions (i.e.,

magnitude, frequency, duration) for joint and overall health must be determined as they are currently unknown.

Studies show that specific joint loading patterns may elicit pain, and/or pain may elicit gait adaptations which in turn can influence joint loading[148]. Evidence suggests that in knee OA, increased pain levels post-walk are related to decreased knee joint moments during walking[56,81,84], and that pain relief (e.g., knee joint analgesia) can lead to increased joint loading[85]. People in pain tend to walk at slower speeds[81], and slower walking speeds are related to smaller peak loading in healthy adults[215–217]. Thus, reducing walking speed may be a mechanism to reduce loading in those with knee OA[218] and walking velocity may be a moderating factor between pain and gait patterns. However, this reduced walking speed may have negative effects on OA progression as it would result in an increased impulse loading[215] and may lead to a more static loading pattern related to OA progression[58,60].

Studies that examine the responses to a continuous walk have been used as a model to understand the link between joint loading and OA processes (i.e., pain, inflammation, structural damage, joint and muscle function)[46,47,49,126–130]. Peeva (2010)[99] found a gradual increase in pain intensity during a 20-minute continuous walk in those with clinical OA and significant knee pain symptoms characterized by having used analgesics for knee pain for at least 15 of the past 30 days. However, only the mean pain intensity scores for the entire group were reported, and so the magnitude of pain increases or decreases for each participant was not known. Emerging evidence shows that there are divergent pain responses to an acute bout of walking in those with knee OA where between 40 and 50% of participants reported an increase in their pain intensity

immediately after 6 to 30 minutes of walking[46–48]. Other continuous walking studies have used the mean or median pain intensity scores without separating participants who increase versus decrease their pain[49,126] which may underestimate pain scores in those with pain increases. However, 2-point clinically meaningful differences were still found with higher pain being reported when walking for one continuous bout compared to multiple shorter intervals with a same total duration[49,126]. These studies suggest that not all individuals, during all walking conditions, experience improved pain with walking, and this may contribute to low physical activity levels in those with knee OA[219].

These continuous walking studies accounted for only one dimension of pain (i.e., pain intensity) despite its multi-dimensional nature. They did not look at baseline measures of pain cognition, assessments for neuropathic pain, or pain sensitization despite up to one third of individuals with knee OA reporting neuropathic pain[107,109,110,152]. Furthermore, no studies assessed whether baseline or changes in endogenous analgesia levels such as BE concentrations, thought to increase with aerobic exercise and contribute to pain relief[139,140] were different in those that increased compared to those that did not increase pain after walking. Since dysfunctional analgesia is reported in those with chronic pain[141], some individuals with OA may not report analgesic effects with exercise. This lack of exercise-induced analgesia may be due to an elevated baseline level of BE in knee OA, and a subsequent inability to further increase BE concentrations or a reduced sensitization to increases in BE. Furthermore, these studies did not examine sex in their analyses, and given that differences between sexes exist in pain[25] and physical activity levels[10] in OA, further evidence is needed to compare

the different dimensions of pain between sexes, and specifically acute pain responses and how they might influence long-term adaptations to walking.

Differences in baseline and post-walk changes in knee joint biomechanics[46,47,49] have been reported and some of these changes in joint mechanics were linked to increases in pain intensity. Specifically, those with post-walk increases in pain had higher first[47] and second peak KAM[46,47] and larger KAM impulse[47] at baseline. Divergent findings for peak KFM measures were identified including lower[47] and higher[80] overall peak KFM, plus lower late-stance KEM[47] and total reaction moments[46,47] at baseline. Pain increases were also associated with greater decreases in peak KAM and KFM in response to 20 minutes of walking[46] and greater increases in knee contact forces in response to 45 minutes of continuous walking[49].

Some baseline joint moments associated with increased pain intensity immediately after walking[46,47] are consistent with joint moments predictive of OA progression (i.e., larger peak and overall magnitude KAM and smaller KFM-KEM range). Thus, pain during walking may be a factor influencing OA progression. The variable results with respect to KFM measures (i.e., higher and lower KFM peaks[46,47]) are consistent with the varying reports of KFM in OA progression[53,54,56,61]. The relationship between KFM magnitude, pain and OA progression is less clear, and the differences between the two studies' designs (within versus between-subjects) and samples (different severity and proportions of males and females) make it difficult to compare their results.

As previously mentioned, there are limitations to interpreting external joint moments. For example, despite reduced KFMs, internal knee joint contact forces[49] may remain high if muscles demonstrate increased co-activation. Furthermore, external

moments cannot assess muscle fatigue, which may contribute to the decreased range of motion at the knee, decreased peak external moments, and greater pain as a continuous walk demonstrated pain increases compared to an interval walk[49]. EMG measured muscle activation can help address these limitations. Boyer and Hafer's (2019)[46] study examined muscle activation patterns and found that pain increases during gait, were associated with greater relative KF-to-KE and medial-to-lateral compartment muscle activation at baseline[46]. The greater pain increases may be due to greater internal joint loading resulting from increased muscle co-activation, and these internal joint loads cannot be determined from external moments alone.

Only a few continuous walking studies have examined the relationships between biomechanical loading and biochemical biomarkers associated with OA. Specific biochemical biomarkers are important tools that have been studied in knee OA for aiding diagnosis, prognosis, and disease management[220]. Common biomarkers include markers associated with collagen II synthesis (e.g., PIIANP) and degradation (e.g. CTX-II), COMP, matrix metalloproteinase[220] and cytokines (e.g., IL-1β)[125]. COMP is a cartilage specific molecule but is also indicative of synovial tissue turnover[221] and has been the focus of several OA studies. COMP reflects cartilage breakdown and synovial inflammation[222] and COMP levels have been related to OA severity[26]. In a recent systematic review[223], the authors summarized the evidence supporting that COMP concentrations are significantly higher in people with OA and that COMP levels can predict OA progression[224].

Studies that used a continuous walking model to examine acute changes in cartilage metabolism during walking[126–130] found that in individuals with knee OA, acute

increases in COMP ranged from 6.3%[127] to 26%[126] post-walk. This large range in COMP increases may be due to differences in the study samples, as Jayabalan (2019)[126] included a greater proportion of females and participants of greater radiographic OA severity than Mündermann's (2009) study. Of interest is that the non-OA group increases (5.6%) were not different from the OA group (6.3%) but there was a large subgroup of participants in both groups who decreased their COMP levels immediately following walking[127]. No clinical or person characteristics were provided to define this subgroup, nor were pre- and post-walk pain assessed to help interpret these differences. Jayabalan (2019)[126] did assess pain and their findings indicated that a greater increase in pain intensity was associated with a greater increase in COMP concentrations following walking. They used a mean pain value for the entire group and did not separate participants into a pain increase and a pain decrease group. Thus, whether individuals who exhibit pain increases with walking also show greater increases in cartilage metabolism (i.e., COMP levels) after walking compared to those that did not experience a pain increase has not been examined. Furthermore, no sex analyses were conducted despite there being known differences between sexes in COMP[24,26,225], so it is unclear whether sex could explain the divergent pain and COMP responses following walking.

Harkey (2017, 2018, 2020)[128–130] used ultra-sound (US) to measure cartilage deformation in healthy adults and found that cartilage deformation was greater following running and walking, compared to sitting[128], and that slower walking speed was associated with greater medial femoral cartilage deformation[129]. Furthermore Harkey et al. (2020)[130] examined whether there were differences between sexes in US measured cartilage responses to walking, and they reported that greater resting COMP

concentrations were associated with less cartilage cross-sectional area in females but not males, and that there was no association between pre- to post-walk changes in cartilage deformation and COMP concentrations in both sexes. Harkey's (2017, 2018, 2020)[128–130] findings were from a cohort of young (18 to 35 years) and healthy individuals and given that healthy cartilage and damaged cartilage respond differently to loading, the results may not link directly with an OA population. These results, however, provide evidence that different loading parameters (e.g., magnitude, frequency) and specific markers of cartilage metabolism (e.g., COMP) are related to acute cartilage structure changes. Thus, there is a need to investigate different pain measures in addition to pain intensity, and to determine whether sex or muscle strength plays a role in changes in gait biomechanics and muscle activation, biomarkers, and cartilage structure in response to an acute bout of walking in a population with knee OA.

There are limitations associated with intervention studies (e.g., high dropout rates[100] and reporting only one dimension of pain[40,104,105]) and the literature reports only a few continuous walking studies[46,47,49,126–130] which also exhibit limitations (e.g., not separating pain increase/decrease groups and reporting only pain intensity[49,126]). There is a need to look beyond pain intensity responses given the discrepancy between symptoms and structural progression[45] and to consider sex given that differences between sexes exist in joint structure[11–15], pain[16–18,226], walking biomechanics[20–23] and muscle activation[21,23], biochemical biomarkers[24–26] and muscle strength[16,23,27–31]. This evidence could help to create individualized walking prescriptions (i.e., duration, frequency, intensity)[37] and to examine differences between sexes given that OA manifests itself differently in males and females and that females are less physically active[10]. If

differences between sexes in responses to walking exist, they could provide evidence to guide sex-specific walking prescriptions and improve our understanding of how males and females with OA respond differently to joint loading. Lower KE muscle strength[27,31,121], and specific gait biomechanics[58] and muscle activation patterns[60] have all been linked to OA progression and there is a direct relationship between these measures[72]. Given that there are differences in KE strength, symptoms, and gait biomechanics and muscle activity between sexes[17,23,27,28,30,31], there is a need for evidence investigating how muscle strength affects acute pain, gait biomechanics and muscle activation responses to walking, and specifically, whether strength can explain the differences between sexes in these responses.

2.8 SUMMARY

The current literature provides evidence that females with OA have lower cartilage volume, higher self-reported pain scores and different biomarker levels compared to males with OA. Pertinent to this study is the evidence that females with knee OA have lower muscle strength, and different knee joint biomechanics, specifically lower KAM magnitude and lower KFM-KEM difference measures. Although lower KAM magnitudes have been associated with less radiographic progression, a lower KFM-KEM range has previously been associated with clinical OA progression. Females with knee OA also recruit higher magnitudes and more prolonged KE and KF muscle activation patterns during walking than males, which have also been linked to OA progression. These gait patterns may be a result of lower muscle strength in females. Evidence shows that knee joint mechanics are altered with pain and structural severity, and pain and structure have

the potential to alter specific joint biomechanics patterns linked to OA disease progression and worsening of symptoms. Given that females with knee OA are less physically active than males, the question is whether there are differences in acute responses to a continuous bout of walking in pain, knee joint moments, and muscle activation patterns between sexes, and if so, how much variance in these responses can be explained by muscle strength. Improving our understanding of this gap in the literature may help to explain the lower physical activity levels in females and the higher prevalence and burden of OA in females and potentially guide sex-specific walking prescriptions.

CHAPTER 3: METHODOLOGY

This chapter provides details on the methods used in this study. First, participant recruitment and inclusion criteria are described. Next, is an overview of the study procedure, followed by detailed descriptions of how each outcome was measured. This includes a description of self-reported clinical outcome measures, PPT testing, NPRS, gait mechanics and muscle activation set-up, acquisition and analysis, and maximal voluntary isometric contraction testing.

3.1 PARTICIPANTS

Data for this thesis were collected as part of an on-going study in the Dynamics of Human Motion (DOHM) Laboratory of older adults that examined differences in responses to a 30-minute continuous walking protocol for individuals with and without OA symptoms. To address the thesis goal, participants were included if they were over 45 years of age, had radiographic evidence of medial compartment knee OA, were not on a waitlist for major lower-limb surgery (e.g., TKA, tibial osteotomy), did not have neurological, cardiovascular, or other musculoskeletal issues including an injury within the past 6 months that could alter gait or place them at risk during walking, did not have an infection or inflammation not related to their OA, and self-reported being able to walk for 30 minutes consecutively. See Appendix 1 for participant inclusion criteria. The aim was to include an equal number of males and females to conduct statistical hypothesis testing between sexes.

Participants were recruited through the DOHM databases, our clinical team members (Dr. Stanish and Dr. Urquhart), the Dalhousie University Notice Digest, and

word of mouth. Potential participants were sent a recruitment letter followed by a phone call 1-2 weeks later. During this phone call, a modified health-screening questionnaire was used to determine the participant's preliminary knee OA status, whether they met inclusion criteria (Appendix 1), and radiograph status. If a participant met inclusion criteria but did not have a lower limb radiograph from within the past year, the individual was given an appointment for the Diagnostic Imaging Department at the Halifax Infirmary within one week of testing to receive a standard anterior and lateral knee radiograph of the test leg which was either the symptomatic limb, or if participants did not have symptoms a random leg was chosen as the test leg. If they had a recent radiograph, it was retrieved from the Diagnostic Imaging Department at the Halifax Infirmary. All radiographs were scored by our orthopedic surgeon team member (Dr. Stanish) using the KL Criteria[227] and the Scott Feature Based Scoring System[228] which have high reliability[228]. Radiographic medial compartment knee OA was based on radiographs showing equal or greater JSN in the medial compared to lateral compartment[228] and a KL grade ≥ 1. Ethics approval was obtained from Nova Scotia Health Authority Research Ethics Board and written informed consent was obtained from all participants prior to study participation.

3.2 STUDY PROCEDURE

3.2.1 Overview

The study procedure below describes how the data analyzed in this study was collected. First, participants were asked to limit their physical activity for 36 hours prior to data collection[127] to minimize the effects of physical activity on response variables including

pain and serum biomarkers. Data collections were conducted in the early morning to minimize the effects of circadian fluctuations on biomarkers. This study does not include an analysis of the biomarker data, and this is included here for completeness. A schematic of the protocol is found in Figure 3.1. Standard operating procedures were followed for all tests and details are listed below. Upon arrival, demographics (age, sex), anthropometrics (mass, height), and current pain and anti-inflammatory medications (dosage, type) were recorded. Clinical tests were performed including passive knee joint range of motion using goniometry and effusion brush test using suprapatellar recess depth[229] but these data were not analyzed in this study. Finally, motion capture markers and surface EMG electrodes were placed on the participant using standard protocols[171,193].

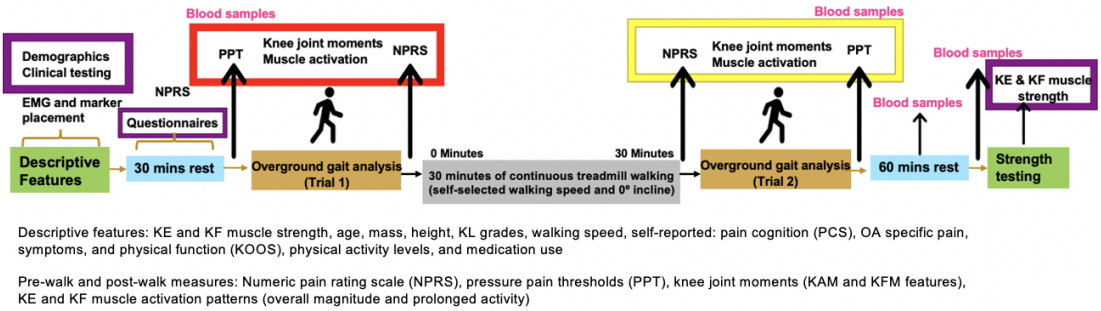

Figure 3.1: Overview of study procedure with muscle strength, demographics and clinical characteristics outlined in purple, pre-walk measures outlined in red, and post-walk measures outlined in yellow. Blood samples (in pink) were collected but not analyzed in this study.

Only details related to the current study objectives are provided and while blood sample collections are indicated on the schematic they will not be described in detail. Participants rested for 30 minutes prior to testing and during that time, the participant

stated an NPRS score[157] and completed a series of questionnaires assessing self-reported measures of pain and physical function (i.e., KOOS, PCS). Then PPTs to test pre-walk pain sensitization[106] were measured using a digital algometer (FPIX50, Wagner Instruments, Greenwich, CT).

Prior to completing the walking intervention, an overground (OG1) gait assessment using a standard gait assessment protocol[171,193] shown to produce reliable joint moment and EMG measures in those with OA[230,231] was performed on the test leg. Participants then completed the walking intervention which consisted of a 30-minute continuous walk on a treadmill (RTM600, Biodex™, Shirley NY) with a 0-degree incline at their self-selected walking speed. Scores for the NPRS for pain intensity were collected immediately pre- and post-walking intervention. Following the walking intervention, participants performed another set of overground walking trials (OG2), had a post-walk blood sample collected and underwent post-walk PPT testing. After the final blood draw, motion caption markers were removed, and participants underwent standardized maximum voluntary isometric contraction (MVIC) testing against an isokinetic dynamometer (Biodex™, Shirley NY) to assess maximal strength for KE and KF muscles, and for EMG amplitude normalization. Details for each test are provided below.

3.2.2 Self-Report Clinical Measures

During the 30 minutes before the pre-walk blood sample draw, participants completed a series of questionnaires. The KOOS provided self-reported measures of OA-specific pain, physical function, and symptoms over the course of the past 7-days. The KOOS[154–156] is an assessment specific for patients with knee OA that includes the widely used WOMAC

pain, function, and stiffness scales which are reliable and valid for participants with knee OA[232]. Participants completed the PCS[116,143] to assess pain catastrophizing and the Walking Club questionnaire, an instrument used specifically in the DOHM lab, to measure self-reported physical activity levels (frequency and intensity) and capture medication use (yes or no). The DOHM self-report physical activity questionnaire has been validated with accelerometry data[233].

3.2.3 Pressure Pain Thresholds

PPT testing was used to measure somatosensory response and pain sensitivity, where a higher PPT indicates a lower pain sensitivity[106]. Participants were instructed to immediately indicate when the pressure stimulus changed to pain by using the word "stop". Using a digital algometer, the probe (1 cm^2) was placed perpendicular to the skin and pressure at the rate of approximately 30 kPa/s (assessed by visual observation) was applied at three sites in the following order: 1) medial joint line of the knee on the test leg, 2) VM muscle of the test knee, and 3) extensor carpi radialis longus (ECRL) muscle of the contralateral forearm. The medial joint line of the knee provides a measure of local pain response whereas the ECRL muscle site provides an assessment of central sensitization and widespread pain[107]. This method has shown high intra-rater reliability[234,235] and the same tester measured all PPTs for the study. The VM test site may provide some indication of local and remote pain sensitization, as it is located close to but not directly on the test knee joint site. The tester covered the algometer screen to ensure that the participant and tester were blinded to the results, and the results were seen only by the technician who recorded the value. After performing the PPT test at all three

sites, the assessment was repeated. If the two readings displayed a greater than 10% difference, the trial was repeated a third time. Approximately one minute of rest was given between each trial and the average of the two closest trials was calculated. PPT testing occurred pre- and post-walk to assess differences between sexes in pain sensitization prior to (Objective 2) and in responses to the walking stimulus (Objective 3).

3.2.4 Numeric Pain Rating Scale

The NPRS is a valid and reliable measure[157] used to capture pain intensity at an instantaneous moment in time. Participants were asked to rate their pain on a scale from 0 to 10, where 10 indicated no pain and 10 indicated the worst pain imaginable. Pre- and post-walk NPRS scores were collected to assess sex differences in pain intensity prior to (Objective 2) and in responses to the walking stimulus (Objective 3).

3.2.5 Electromyography and Motion Capture Marker Set-Up

Following clinical testing, skin was shaved, alcohol wiped, and silver/silver chloride surface electrodes (3M Red Dot) were placed using standard procedures[171] on the KE (RF, VL, VM) and KF (LH, MH) muscles, and the tibial shaft (ground electrode) of the test leg to measure muscle activity. See Figure 3.2 for electrode placement of the KE and KF muscles. Correct electrode placement and EMG signal acquisition were verified or adjusted as the participant performed movements eliciting muscle contraction (e.g., knee extension (RF, VL, VM), knee flexion (LH, MH)). EMG gains were set to ensure good signal-to-noise ratio without saturation[236].

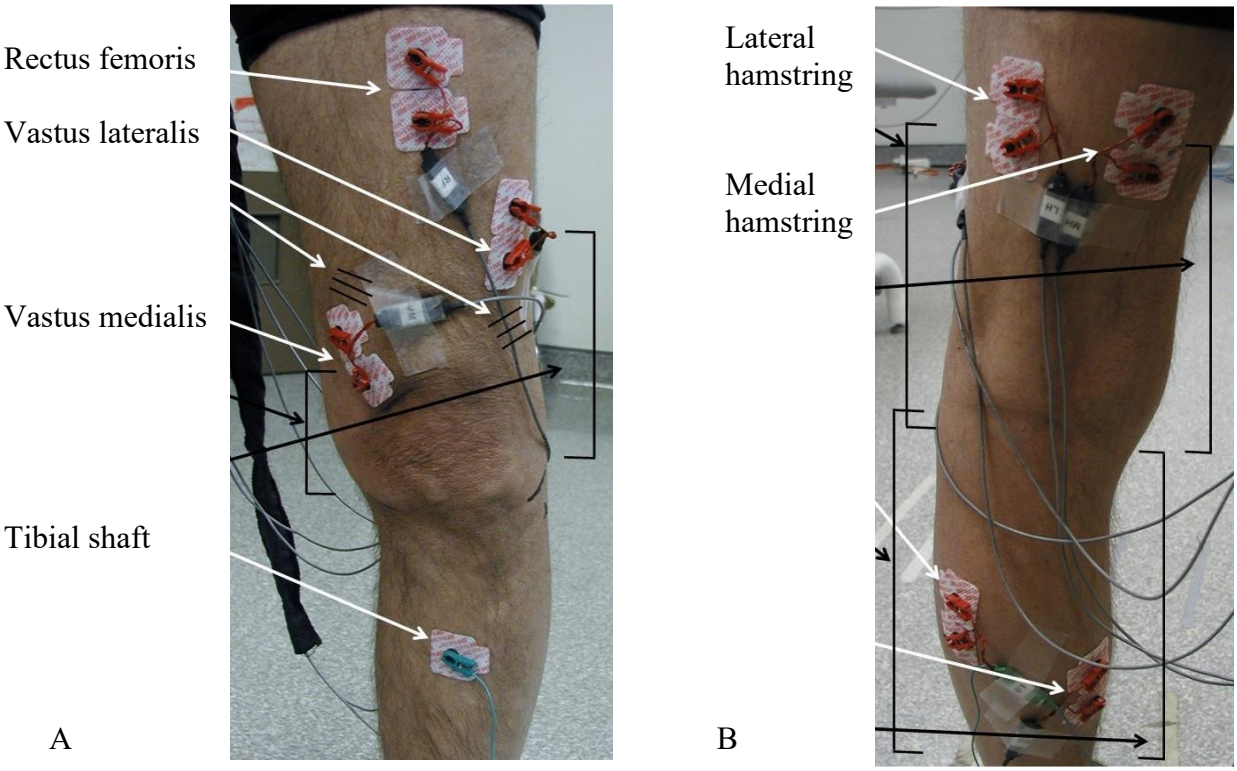

Figure 3.2. Anterior (A) and posterior (B) view of the electrode placement on the knee extensor muscles and tibial shaft (A) and the knee flexor muscles (B) on the test leg.

Infrared emitting diode (IRED) motion capture markers were attached using standard protocols[193]. As illustrated in Figure 4.3, all IREDs were placed on the side of the test leg with triads placed on the pelvis, thigh, shank, and foot and individual IREDs placed on the greater trochanter, lateral epicondyle, lateral malleolus, and shoulder according to standard protocols[193]. Once IREDs were placed, the participant remained static during a standing calibration trial and the digitization of eight virtual points (right and left ASIS, medial epicondyle, fibular head, tibial tuberosity, medial malleolus, second metatarsal, and heel). Finally, a hip joint centre of rotation trial was collected.

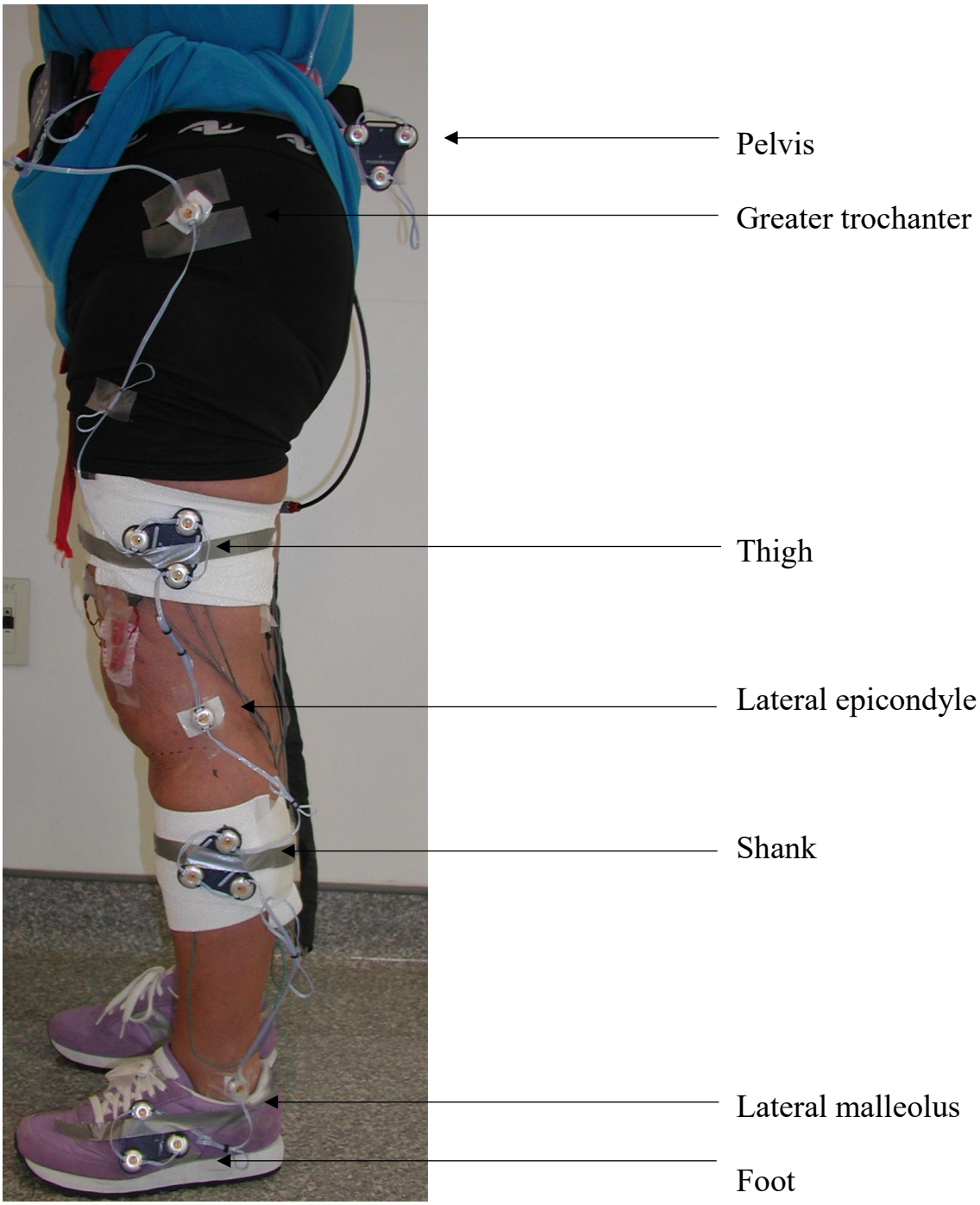

Figure 3.3. Lateral view of infrared emitting diode motion capture marker placement on the test leg including marker triads placed on the pelvis, thigh, shank, and foot, and individual markers placed on the greater trochanter, lateral epicondyle, and lateral malleolus. The shoulder marker is not captured in this image.

3.2.6 Knee Joint Moments and Electromyography Analysis

Participants performed 5-7 walking trials at self-selected speed prior to (OG1) and following (OG2) the 30-minute walk while three-dimensional motion sampled at 100 Hz (Optotrak™ Certus, Northern Digital Inc. Waterloo ON), 3D ground reaction forces sampled at 2000 Hz (AMTI™, Walkerton MA), and surface electromyograms (AMT-8, Bortec, Inc., Calgary, AB) eight-channel EMG system (Input Impedance: ~10GΩ, CMRR:115dB at 60 Hz, Band-pass (10-1000 Hz)) were simultaneously recorded using the Optotrak software. The walking intervention consisted of a 30-minute continuous walk at a self-selected walking speed and 0° incline on a treadmill (RTM600, Biodex™, Shirley NY). Individuals were instructed to not use handrails, if possible, but those who required the use of handrails for comfortability were permitted to do so and this was recorded.

External knee joint moments were calculated over the gait cycle using standard procedures[237,238] and were reported about the anatomical joint coordinate system[239]. All knee joint moments were time-normalized to percent of stance phase (heel-strike to toe-off) using linear interpolation[195,239] and amplitude-normalized to body mass[195,239]. External knee joint moments were calculated using inverse dynamics[240,241] through a custom MATLAB (MathWorks Inc., Natick, MA) code using kinematic (i.e., position, velocity, acceleration) and inertial properties data (i.e., mass, centre of gravity, mass distribution)[200].

Briefly, inverse dynamics modelling started at the foot, where the entire lower limb was modelled as linked segments. Ground reaction forces were obtained from force plate data and forces at the foot (F) were calculated from the segment mass (m) and segment acceleration (a) which were determined from previously published body

segment parameters[242] and motion data respectively [3.1]. The same process was followed to determine the knee forces, based on the calculated foot forces.

$$\sum F(N) = m(kg) * a(m/s^2) \quad [3.1]$$

Using these calculated forces, external joint moments (M) about the centre of mass for each segment were calculated from the segment moment of inertia (I) and segment angular acceleration (α) [3.2]. Segment moments of inertia were determined using an optimization method by Vaughan et al. (1992)[243] and angular accelerations (α) were determined from motion data. Joint reaction forces were converted to joint moments using segment lengths obtained from motion data, and previous data on locations of segment centres of mass[242]. Consistent with the forces modelling, modelling for moments started at the foot and continued upwards to the knee joint.

$$\sum M(Nm) = I(kg*m^2) * \alpha(rad/s^2) \quad [3.2]$$

EMG signals were band pass filtered at 20-500 Hz, full wave rectified, low pass filtered at 6 Hz, amplitude normalized to percent of MVIC, and time-normalized to one gait cycle, and these knee joint moment and EMG protocols have shown high reliability[230,231].

Specific discrete knee joint moments features analyzed were chosen based on measures previously linked to OA progression. The two key moment features were the KAM impulse calculated as the integral of KAM over the stance phase, and the KFM-

KEM difference calculated as the difference between KFM early-stance peak and KEM late-stance peak, as these were the greatest predictors of clinical OA progression[58]. Secondary variables included the KAM 1st peak (maximum KAM over 0-40% stance phase) and KAM 1st peak to mid-stance minimum difference (KAM 1st peak - minimum KAM over 40-70% stance phase) due to evidence of links to OA progression[58] and the KFM peak (maximum KFM over 0-100% stance phase) and KEM late-stance peak (maximum KEM absolute magnitude over 50-80% stance phase) as explanatory variables for the KFM-KEM difference measure. Table 3.1 provides of summary of the discrete knee joint moments analyzed in this study.

Table 3.1. Descriptions of discrete knee joint moments

Discrete knee joint moments	Description
Primary moments	
KAM impulse	Integral of KAM over the stance phase
KFM-KEM difference	KFM early-stance peak - KEM late-stance peak
Secondary moments	
KAM 1st peak	Maximum KAM over 40% stance phase
KAM 1st peak to mid-stance minimum difference	KAM 1st peak - minimum KAM over 40-70% stance phase
KFM peak	Maximum KFM over 0-100% stance phase
KEM late-stance peak	Maximum KEM absolute magnitude over 50-80% stance phase

EMG measures related to OA progression included in this study were based on PCA studies, as previous literature has more often linked OA progression to muscle

activation patterns using PCA[54,60,61] whereas few papers have reported discrete metrics[62]. PCA reduces large volumes of data into a smaller number of features (i.e., PCs) that capture the waveforms' amplitude, difference operators, and phase shifts. PCA has been shown to have high between-day reliability in participants with knee OA in EMG variables[178]. To produce PCs representative of key features and to avoid extracting erroneous features and "overfitting"[244], a standard PC data set was formed for each muscle group using a large data set (n = 428, 221 males/207 females) of previous DOHM lab participants including asymptomatic (n = 188, 62 males/126 females) and moderate OA (n = 240, 159 males/81 females) participants.

Standard muscle activation PCs were formed using a standard procedure[60,171]. Two data matrices (X) were formed for the EMG waveforms based on each muscle group: 1) KE (3 muscles, 1284 waveforms), 2) KF (2 muscles, 856 waveforms). PCAs were performed for each matrix by calculating the eigenvector decomposition of the cross-product matrix ($[S] = [X^T] \times [X]$) and this resulted in orthonormal eigenvectors or PCs for the KE and KF muscle groups. PCs accounting for at least 90% of the total variance of the data set (with no individual PCs contributing <1% of the variance) were used for statistical hypothesis testing and these corresponded with PC1 and PC2 for the KE (VL, VM, RF) and KF (MH, LH) muscles which were also of greatest clinical relevance based on their association with OA progression[54,60,61]. PC1 captured overall muscle activation magnitude with higher PC1 scores indicating higher muscle activation magnitude. PC2 captured prolonged muscle activation with higher PC2 scores indicating more prolonged muscle activity. Although VL, VM, and RF overall magnitude (PC1) has not been directly linked to clinical OA progression, it was included as a primary muscle

activation measure given its association with OA severity and the limited number of EMG studies on OA progression.

Each original muscle waveform was compared to the standard PC of the related muscle grouping (e.g., vastus lateralis was scored against the KE eigenvector). A PC score was calculated for each participant's original waveform compared to the standard PC. Original waveforms for participants within the 5th and 95th percentiles for each PC were examined to interpret extracted patterns. Standard PCs for EMG waveforms of the KE and KF muscles, and waveforms of participants within the 5th and 95th percentile for each PC are presented in Appendix 2 and Appendix 3. All gait data and PCA processing was completed through a custom MatLab^{TM} version 7.1 written program (The Mathworks Inc., Natick, Massachusetts, USA).

To better interpret EMG PC scores, discrete metrics were calculated for measures previously correlated with EMG PC scores[60]. Discrete features assisted with identifying the magnitude of EMG differences or changes. Correlations between PCs and discrete features were calculated to better interpret which features the PCs were best capturing.

3.2.7 Maximal Voluntary Isometric Contractions

Maximal muscle torques for the KF and KF muscles were assessed through MVIC testing on an isokinetic dynamometer (Biodex^{TM}, Shirley NY). Participants were positioned onto the dynamometer, and the seat and moment arm were adjusted as needed to ensure that the knee joint center was aligned with the axis of the dynamometer. Participants were given detailed instructions on how to perform two sets of exercises on the dynamometer (knee flexion at 45°, knee extension at 45°). A gravity correction trial was recorded at

each position prior to the dynamometer contractions. Participants were then asked to perform one practice trial (approximately 50% effort) and two test trials with at least 60 seconds of rest in between. During test trials, participants were instructed to push as hard as possible for three full seconds and to try to hold a steady level of force throughout.

Torque data from the dynamometer was processed through a custom MatLab™ version 7.1 written program (The Mathworks Inc., Natick, Massachusetts, USA). First, a calibration constant (Cal_cons) was determined by calculating the torque/voltage difference between a known mass and a known distance (Ma) with the lever of the Biodex parallel to the ground, and no mass with the lever of the Biodex perpendicular to the ground [3.3].

Cal_cons (Nm/V) = (((known mass*9.8)*Ma +1)) / (known mass (V) - 0kg (V))) [3.3]

Next, raw voltage signals were transformed to torque (Nm) values and were gravity corrected [3.4]. Gravity-corrected torques were additive when the torque produced was against gravity (e.g., knee extension) and were reductive when the torque produced was assisted by gravity (e.g., knee flexion).

Torque (Nm) = ((Trial (V) - 0kg (V))*Cal_cons (Nm/V)) $\pm$ Gravity correct (Nm) [3.4]

A 500ms moving-average window algorithm was used to capture the maximum torque over the three-second steady-state contraction. The average value of the two trials for each exercise was recorded as the maximal strength in Nm. EMG data was

simultaneously recorded during MVIC trials and maximal EMG amplitudes for each muscle were calculated using a 100ms moving-average window. The maximal EMG amplitude for each muscle regardless of the exercise performed was selected, and EMG amplitudes were normalized as a percentage of this maximum (%MVIC)[202,230]. This method has shown high reliability in individuals with knee OA[230,245]. MVICs generally indicate a close to maximal effort as torque values are typically over 90% of the values elicited during superimposition testing for both healthy controls and knee OA participants[168,246,247].

3.3 STATISTICAL ANALYSIS

All statistical analyses were completed in IBM SPSS Statistics software, version 26 (IBM, NY, USA). Independent t-tests were used to test for differences between sexes in muscle strength, demographics, and clinical characteristics (Objective 1) and pre-walk pain, knee joint moment, and muscle activation features (Objective 2). Independent t-tests were also used to test for differences between males and females in OG1 and OG2 walking speeds, and for differences between OG1 and OG2 walking speeds, as greater walking speed affects gait parameters including higher knee joint moments[193,194,218,248,249] and muscle activation[250,251]. Assumptions of equal variances were evaluated using the Levene's test, and if violated, the adjusted test statistic for equal variances not assumed provided by SPSS was used. Assumptions of normality were examined using 3 criteria: 1) the Shapiro-Wilk test, 2) graphical analysis of histograms and q-q plots, and 3) skewness and kurtosis values. Variables exhibiting non-normal data (i.e., not normal

based on violating ≥2/3 criteria) were still analyzed with independent t-tests for consistency but were additionally analyzed using Mann-Whitney U tests. The Mann-Whitney U test also tested for differences in KL grades, given that KL grades are measured on an ordinal scale. For categorical variables, Pearson's chi-squared tests were used to test for differences between sexes in clinical characteristics (Objective 1) and pre-walk pain, knee joint moment, and muscle activation features (Objective 2) if the sample size assumption was met (i.e., expected count ≥5 in each cell). If the sample size assumption was not met, Fischer's exact tests were instead conducted. Statistical significance was set to α= 0.05.

Pearson's chi-squared test was used to test for differences between sexes in the percentage of individuals who experienced a change in NPRS score post-walk. Two-way mixed analyses of variance (ANOVAs) (sex, time) tested for significant interaction and main effects for pain intensity, pain sensitization, knee joint moments and muscle activation variables (Objective 3). Given the small sample size and exploratory nature of the study, statistical significance was set to α= 0.1 for interaction and main effects. Assumptions of normality were examined using 1) Shapiro-Wilk tests, 2) graphical analysis of histograms and q-q plots, and 3) skewness and kurtosis values and data were classified as non-normal if they did not pass ≥2/3 criteria. Assumptions of equal variances and equal covariances for all continuous variables were examined using Levene's and Box's tests. If assumptions were violated, appropriate transformations (i.e., log10 transformation) were applied and/or outliers (i.e., +/- 3 studentized residuals) were removed. ANOVAs for variables not meeting the necessary assumption were performed on both original and transformed data. Post-hoc analyses for variables with significant

interaction effects were used to determine significant between-group (sex) or within-group (time) differences with statistical significance set to α= 0.05.

Linear regression models for response variables with significant sex by time interactions (Objective 3) were developed. A pre-post-walk response score for each response variable was calculated as the difference between the post-walk and pre-walk scores [3.5] and this pre-post-walk response score was the dependent variable for each model.

Pre-post-walk response score = Post-walk score – pre-walk score [3.5]

Linear regression models were developed to address the study's overall objective to determine where muscle strength can explain the differences between sexes in acute pain, knee joint moment and muscle activation responses to walking. First, Pearson's product-moment correlations were calculated for normalized strength and the pre-post-walk response scores for variables with significant sex by time interactions (from Objective 3) for the total group, and then for males and females separately to determine whether muscle strength was correlated to pre-post-walk response scores within male and female groups separately. Linear regression models were developed for those response variables that were significantly correlated with normalized muscle strength to determine the predictive equation and how much variance muscle strength explained in pre-post-walk response scores.

CHAPTER 4: SEX DIFFERENCES IN MUSCLE STRENGTH, DEMOGRAPHICS, CLINICAL CHARACTERISTICS AND PAIN, KNEE JOINT MOMENT AND MUSCLE ACTIVATION MEASURES (OBJECTIVES 1 & 2)

This chapter presents the results related to Objectives 1 and 2 and a discussion of the key findings. The two main hypotheses were that females with radiographic medial compartment knee OA 1) would have significantly lower KF and KE muscle strength, higher pain catastrophizing, and worse OA-specific pain, physical function, symptoms, and physical activity levels (**Hypothesis 1**) and 2) higher pain intensity and pain sensitization, a lower KAM magnitude, a smaller KFM-KEM difference, and higher and more prolonged muscle activation magnitude (**Hypothesis 2)** than males with radiographic medial compartment knee OA.

4.1 RESULTS

Forty-five participants (22 males, 23 females) with evidence of medial compartment radiographic OA (KL grade $\geq$ 1) were included in this study. 15/22 males and 22/23 females had greater JSN in the medial compared to the lateral compartment and 7/22 males and 1/23 females had equal JSN in the medial and lateral compartments. Twenty-four participants (53%) reported OA symptoms (12 males, 12 females). One symptomatic female participant did not complete MVIC testing and was excluded from the muscle strength and EMG analyses.

4.1.1 Muscle Strength, Demographics, and Clinical Characteristics (Objective 1)

The descriptive statistics for muscle strength, demographics and clinical characteristics by sex are found in Tables 4.1 and 4.2. All continuous variables met the t-test assumptions, except for the KOOS pain, symptom, ADL function, and sport function scores which did not meet the assumption of normality. These scores were additionally examined using a Mann-Whitney U test. For the four KOOS measures, there was no difference between the findings of the t-test and Mann-Whitney U test as both indicated no significant difference. For the categorical variables, medication use met the sample size assumption and therefore a Pearson's chi-squared test was used to test for differences between sexes. Physical activity frequency and intensity did not meet the sample size assumption and were examined using Fischer's exact tests.

There were statistically significant differences ($p<0.05$) between males and females in muscle strength where males had greater KE absolute (110. 7 $\pm$ 31.0 Nm) and normalized (1.3 $\pm$ 0.4 Nm/kg) muscle strength than females (74.1 $\pm$ 29.9 Nm, 1.0 $\pm$ 0.3 Nm/kg) (Table 4.1) as illustrated graphically in Figure 4.1. Males also had greater KF absolute (62.6 $\pm$ 20.3 Nm) and normalized (0.8 $\pm$ 0.2 Nm/kg) muscle strength than females (34.5 $\pm$ 14.4 Nm, 0.5 $\pm$ 0.2 Nm/kg) (see Table 4.1 and Figure 4.1). There were no significant differences ($p>0.05$) between males and females in any other demographic or clinical characteristic (Tables 4.1 and 4.2).

Table 4.1: Muscle strength, demographics, and clinical characteristics for males and females with radiographic knee OA reported as mean (standard deviation) or *median (range),* 95% confidence intervals and *p*-values

Descriptive characteristic	Males (n=22)	Females (n=23)	95% CI	*P*-value (t-test)	*P*-value (Mann-Whitney U)
Age (years)	63.3 (6.7)	62.4 (7.9)	(-3.5, 5.3)	0.690	
Mass (kg)	84.2 (12.9)	76.9 (16.5)	(-1.7, 16.1)	0.109	
BMI (kg/m^2)	27.8 (4.6)	30.6 (6.0)	(-6.1, 0.4)	0.082	
KL grade (0-4)	*2 (1-4)*	*2 (1-4)*			0.589
KL grade distribution	1=2, 2=10, 3=6, 4=4§	1=2, 2=12, 3=7, 4=2§			
KOOS pain (/100)	82.1 (18.3)	79.1 (20.0)	(-8.6, 14.5)	0.607	0.512
KOOS symptoms (/100)	80.8 (19.5)	77.6 (18.7)	(-8.3, 14.7)	0.576	0.457
KOOS ADL (/100)	86.9 (15.3)	85.7 (17.2)	(-8.6, 11.0)	0.803	0.710
KOOS sport (/100)	70.7 (29.5)	67.0 (32.6)	(-15.0, 22.4)	0.690	0.574
PCS (/52)	6.6 (6.1)	10.9 (10.2)	(-9.4, 0.7)	0.091	
Knee extensor strength (Nm)	110.7 (31.0)	74.1 (29.9)*	(18.0, 55.1)	**<0.001**	
Knee extensor strength (Nm/kg)	1.3 (0.4)	1.0 (0.3)*	(0.1, 0.6)	**0.002**	
Knee flexor strength (Nm)	62.6 (20.3)	34.5 (14.4)*	(17.4, 38.8)	**<0.001**	
Knee flexor strength (Nm/kg)	0.8 (0.2)	0.5 (0.2)*	(0.2, 0.4)	**<0.001**	

Bold = Statistically significant differences between sexes ($p<0.05$)
*Female muscle strength values where n=22
§Number of participants in each KL grade

Table 4.2: Exercise frequency, exercise intensity, and medication use for males and females with radiographic knee OA reported as frequency count (percentage) and *p*-values

Descriptive characteristic		Males (n=22)	Females (n=23)	*P*-value Chi-Squared	*P*-value Fischer's Exact
Frequency of exercise causing sweating and a rapid heart rate over the past 7-days	3+ times/week	13 (59.1%)	11 (47.8%)		
	1-2 times/week	7 (31.8%)	5 (21.7%)		
	Rarely or never	2 (9.1%)	7 (30.4%)		0.228
Perceived intensity of exercise over the past 7-days	Intense effort	4 (18.2%)	6 (26.1%)		
	Moderate effort	17 (77.3%)	13 (56.5%)		
	Light effort	1 (4.5%)	4 (17.4%)		0.257
Current medication use for knee pain	Yes	8 (36.4%)	7 (30.4%)		
	No	14 (63.6%)	16 (69.6%)	0.758	

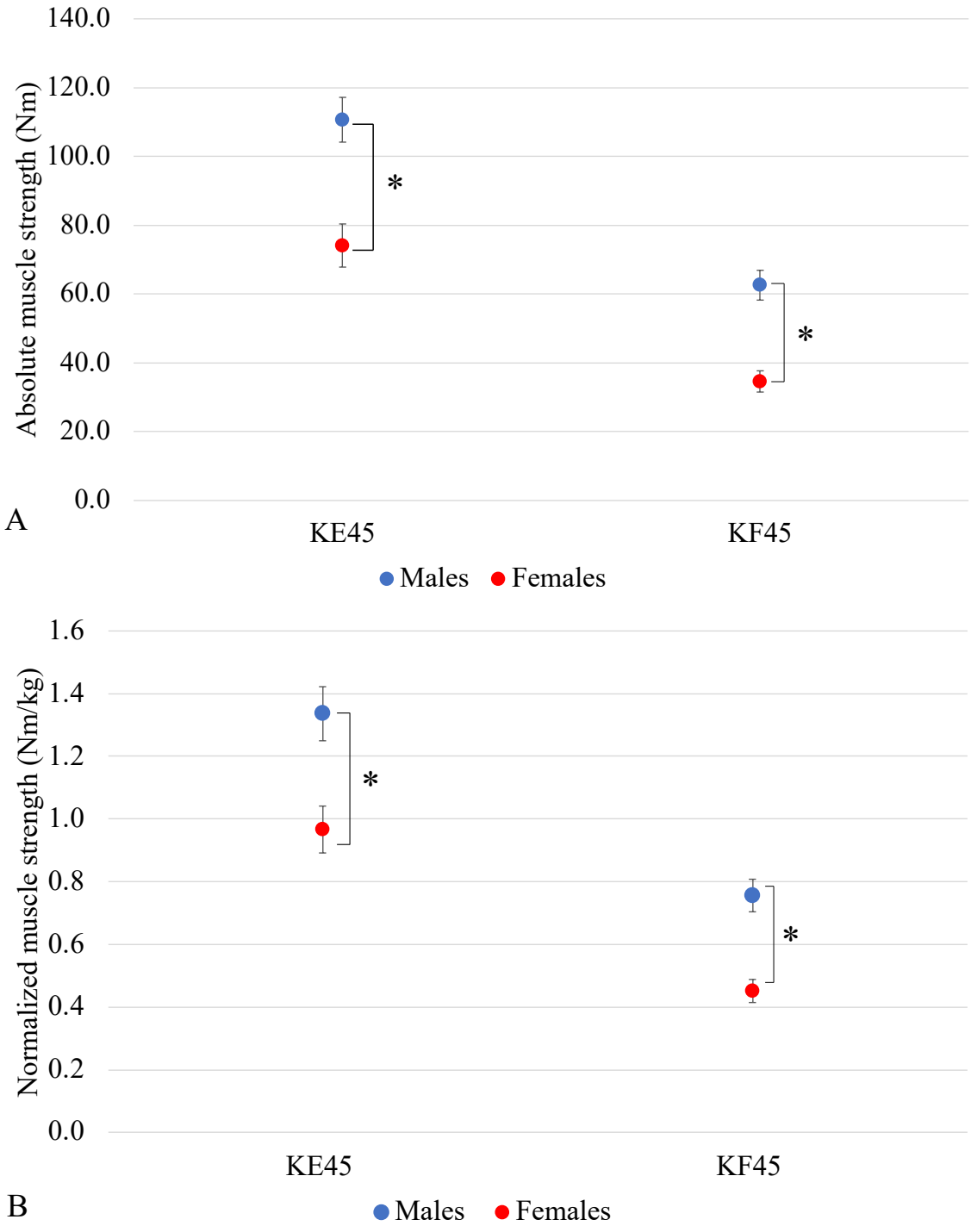

Figure 4.1. Mean absolute (A) and body-mass normalized (B) knee extensor (KE) and knee flexor (KF) muscle strength measured at a 45° knee flexion angle for males (n=23) and females (n=22). *Males had significantly greater KE and KF strength than females (p<0.05). Error bars indicate ± 1 standard error of the mean.

4.1.2 Pain Intensity, Pain Sensitization, Knee Joint Moments, and Muscle Activation Patterns (Objective 2)

The descriptive statistics for pre-walk pain intensity and PPT scores by sex are found in Table 4.3. The VM and ECRL PPTs met the t-test assumptions. Pain intensity and the knee joint PPT did not meet the assumption of normality and were additionally examined using the Mann-Whitney U test. There was no difference between the findings of the t-test and Mann-Whitney U test as both indicated no significant difference for the NPRS and a significant difference for the knee joint PPT. There were statistically significant differences ($p<0.05$) between males and females in all three pre-walk PPTs, where females had lower knee joint (M = 3.59 $\pm$ 2.03 kgf/cm^2, F =1.82 $\pm$ 1.15 kfg/cm^2), VM (M = 3.94 $\pm$ 2.05 kgf/cm^2, F = 1.97 $\pm$ 0.96 kgf/cm^2), and ECRL (M = 2.21 $\pm$ 1.24 kgf/cm^2, F =1.49 $\pm$ 0.79 kgf/cm^2) PPTs (i.e., higher pain sensitization) (Table 4.3) as illustrated graphically in Figure 4.2. There were no statistically significant ($p>0.05$) differences between sexes in pain intensity scores (Table 4.3).

Table 4.3: Pain intensity and sensitization measures for males and females with radiographic knee OA presented as mean (standard deviation), *median,* mean difference, 95% confidence interval (CI), and *p*-value

Pain measure	Males (n=22)		Females (n=23)		Mean difference (M-F)	95% CI	*P*-value (t-test)	*P*-value (Mann-Whitney U)
NPRS (/10)	0.3 (0.9)	*0*	0.3 (0.7)	*0*	0.0	(-0.5,0.5)	0.895	0.705
Knee PPT (kgf/cm^2)	3.59 (2.03)	*3.43*	1.82 (1.15)	*1.79*	1.77	(0.76, 2.78)	**0.001**	**0.003**
Vastus medialis PPT (kgf/cm^2)	3.94 (2.05)	*3.90*	1.97 (0.96)	*2.02*	1.96	(0.98, 2.95)	**<0.001**	
Extensor carpi radialis longus PPT (kgf/cm^2)	2.21 (1.24)	*1.93*	1.49 (0.79)	*1.48*	0.72	(0.10, 1.34)	**0.025**	

Bold: Statistically significant sex difference ($p<0.05$)

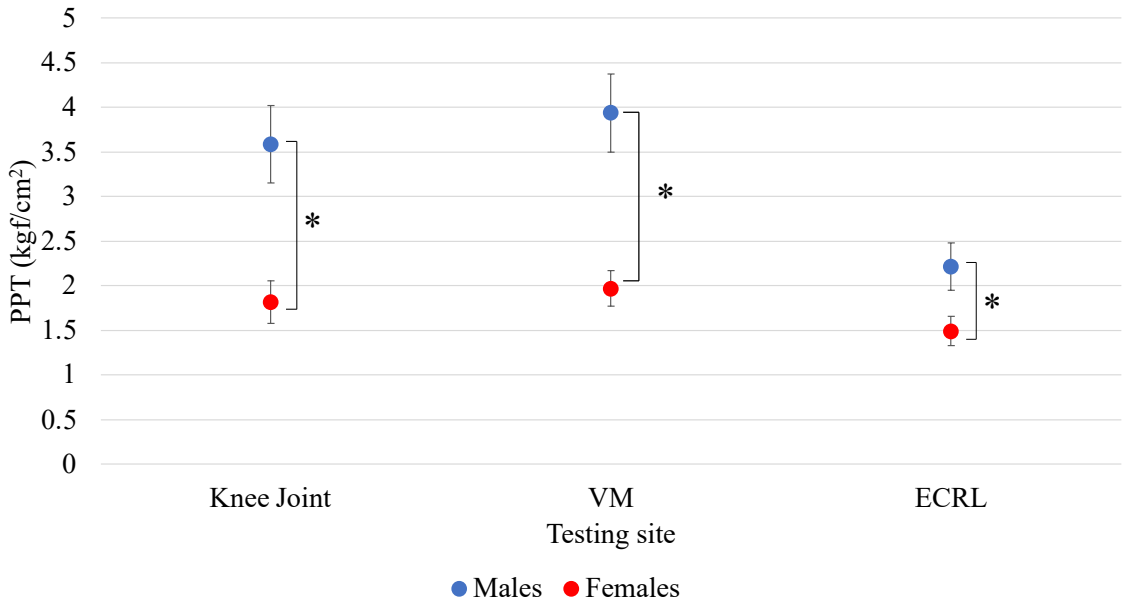

Figure 4.2. Mean pressure pain thresholds at the knee joint, vastus medialis (VM), and extensor carpi radialis longus (ECRL) separated by sex (males = blue, females = red). Error bars indicate ± 1 standard error of the mean.

For the overground pre-walk gait trials, there was no significant difference (p=0.143) in overground walking speed between males (1.3 m/s) and females (1.2 m/s). The descriptive statistics by sex for pre-walk knee joint moments are presented in Table 4.4 and ensemble average waveforms are presented in Figure 4.3. All variables met the t-test assumptions except for the KEM late-stance peak which did not meet the assumption of normality. A Mann-Whitney U test was additionally performed on the KEM late-stance peak. Females had a significantly smaller (p<0.05) KFM-KEM difference (i.e., KFM peak - KEM late-stance peak) than males (M = 0.92 $\pm$ 0.32 Nm/kg, 0.73 $\pm$ 0.32 Nm/kg) (Table 4.4) as illustrated in Figure 4.3. There was a significant difference (p<0.05) between sexes based on the Mann-Whitney U test for the KEM late-stance peak where females had a median peak of smaller absolute magnitude (-0.22 Nm/kg) than males (-0.33 Nm/kg) (Table 4.4.). The t-test p-value was not statistically significant for the KEM late-stance peak (p=0.093), likely due to the variability and the larger difference in medians than means between sexes. Both tests are reported, and the difference is illustrated in Figure 4.3. There were no statistically significant differences between sexes in the other knee joint moment features (Table 4.4).

Table 4.4: Knee joint moment discrete metrics for males (M) and females (F) with radiographic knee OA presented as mean (standard deviation), *median*, mean difference, 95% confidence interval (CI), and *p*-value

Knee joint moment	Males (n=22)		Females (n=23)		Mean difference (M-F)	95% CI	*P*-value (t-test)	*P*-value (Mann-Whitney U)
Primary moments								
KAM impulse (Nm/kg*s)	0.21 (0.07)	*0.2*	0.19 (0.05)	*0.19*	0.02	(-0.02, 0.05)	0.326	
KFM-KEM difference (Nm/kg)	0.92 (0.32)	*0.94*	0.73 (0.32)	*0.70*	0.19	(0.00, 0.39)	**0.046**	
Secondary moments								
KAM 1st peak-mid-stance minimum difference (Nm/kg)	0.26 (0.15)	*0.26*	0.24 (0.13)	*0.22*	0.02	(-0.07, 0.105)	0.656	
KAM 1st peak (Nm/kg)	0.55 (0.13)	*0.53*	0.54 (0.14)	*0.55*	0.01	(-0.07, 0.09)	0.756	
KFM peak (Nm/kg)	0.66 (0.27)	*0.65*	0.56 (0.21)	*0.51*	0.10	(-0.04, 0.25)	0.167	
KEM late stance peak (Nm/kg)	-0.26 (0.19)	*-0.33*	-0.17 (0.17)	*-0.22*	-0.09	(-0.02, 0.20)	0.093	**0.021**

Bold: Statistically significant sex difference ($p<0.05$)

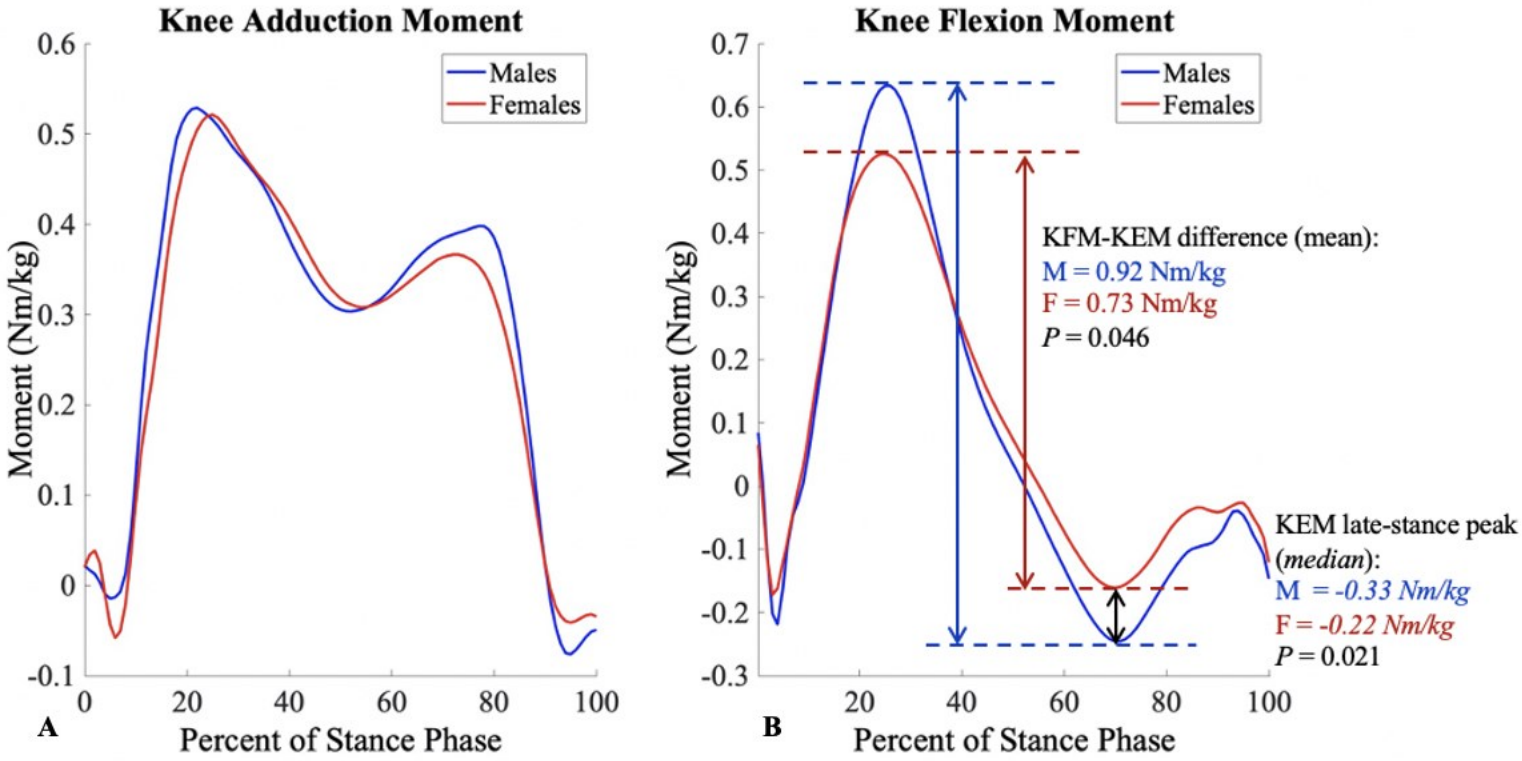

Figure 4.3. Ensemble average knee adduction (A) and knee flexion (B) joint moment waveforms (amplitude normalized to body mass) during self-selected speed overground walking by sex (red = females, blue = males). (A) There were no significant differences between males and females for all KAM measures (p>0.05). (B) There was a significant difference between males and females in the KFM-KEM difference measure with females having a smaller difference (p=0.046) and in the KEM late-stance peak (p=0.021) with females having a *median* KEM late-stance peak of smaller absolute magnitude.

The descriptive statistics for pre-walk muscle activation variables by sex are presented in Table 4.5 and ensemble average waveforms are presented in Figure 4.4. Only VL PC2 met the t-test assumptions. All other muscle activation variables did not meet the assumption of normality and Mann-Whitney U tests were additionally performed on these variables. There was no difference between the findings of the t-test and Mann-Whitney U test as both were consistent with respect to whether a variable was significantly different between sexes or not. Females had a significantly higher ($p<0.05$) RF PC1 (i.e., overall activation magnitude) score than males (M = 80.8 $\pm$ 58.0, F = 179.3 $\pm$ 112.5) (Table 4.5) as illustrated in Figure 4.4C. Despite females having higher PC1 scores for all muscle sites (Table 4.5 and Figure 4.4), there were no significant differences between sexes ($p>0.05$) in the other muscle activation variables (Table 4.5). To better interpret these PC scores, discrete muscle activation measures previously associated with PC scores[72] including stance-phase root mean squared (RMS), mid-stance RMS, and early-stance RMS to mid-stance RMS difference were calculated and are presented in Appendix 4 for comparison and interpretation purposes only. Correlations between discrete muscle activation values and PC scores are presented in Appendix 5.

Table 4.5: Knee extensor and knee flexor muscle activation principal component (PC) scores for males (M) and females (F) with radiographic knee OA presented as mean (standard deviation), *median*, mean difference, 95% confidence interval (CI) and *p*-value

Muscle activation pattern	Males (n=22)		Females (n=22)		Mean difference (M-F)	95% CI	*P*-value (t-test)	*P*-value (Mann-Whitney U)
Knee extensors								
VLPC1	165.0 (89.6)	*169.7*	223.3 (141.2)	*187.4*	-58.32	(-130.26, 13.63)	0.109	0.231
VLPC2	-27.3 (31.9)	*-21.7*	-22.3 (41.7)	*-24.8*	-4.98	(-27.57, 17.61)	0.659	
VMPC1	146.3 (74.1)	*126.7*	222.1 (171.6)	*173.1*	-75.78	(-157.33, 5.77)	0.067	0.139
VMPC2	-30.7 (30.9)	*-27.1*	-24.0 (52.6)	*-18.8*	-6.69	(-32.95, 19.57)	0.610	0.453
RFPC1	80.8 (58.0)	*59.7*	179.3 (112.5)	*135.4*	-98.54	(-153.54, -43.54)	**0.001**	**<0.001**
RFPC2	13.3 (31.2)	*4.0*	19.7 (47.2)	*7.6*	-6.39	(-30.74, 17.96)	0.599	0.690
Knee flexors								
LHPC1	168.3 (102.9)	*142.0*	232.5 (181.2)	*178.5*	-64.21	(-153.85, 25.44)	0.156	0.260
LHPC2	-10.0 (67.2)	*-11.6*	-13.5 (61.7)	*-23.8*	3.49	(-35.75, 42.73)	0.858	0.606
MHPC1	162.8 (73.9)	*152.5*	207.5 (171.8)	*156.6*	-44.66	(-126.24, 36.92)	0.272	0.725
MHPC2	-46.1 (54.2)	*-27.8*	-54.7 (61.7)	*-52.9*	8.57	(-26.77, 43.90)	0.627	0.133

Bold: Statistically significant sex difference ($p<0.05$)
PC1: A higher score indicates greater overall muscle activity magnitude
PC2: A more positive score indicates greater mid-stance compared to early-stance muscle activation amplitude (i.e., more prolonged activation)

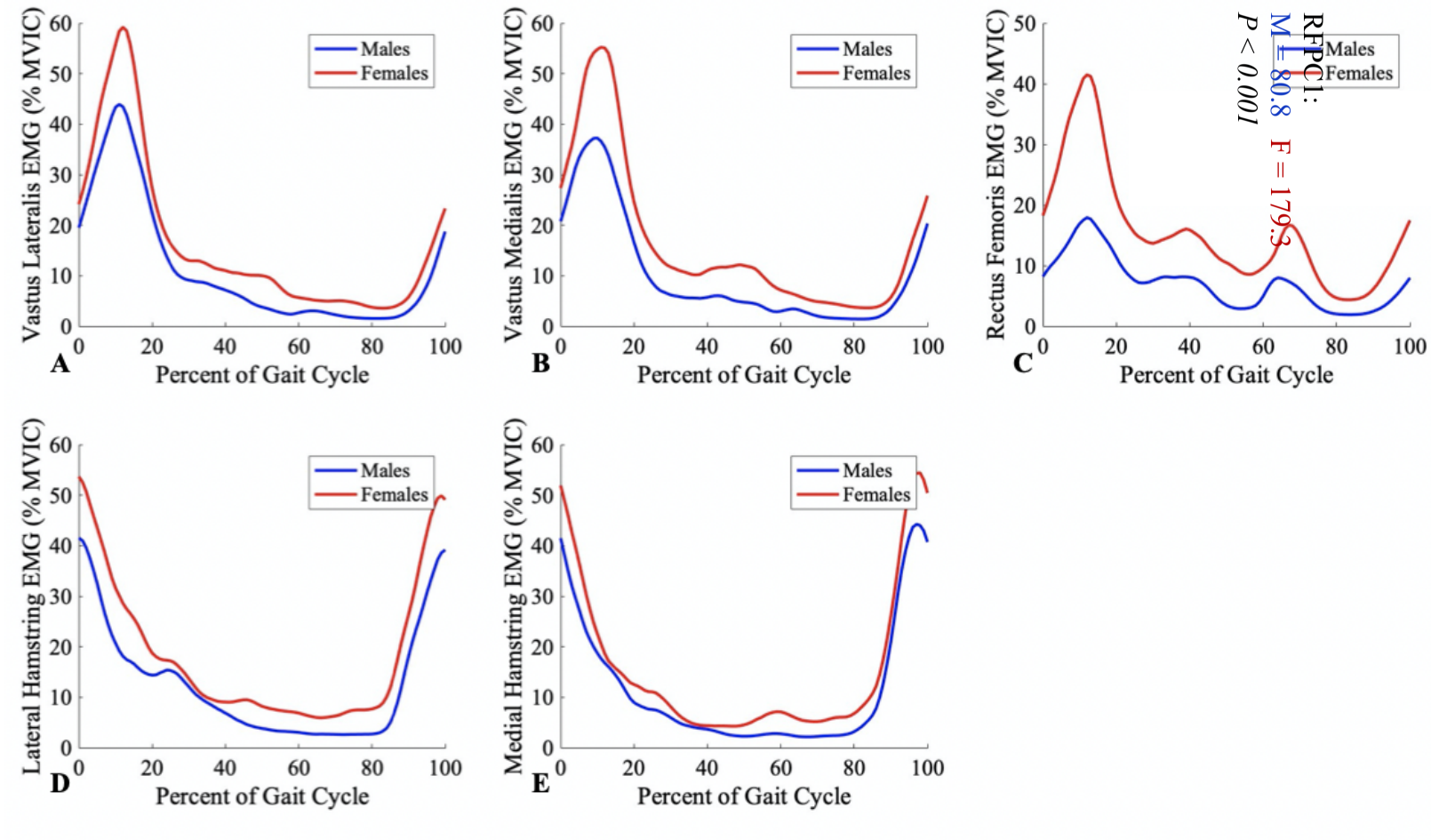

Figure 4.4. Ensemble average electromyography waveforms (amplitude normalized to percent maximum voluntary isometric contraction) during gait for the knee extensor (A-C) and knee flexor (D, E) muscles by sex (red = females, blue = males). There were no significant differences between males and females for all knee flexor muscle activation patterns. (C) There was a significant difference between males and females in the rectus femoris overall activation magnitude pattern with females having a higher muscle activation magnitude.

4.2 DISCUSSION

This chapter tested whether there were differences between males and females with radiographic medial compartment OA in 1) muscle strength, demographic, and clinical characteristics (Objective 1) and in 2) pain intensity, pain sensitization, knee joint moments, and muscle activation patterns during walking linked to OA progression (Objective 2).

The key findings of this study are the significant differences between males and females with radiographic medial compartment knee OA in KE and KF muscle strength, pain sensitization at all three sites (i.e., knee joint, VM, and ECRL), KFM features, and RF muscle overall activation amplitude (PC1) during overground (OG1) walking. These findings partially support the hypotheses. Unique to this study is that multiple variables associated with OA processes including multiple dimensions of pain, knee joint moments and muscle function were assessed allowing for a comprehensive examination of differences between sexes and a better understanding of the interactions among multiple OA-specific variables.

4.2.1 Muscle strength

Females had lower absolute and body mass normalized muscle strength for both the KF and KE muscles supporting Hypothesis 1. In general, the lower KE and KF strength in females in the current study is consistent with studies of asymptomatic, moderate OA and severe OA samples[20,23,29,30,176] . When interpreting these findings, it is important to consider the many variables previously shown to affect muscle strength including age[252–254], body mass[252,255], radiographic knee OA severity[256], patient-reported knee OA

outcomes (i.e., pain and function)[122,161], pain catastrophizing[162], pain intensity[163], pain sensitivity[164], and physical activity levels[86]. The two sex groups were not significantly different in age, radiographic severity, body mass, BMI, patient-reported pain and function outcomes from the KOOS questionnaire, pain catastrophizing, pain intensity, or physical activity levels. Approximately half of the males and half of the females did not report symptoms and both males and females reported similar medication use. Together these findings support that the two sex groups were well matched and that these variables do not fully explain the differences between sexes found in muscle strength. However, two variables that influence muscle strength are further discussed below including body mass and pain, given that the proportion of lean body mass needs to be considered as do the multiple dimensions of pain measured in this study.

With respect to body mass, studies show a positive relationship between body mass and muscle strength[252,255], but this relationship is based on the assumption that higher mass is associated with higher lean body mass and does not account for mass increases due to adipose tissue.

Differences between sexes in absolute muscle strength despite no significant differences in body mass, and differences in normalized strength values support that strength differences between sexes are not dependent on the lower body mass in females. Females had a 2.8 kg/m^2 higher BMI than males which was not statistically significant (p=0.081) but this 10% difference is consistent with previous studies reporting similar differences[20,59] or no significant differences[23,257] between sexes in knee OA samples. BMI is not a direct measure of body fat, but females on average, have a higher total body fat percentage than males, even with equal BMIs[258,259]. Thus, it is possible that the females

in this sample had a higher proportion of fat mass than the males, and this could help explain the lower strength values given that intermuscular fat is predictive of lower KE strength in females with or at risk of radiographic knee OA[260].

Absolute KE and KF strength values from the current study were lower than values from an asymptomatic group who had a lower mean body mass and a lower BMI than the current sample[20] and absolute KE strength was lower for both sexes whereas absolute KF strength was lower for males and higher for the females compared to a moderate knee OA group with a higher mean body mass and a similar BMI to the current sample[20]. These absolute muscle strength values are difficult to compare across studies given that study samples have varying body masses, but it is clear from these findings that a higher body does not always equate to higher muscle strength. To address this limitation, normalized to body mass strength values provide a measure of muscle strength relative to body mass, partially addressing the differences in mass among studies, patient groups, and sexes.

The KE normalized strength values were comparable to values from a sample with or at risk of knee OA[29] and both KE and KF normalized strength values for both sexes were between values for asymptomatic and moderate OA groups[20,176]. These findings were expected given the similar age and function level compared to the with or at risk of OA sample[29] and that all participants in this study had radiographic knee OA with only half the sample, and an equal number of males and females, reporting symptoms. Together, this indicates that this sample is between an early mild to moderate knee OA group with other characteristics similar to mild to moderate OA groups including median KL grades of 2[227,261], self-reported pain and function scores[262,263], and

overground walking speeds[193] and was likely between the clinical severity of asymptomatic and moderate OA participants[20,176].

The second variable discussed is pain as its presence is associated with lower KE and KF muscle strength[90–92] and experimentally induced pain reduces strength[87] whereas pain relief increases strength[88,89]. The males and females in this sample did not differ in self-reported pain intensity, OA-specific pain, and pain catastrophizing. Only 3/22 males and 4/23 females had an NPRS score greater than zero and of these individuals, only 1 male and 3 females had an NPRS score greater or equal to 2 which is a clinically meaningful pain score[157,264]. All of these pain measures are self-reports that measure different dimensions of pain intensity and pain cognition[116,143,154–156] and the current findings do not support the general consensus that females with knee OA have higher pain levels than males with knee OA[5–9,17].

The only pain measure that differed between sexes was the PPT values that provide an objective assessment of pain sensitization. Females had higher pain sensitization at both the local knee joint and remote VM and ECRL sites, despite similar self-reported pain intensity at the time of PPT testing. It is plausible that physiological mechanisms that are not captured in self-reported pain measures can impact and partially explain the strength differences between sexes.

4.2.2 Pain Sensitization

The lower PPTs in females indicate higher pain sensitization at all three test sites supporting Hypothesis 2. The magnitude of PPT differences between sexes was over two times greater at the knee joint and the VM sites compared to the ECRL site indicating

that differences in pain sensitivity were greatest at sites closest to the knee joint. PPTs at sites closest to the affected site, in this case the knee joint, are likely a result of nociceptive pain whereas pain sensitization at a remote site such as the ECRL is more likely to be a result of neuropathic pain mechanisms and indicates a more central sensitization to pain[150].

The higher sensitization (i.e., lower PPTs) at the local and the remote sites in females compared to males is consistent with previous reports of higher peripheral and central pain sensitivity to multiple stimuli (i.e., pressure, heat, cold) in females in the general population[146,265–268] and females with symptomatic knee OA[9,19] and with knee pain[269]. Central sensitization is often a result of neuropathic pain[134], and there is a higher prevalence of neuropathic pain reported in females[110]. Thus, the higher generalized pain sensitization may reflect a higher prevalence of neuropathic pain and a more systemic disease in females with radiographic knee OA.

The knee joint PPT values are similar to values from males and females with symptomatic knee OA[19]. However, their quadricep muscle PPTs were higher for both the males and females compared to the current sample, but the exact location of measurement was not specified nor was the location of the OA (i.e., medial or lateral compartment)[19]. The location of testing and OA compartment might affect the PPT values as the VM site in the current study is close to the affected medial compartment of the knee. Compared to other reports in both healthy samples and individuals with knee pain[268,269], the PPT scores in this study were lower at all sites. This may be due to these samples being between 10 to 25 years younger than the current sample, as PPTs have been shown to decrease with age[270,271].

Though the current findings show overall lower PPTs than previous reports, the PPT differences between sexes are consistent with reports that asymptomatic and knee OA females have lower PPT values than males at both the affected local and remote sites[19,268,269]. Furthermore, the differences between sexes at the knee joint, VM, and ECRL test sites are greater than the standard error of measurement and minimal detectable change previously reported for PPT testing[234,272], with the exception of the ECRL whose mean difference value between sexes was greater than the minimal detectable change from one study who measured minimal detectable change from meaningful changes after 4 weeks of regular physiotherapy[234] but not another whose minimal detectable change value was calculated based on the standard error of measurement[272]. Overall, these findings suggest that there are meaningful PPT differences between sexes but that the small magnitude of difference at the ECRL must be interpreted with caution.

The current finding of greater sex differences at the sites closest to the knee joint compared to the remote ECRL site suggests greater peripheral sensitization in females with radiographic OA. The radiographic evidence suggests similar cartilage damage between sexes in this study, but females typically have higher inflammation[26,273] that can impact nociceptive pain mechanisms potentially accounting for these sex differences.

Assessing multiple pain variables is important given that pain is multidimensional with physiological and psychological components[50]. Though previous studies often report self-reported pain measures, the current findings suggest that these may not be sensitive enough to capture physiological changes in pain given that the PPT values, but not self-reported pain measures differed between sexes. This highlights the importance of

assessing different dimensions of pain to better understand physical function decline in knee OA, as the presence of pain has been associated with alterations in multiple gait parameters including slower walking speed[78], lower peak KFM and KEMs[82], and higher muscle activation magnitudes[82]. Females in this study had greater pain sensitization, and a stiffer knee gait pattern with higher overall muscle activation magnitude than males, consistent with a painful gait pattern.

4.2.3 Knee Joint Moments

Walking speed influences both frontal (KAM) and sagittal (KFM) plane knee joint moment features where slower speeds have been linked to lower knee joint moment peaks and difference measures[193,194,218,248,249]. However, no differences in overground walking speed during the gait analysis test trials were found between sexes. Supporting Hypothesis 2, females walked with a lower KFM-KEM difference feature indicative of a stiffer-knee gait pattern, and this is consistent with findings from previous moderate and severe OA groups[20,59]. Similar to these studies[20,59], there was no significant difference between sexes in walking speed. Thus, a slower overground walking speed does not explain this difference between sexes in the stiff-knee gait pattern. A stiffer-knee gait pattern results in a more sustained loading pattern[72] and more sustained loads have been shown to induce catabolic cartilage changes in cartilage explant[124] and animal models[182,183]. This stiffer-knee gait pattern was expected based on the lower muscle strength and higher pain sensitization found in the females compared to the males in this study. Lower KE muscle strength and to a lesser extent KF strength have been correlated with a stiff-knee gait pattern[72,82] and individuals with symptoms have a stiffer-knee gait

pattern than those without symptoms[82]. Importantly, this stiff-knee gait pattern is a feature unique to predicting clinical progression[58] and may be indicative of a more systemic disease.

There was no difference between sexes in the three KAM features which was somewhat expected based on the similar overground walking speeds between sexes. The KAM is a ratio of medial-to-lateral joint loading[68], and males and females had similar medial compartment disease with most having greater JSN in the medial compared to the lateral compartment. The lack of significant difference between sexes in KAM features is consistent with results from a moderate knee OA cohort[20] who did not report the OA compartment. Studies on more severe OA cohorts have reported lower KAM magnitudes in females[59,77], but only one study included only medial compartment knee OA[58], whereas the other did not specify OA compartment[77] and so it is unknown whether males and females had similar medial-to-lateral radiographic severity. Based on the current findings and previous literature, females with radiographic knee OA (mean KL grade = 2) and mild symptoms walk with different sagittal but not frontal plane moment patterns compared to males of similar radiographic and symptom severity. Thus, with respect to the frontal plane moments, there is no difference in risk of structural or clinical progression between sexes given that a higher KAM is associated with structural and clinical progression outcomes[52–56,58].

The knee joint moments provide an estimate of knee joint forces, however, they are calculated from external forces and assumptions that are not exact (e.g., generalized body segment parameters, assuming no co-activation of muscles), and have limitations in estimating internal contact forces[243]. Muscle activation patterns provide additional

information that can help to interpret these knee joint moments since muscles are key contributors to joint stability[70] and loading[178,179].

4.2.4 Muscle Activation Patterns

Females had significantly higher RF overall muscle activation (PC1) than males partially supporting Hypothesis 2. Although females had higher overall muscle activation (PC1) in all KE, and to a lesser degree KF muscles as illustrated in Figure 4.4, the large variability and the relatively small sample size potentially contribute to the lack of statistically significant differences. A power analysis indicated 36% and 46% power for the overall activation magnitude of the VL and VM respectively. To place the magnitude of differences into context, the females had overall stance phase RMS values between 4 (MH) to 6 (LH) % MVIC higher than males for the KF muscles and between 7 (VL) to 12 (RF) % MVIC higher than males for the KE muscles (see Appendix 4). Thus, the magnitude of difference between sexes was greater for the KE muscles, suggesting that the KE muscles may fatigue more quickly for the females, and this is important given that KE muscle fatigue has been shown to result in a decreased KFM-KEM difference[51]. Furthermore, low levels of muscle activity over a prolonged period have been shown to increase cartilage cell death in animal models[182,183] and they have been associated with OA severity[78,82,170,171,196,201–204] and progression[60,61].

Since overground walking speeds during the gait analysis test trials were not significantly different between sexes and slower walking speeds have been associated with lower muscle activity[250,251], speed does not explain the significantly higher RF activity or the higher overall activity. As reported above, the lower muscle strength and

higher PPTs in females may have required females to activate their KE muscles to a higher percentage of their maximal activation to produce the forces necessary to maintain similar walking speeds and to produce sufficient joint stiffness to minimize pain.

For both males and females, the prolonged activity (PC2) scores were negative for all muscles except the RF, indicating minimal prolonged activity compared to the asymptomatic and moderate OA samples used to generate the standard PC. More prolonged muscle activity, or significantly greater KF overall muscle co-activity, was expected in females given that the stiff-knee gait pattern has been previously correlated with more prolonged KE and KF muscle activity and higher KF muscles overall activation magnitude in individuals with moderate medial compartment knee OA[72]. Higher RF muscle activity and a smaller KFM-KEM difference feature have both been reported in symptomatic compared to asymptomatic individuals with the same radiographic evidence of knee OA[82] supporting the link between muscle activity and a stiff knee gait. While there is support that pain and muscle strength could contribute to the significantly higher RF muscle activity, and the higher but non-significant activity in the VM and VL, other factors like joint instability can also result in higher muscle activity[274] but this was not directly measured. The overall muscle activation magnitude of both the KE and KF muscles requires further examination to better understand the role of muscle activity in relation to the knee joint moment, muscle strength and pain sensitivity differences found between sexes, given the lack of significant findings in this small sample.

The higher KE muscle activation may in part be a mechanism to compensate for higher pain sensitization, as higher activation has been found in individuals with OA

symptoms, compared to individuals with the same radiographic severity but no symptoms[82]. Higher muscle activation is reported in individuals with the presence of knee OA and in individuals with more severe knee OA, which is typically associated with worse symptoms[78,82,170,171,196,201–204]. This increased overall muscle activity may be a mechanism to increase joint stiffness[274], and this is supported by the stiffer knee gait pattern in females. These compensatory mechanisms, however, can result in long-term consequences given evidence from animal studies that show knees with higher muscle activity over a prolonged period of time have increased cartilage cell death[182,183]. Higher muscle activation may also lead to muscle fatigue more quickly, and KE fatigue has been linked to a stiffer-knee gait pattern[51] and therefore more static loading.

Previous studies have reported higher KE overall activation magnitudes in females compared to males with severe OA[59,176] and to a lesser degree in those with moderate OA[176], but not in asymptomatic participants[176]. Given the uniqueness of the current sample that included those with radiographic OA and minimal symptoms, the magnitude of muscle activation differences between sexes likely falls between those of previous asymptomatic and moderate OA samples.

This study investigated differences between sexes in pain and gait metrics at one point in time. However, it is equally important to investigate how males and females respond differently to walking given that this is a frequently performed daily activity and is a highly recommended activity for knee OA management[37]. There is limited direct evidence on how walking influences OA processes[37], and the limited studies that have studied these responses to walking, have not separated participants by sex[46,47,49,126–130]. To better understand how pain intensity and sensitization, knee joint moments, and muscle

activation patterns change in response to a standard walking prescription, the following chapter examined these responses and determined if they differ between males and females with radiographic knee OA.

4.3 CONCLUSION

The results of this study showed that females with radiographic medial compartment knee OA have lower absolute and normalized KE and KF muscle strength, higher pain sensitization at local and remote sites, a stiffer-knee gait pattern based on the KFM-KEM difference measure, and higher overall RF muscle activation despite similar self-reported pain, symptoms, physical function, and physical activity levels to males. This combination of features provides a unique profile for females distinct from males that is consistent with a higher risk of clinical OA progression.

CHAPTER 5: SEX DIFFERENCES IN PRE-POST-WALKING RESPONSES AND THE ROLE OF MUSCLE STRENGTH IN PREDICTING PRE-POST-WALKING RESPONSES (OBJECTIVES 3 & 4)

This chapter presents the results related to Objectives 3 and 4 and a discussion of the key findings. The two main hypotheses were that 1) females with radiographic medial compartment knee OA would have significantly greater increases in pain intensity, pain sensitization, KAM magnitude, muscle activity magnitude and prolonged muscle activity responses, and decreases in the KFM-KEM difference measure than males (**Hypothesis 3**) and 2) muscle strength would explain significant variance in pain intensity, pain sensitization, the KFM-KEM difference measure, KE and KF muscles overall activity magnitude and prolonged activity responses (**Hypothesis 4).**

5.1 RESULTS

Forty-five participants (22 males, 23 females) with evidence of medial compartment radiographic knee OA were included in this study. Twenty-four participants (53%) reported OA symptoms (12 males, 12 females). The descriptive statistics for muscle strength, demographic, and clinical characteristics are presented in Tables 4.1 and 4.2 for each sex group. One symptomatic female participant did not complete strength testing and was excluded from muscle activation pre-post-walk response analyses only. Post-walk gait analysis for one asymptomatic female could not be processed, resulting in this participant's data being excluded from knee joint moment pre-post-walk response analyses only. There was no statistically significant difference between males and

females in pre-walk (M = 1.3 ± 0.2 m/s, F = 1.2 ± 0.2 m/s, p=0.143) or post-walk (M = 1.3 ± 0.2 m/s, F = 1.2 ± 0.2 m/s, p=0.200) over-ground walking speeds (OG1, OG2), or between OG1 and OG2 walking speeds (OG1 = 1.3 ± 0.2 m/s, OG2 = 1.3 ± 0.2 m/s, p=0.818). For the 30-minute walking intervention stimulus, there was no significant difference between males and females in average treadmill walking speed (M = 1.2 ± 0.2 m/s, F = 1.1 ± 0.3 m/s, p=0.068) or in the number of steps taken over the 30 minutes (M = 3272 ± 306 steps, F = 3318 ± 432 steps, p=0.678).

5.1.1 Pre-Post-Walk Pain Response Measures (Objective 3)

Descriptive statistics for pre- and post-walk pain intensity and pain sensitization by sex are found in Table 5.1. Post-hoc results are presented in Table 5.4. The knee joint PPT did not meet the assumptions of normality and homogeneity of variance, the VM PPT did not meet the assumption of homogeneity of variance, and the ECRL did not meet the assumption of normality. There were no outliers for any PPT measures. Data for each PPT measure was square root transformed, after which all PPT measures met all ANOVA assumptions. The transformations did not change the significant effects from the non-transformed data, and the p-values from the ANOVAs on the transformed data are presented in Appendix 6. Transformations change the values of the data and given that this then makes values difficult to interpret, only the non-transformed results will be presented and discussed.

There was a statistically significant sex by time interaction effect (p<0.1) for the knee joint PPT as illustrated graphically in Figure 5.1 capturing a moderate post-walk decrease in males (partial η^2 = 0.072), and a large increase in females in knee joint PPT

(partial $\eta^2 = 0.173$), i.e., increased, and decreased pain sensitization respectively (see Tables 5.1 and 5.4). Post-hoc analysis revealed that males had significantly ($p<0.05$) higher knee joint PPTs than females at both pre-walk and post-walk time points, and post-walk females had significantly higher PPTs than pre-walk females (see Figure 5.1 and Table 5.4). There was a significant sex main effect ($p<0.1$) for the VM and ECRL PPTs, where females had lower PPTs than males as illustrated in Figure 5.2. For pain intensity, there was no significant difference ($p>0.1$) in the proportion of males and females who increased (≥1 point increase) (M=27%, F=26%) versus those who did not change (M=73%, F=74%) their NPRS post-walk scores ($\chi^2=0.928$). NPRS scores did not meet the assumption of normality and could not be transformed but ANOVAs were still conducted, and statistics were carefully interpreted based on descriptive data (i.e., means and SD). NPRS scores showed a significant time effect ($p<0.1$) with greater scores post-walk compared to pre-walk.

Table 5.1: Pre-post-walk pain intensity and pain sensitization responses to a 30-minute walk for males and females with radiographic knee OA presented as mean (standard deviation) and *p*-value

Pre-post-walk pain response measure	Pre-Walk		Post-Walk		Sex Effect	Time Effect	Interaction Effect
	Male (n=22)	Female (n=23)	Male (n=22)	Female (n=23)	*P*-value	*P*-value	*P*-value
NPRS (/10)	0.4 (1.0)	0.7 (1.0)	0.7 (1.6)	1.0 (1.6)	0.420	**0.030**	0.920
Knee PPT (kgf/cm^2)	3.59 (2.03)	1.82 (1.15)	3.39 (1.81)	2.03 (1.44)	**0.002**	0.940	**0.030**
VM PPT (kgf/cm^2)	3.94 (2.05)	1.97 (0.96)	4.06 (2.31)	2.19 (1.13)	**<0.001**	0.182	0.700
ECRL PPT (kgf/cm^2)	2.21 (1.24)	1.49 (0.79)	2.15 (1.29)	1.56 (0.84)	**0.039**	0.945	0.375

Bold = Statistically significant difference ($p<0.1$)

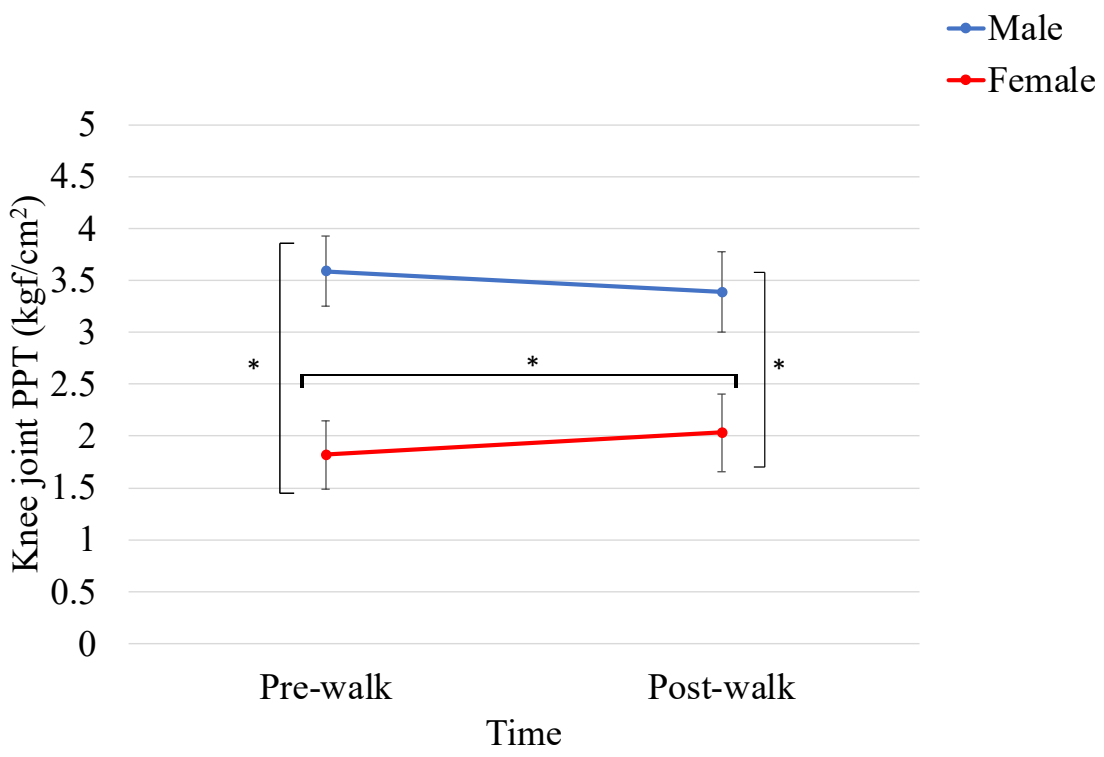

Figure 5.1. Interaction plot for the knee joint pressure pain threshold (PPT) at two time points (pre-walk, post-walk) separated by sex (males = blue, females = red). * Males had significantly higher knee joint PPTs than females at both pre-walk and post-walk time points and post-walk females had a significantly higher knee joint PPT than pre-walk females (p<0.05). Error bars indicate $\pm$ 1 standard error of the mean.

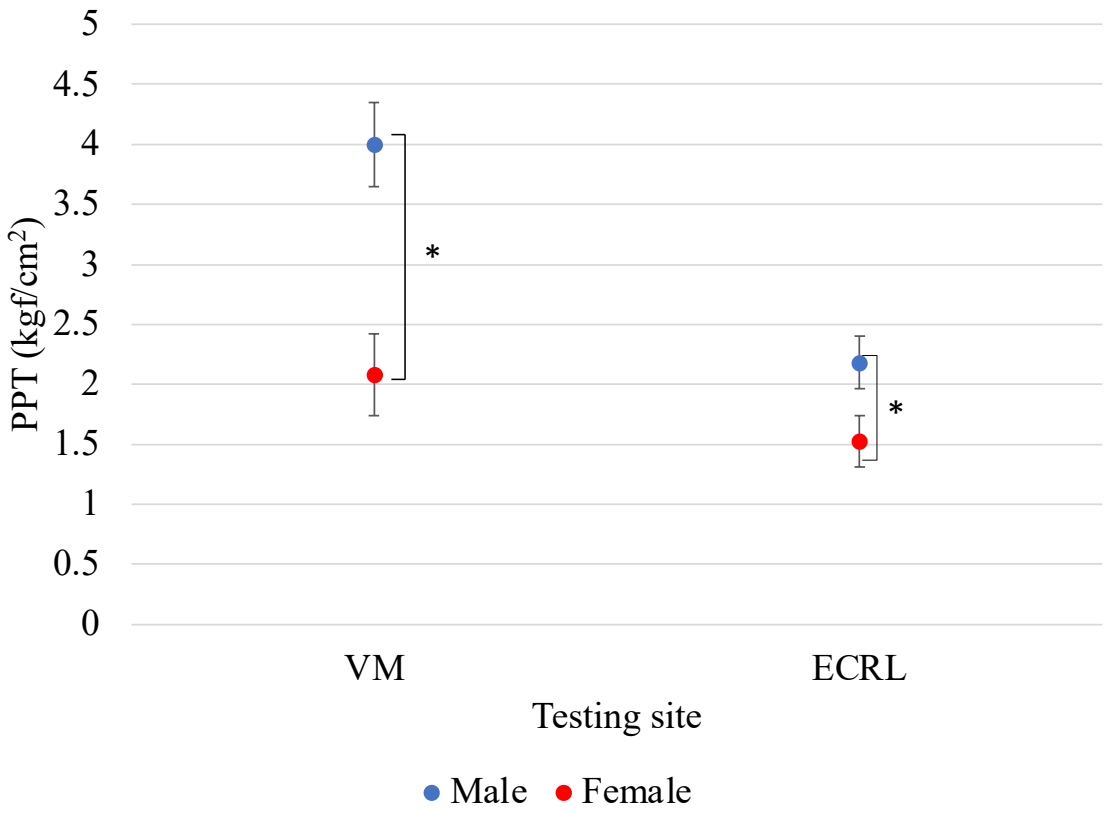

Figure 5.2. Sex main effect plot for the pressure pain thresholds (PPTs) for the vastus medialis and extensor carpi radialis longus PPT separated by sex (males = blue, females = red). * Males had significantly higher PPTs at both testing sites (p<0.1). Error bars indicate ± 1 standard error of the mean.

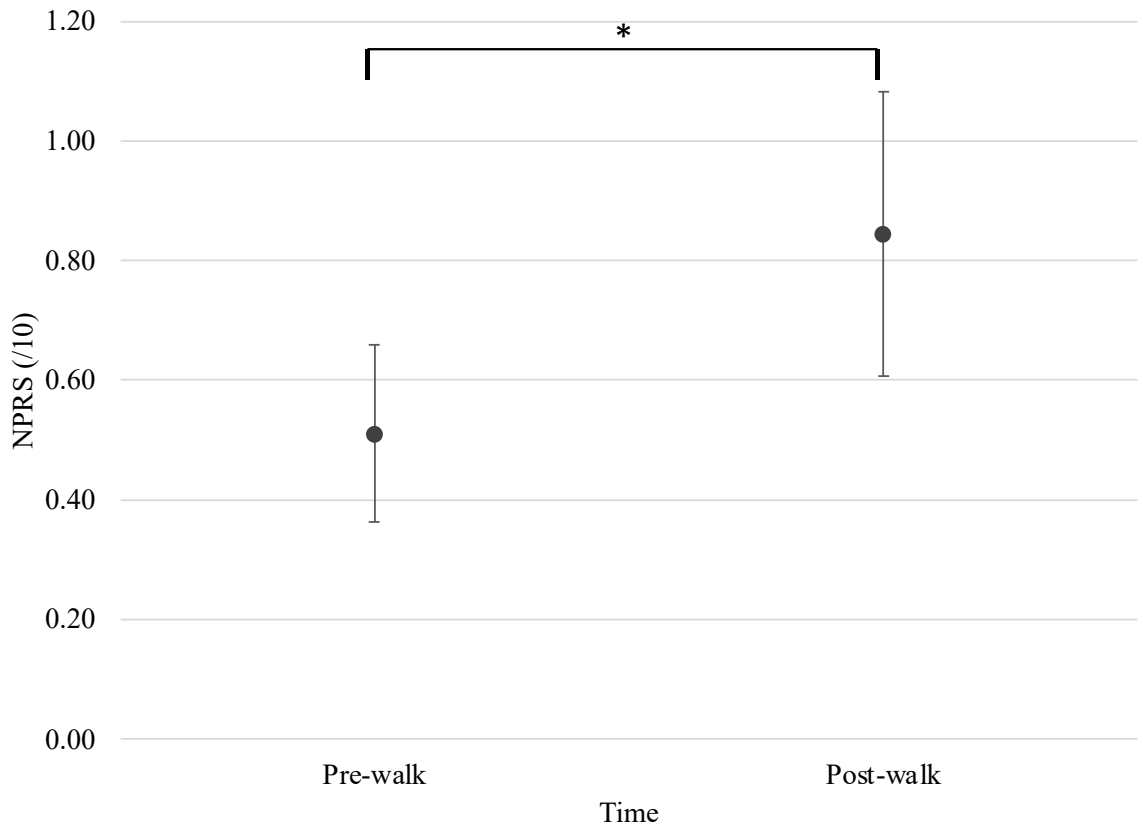

Figure 5.3. Time main effect plot for the Numeric Pain Rating Scale (NPRS) score at two time points (pre-walk, post-walk). * Post-walk NPRS scores were significantly higher than pre-walk NPRS scores (p<0.1). Error bars indicate $\pm$ 1 standard error of the mean.

5.1.2 Pre-Post-Walk Knee Joint Moment Response Measures (Objective 3)

Descriptive statistics for pre- and post-walk knee joint moment data by sex are found in Table 5.2. Post-hoc results are presented in Table 5.4. Ensemble average waveforms for the KAM and KFM are illustrated in Figure 5.4. Mixed model ANOVAs were run on all knee joint moment features. The KAM impulse and KFM late-stance peak extension did not meet the assumptions of homogeneity of variances and covariances, and the assumption of normality respectively. The raw data were transformed for the KAM impulse and the outliers were removed for the KFM late stance to meet the assumptions, and the ANOVAs were rerun on the transformed data. The transformations did not change the significant effects for the KAM impulse results but did result in a significant sex main effect for the KFM late stance peak extension not found in the non-transformed data. The *p*-values from the ANOVAs on the transformed data are presented in Appendix 7. For ease of interpretation, the non-transformed results will be presented and discussed in this chapter.

The results for the frontal plane knee joint moments showed a statistically significant sex by time interaction ($p<0.1$) for the KAM impulse where males had a medium increase (partial $\eta^2 = 0.068$), and females a medium decrease post-walk (partial $\eta^2 = 0.076$), as illustrated in Figures 5.4 and 5.5 and indicated in Tables 5.2 and 5.4. There was a significant sex by time interaction ($p<0.1$) for the KAM 1st peak and KAM 1st peak to mid-stance difference as illustrated in Figures 5.4, 5.6 and 5.7 where males had a large post-walk increase in KAM 1st peak (partial $\eta^2 = 0.438$) and in 1st peak to mid-stance difference (partial $\eta^2 = 0.351$), whereas females had a small post-walk decrease in KAM 1st peak (partial $\eta^2 = 0.012$), and a small increase in the KAM

difference measure (partial $\eta^2 = 0.014$) (Table 5.4). Post-hoc analysis revealed that post-walk males had significantly ($p<0.05$) higher KAM 1st peak and 1st peak to mid-stance difference compared to pre-walk males as illustrated in Figures 5.6, and 5.7 and indicated in Table 5.4.

There was a statistically significant time main effect ($p<0.1$) for the KFM-KEM difference with moderately higher values post-walk than pre-walk (partial $\eta^2 = 0.073$), as shown in Figures 5.4 and 5.8. This increase in the KFM difference measure post-walk seemed to be mainly a result of the KFM peak, which also had a significant time main effect ($p<0.1$) and a large overall increase post-walk (partial $\eta^2 = 0.123$) (see Figure 5.4 and 5.8).

Table 5.2: Pre-post-walk knee joint moment discrete metric responses to a 30-minute walk for males and females with radiographic knee OA presented as mean (standard deviation) and *p*-value

Pre-post-walk knee joint moment response measure	Pre-Walk		Post-Walk		Sex Effect	Time Effect	Interaction Effect
	Male (n=22)	Female (n=22)	Male (n=22)	Female (n=22)	*P*-value	*P*-value	*P*-value
Primary moments							
KAM impulse (Nm/kg*s)	0.21 (0.07)	0.20 (0.04)	0.21 (0.07)	0.19 (0.04)	0.316	0.744	**0.086**
KFM-KEM difference (Nm/kg)	0.92 (0.32)	0.75 (0.31)	0.93 (0.34)	0.79 (0.36)	0.117	**0.075**	0.266
Secondary moments							
KAM 1st peak (Nm/kg)	0.55 (0.13)	0.56 (0.13)	0.59 (0.14)	0.55 (0.13)	0.671	**0.007**	**0.001**
KAM 1st peak to mid-stance minimum difference (Nm/kg)	0.26 (0.16)	0.25 (0.13)	0.30 (0.17)	0.25 (0.14)	0.529	**0.005**	**0.027**
KFM peak (Nm/kg)	0.66 (0.27)	0.57 (0.22)	0.67 (0.28)	0.61 (0.26)	0.330	**0.019**	0.124
KEM late stance peak (Nm/kg)	-0.27 (0.19)	-0.19 (0.16)	-0.26 (0.21)	-0.18 (0.17)	0.141	0.427	0.546

Bold = Statistically significant difference ($p<0.1$)

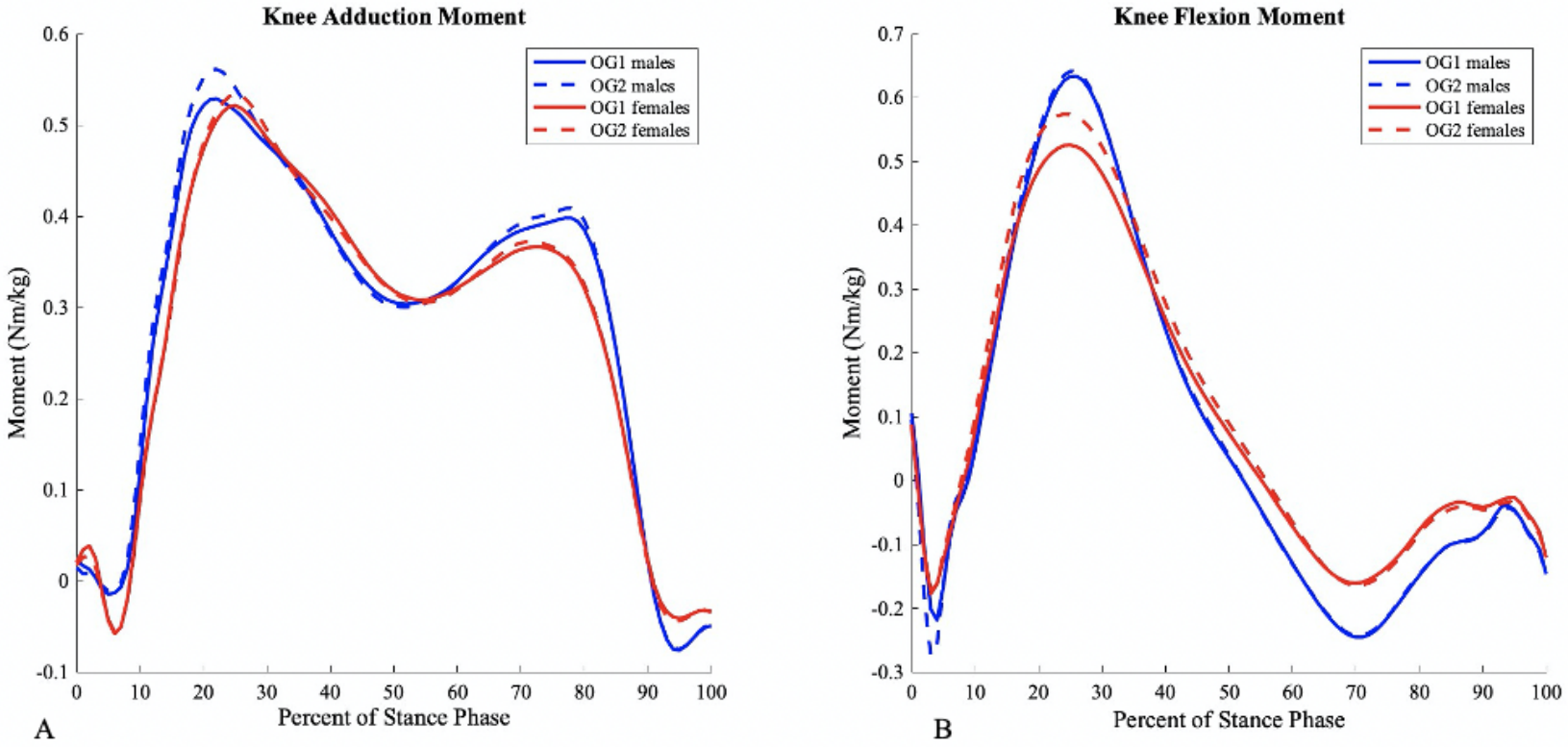

Figure 5.4. Ensemble average waveforms. Knee joint moments (amplitude normalized to body mass) during gait include the knee adduction moment (A) and knee flexion moment (B) by time (solid = over-ground walk 1, dashed = over-ground walk 2) and sex (red = females, blue = males). There was a sex by time interaction (p<0.1) for the KAM impulse, KAM 1st peak, and KAM 1st peak to mid-stance minimum difference and a time main effect (p<0.1) for the KFM-KEM difference and KFM peak.

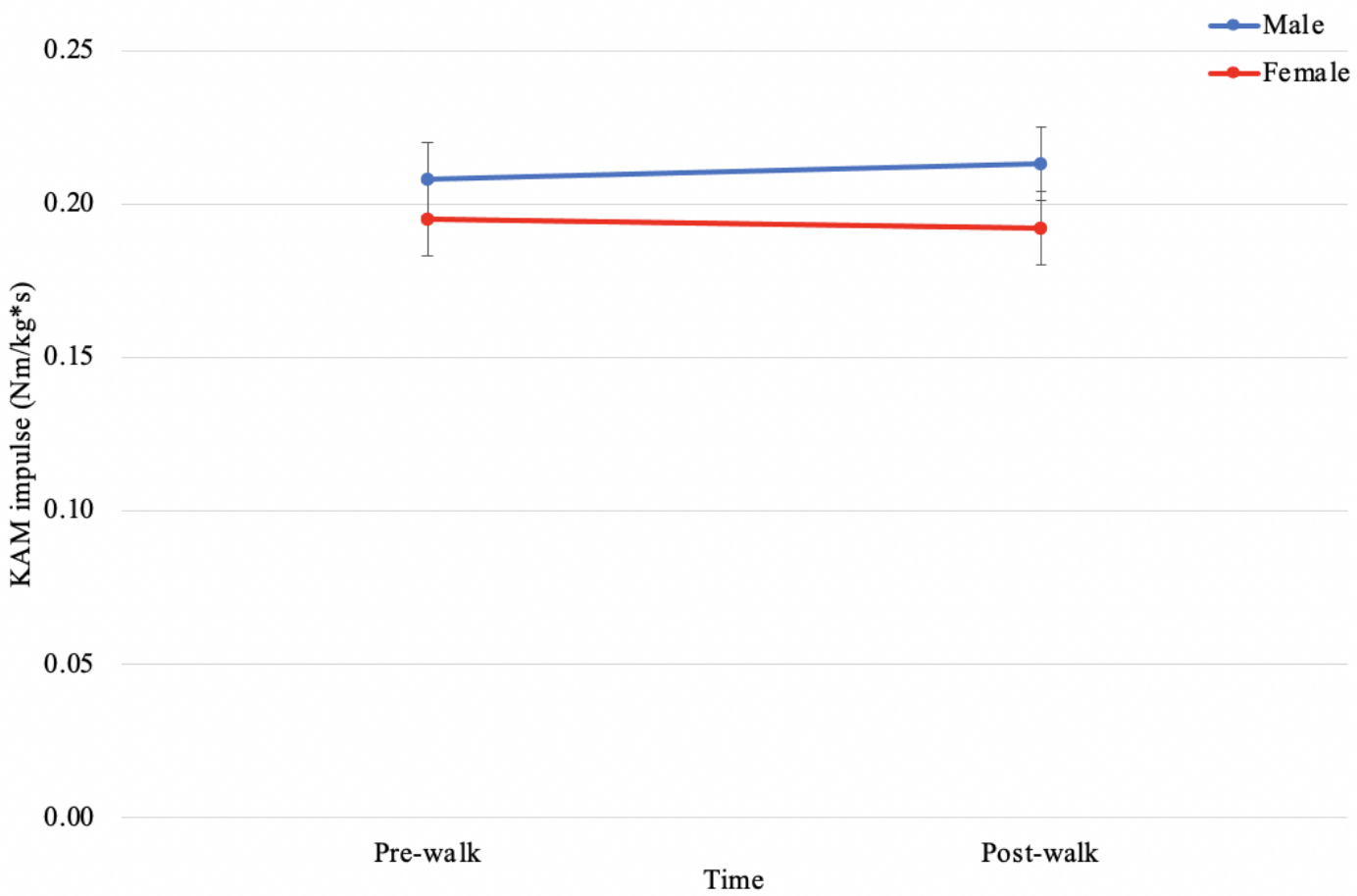

Figure 5.5. Interaction plot for the knee adduction moment (KAM) impulse at two time points (pre-walk, post-walk) separated by sex (males = blue, females = red). Error bars indicate $\pm$ 1 standard error of the mean.

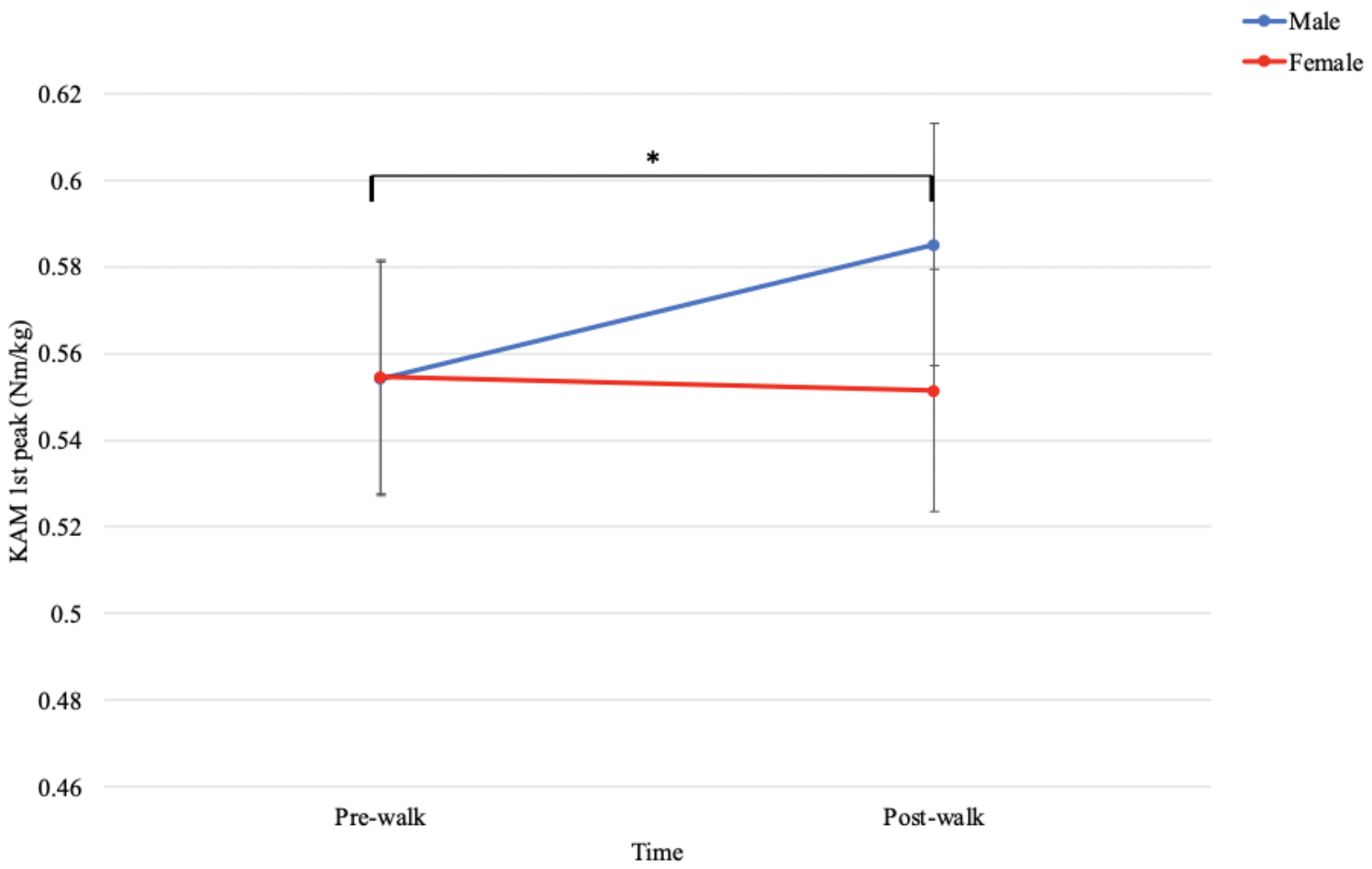

Figure 5.6. Interaction plot for the knee adduction moment (KAM) 1st peak at two time points (pre-walk, post-walk) separated by sex (males = blue, females = red). * Post-walk males had a significantly higher KAM 1st peak than pre-walk males (p<0.05). Error bars indicate ± 1 standard error of the mean.

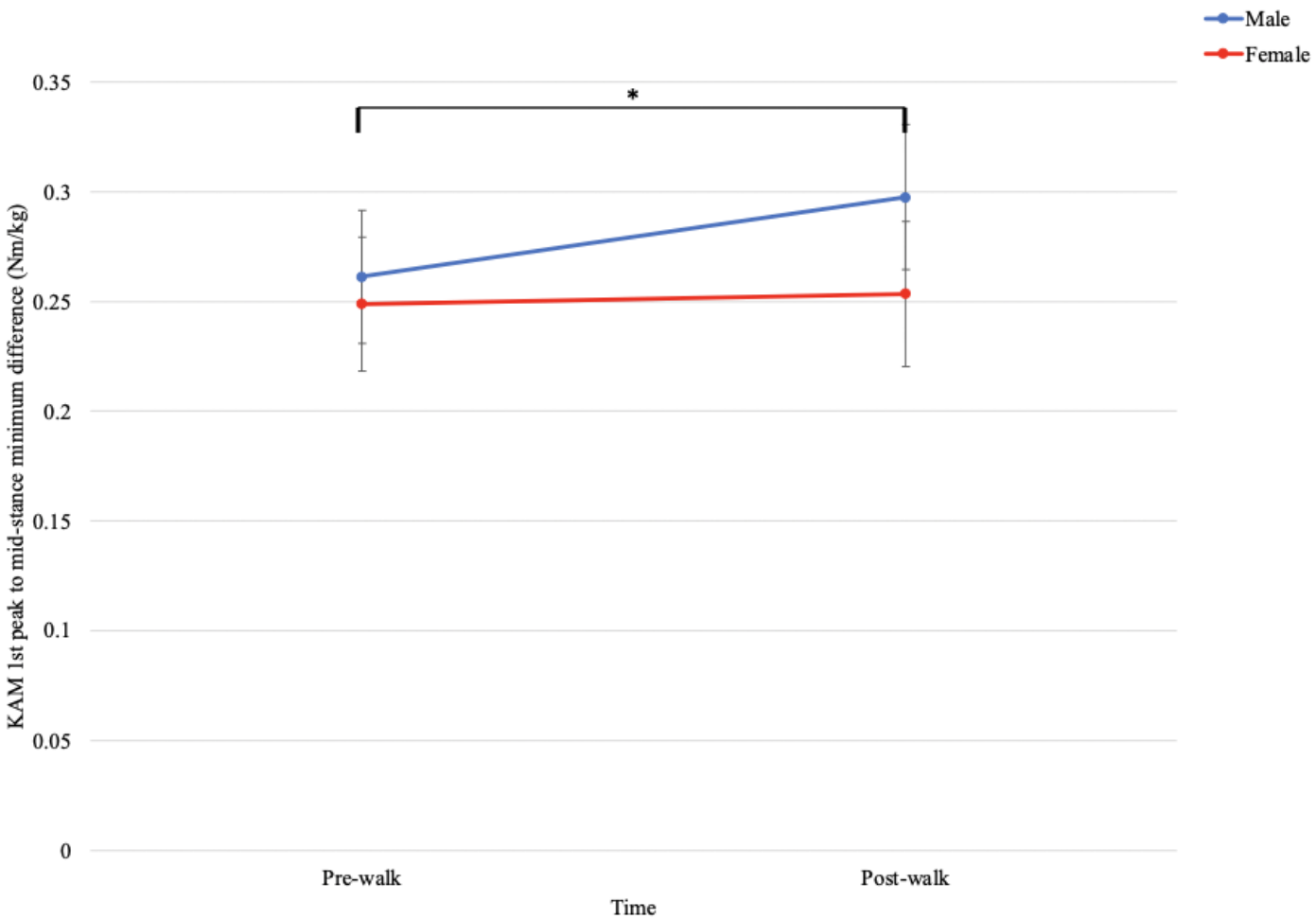

Figure 5.7. Interaction plot for the knee adduction moment (KAM) 1st peak to mid-stance minimum difference at two time points (pre-walk, post-walk) separated by sex (males = blue, females = red). * Post-walk males had a significantly higher KAM 1st peak to mid-stance minimum difference than pre-walk males ($p<0.05$). Error bars indicate ± 1 standard error of the mean.

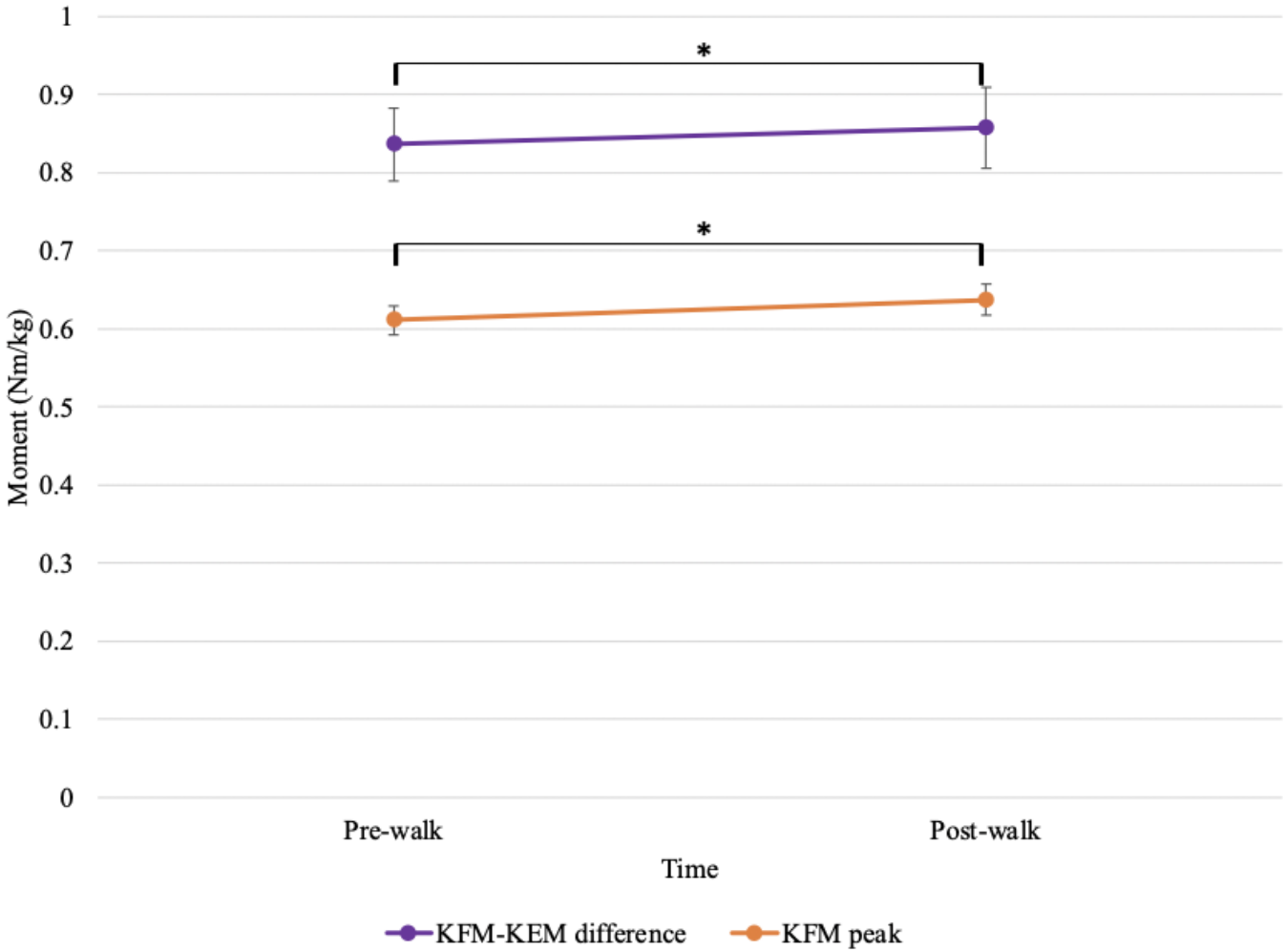

Figure 5.8. Time main effect plot for the knee flexion moment-knee extension moment (KFM-KEM) difference and the KFM peak at two time points (pre-walk, post-walk). * The KFM-KEM difference and KFM peak had significant main time effects (p<0.1). Error bars indicate ± 1 standard error of the mean.

5.1.3 Pre-Post-Walk Muscle Activation Response Measures (Objective 3)

Descriptive statistics for pre- and post-walk muscle activation measures by sex are found in Table 5.3. Post-hoc results are presented in Table 5.4. Ensemble average waveforms for all muscles are illustrated in Figure 5.9. Nine out of the ten muscle activation measures (all but VL PC2) did not meet the ANOVA assumptions. For these nine measures, either the data was transformed, or outliers were removed resulting in seven of the nine muscle activation measures meeting the assumptions. The ANOVAs were performed on the non-transformed and the transformed/outlier-removed data. For comparative purposes, the results for the two analyses are presented in Appendix 8. The statistical significance of the ANOVAs on transformed or outlier-removed data did not differ from the non-transformed data for all variables except for the VM PC1 interaction effect (see Appendix 8). Only the non-transformed data will be presented in the tables and figures.

There was a statistically significant sex by time interaction (p<0.1) for the VL PC1 (i.e., overall activation magnitude) (Figure 5.10) and MH PC2 (i.e., prolonged activity) (Figure 5.11) scores (Table 5.3). Males had a greater decrease in VL PC1 scores than females as illustrated in Figures 5.9 and 5.10, though both had large decreases post-walk (M: partial η^2= 0.465, F: partial η^2 = 0.333) (Tables 5.3 and 5.4). Males had a medium decrease (partial η^2 = 0.108) whereas females had a small increase (partial η^2 = 0.036) in MH PC2 scores post-walk, meaning that males moved towards less prolonged activity, whereas females moved towards more prolonged activity as shown in Figures 5.9 and 5.11 and indicated in Tables 5.3 and 5.4. Post-hoc analysis revealed that post-walk, males had significantly (p<0.05) lower VL PC1 scores (i.e., overall magnitude)

than females and that both males and females post-walk had lower VL PC1 scores than their pre-walk scores (see Figure 5.10 and Table 5.4).

There was a significant time effect (p<0.1) for VM, RF, LH, and MH PC1 scores between pre- and post-walk, with larger decreases in scores post-walk (partial η^2: VM = 0.309, RF = 0.228, LH = 0.150, MH = 0.303), meaning lower overall activity post-walk (see Figure 5.12). The magnitude of decreases ranged from 2-5% stance-phase RMS amplitude as illustrated in Appendix 9. There was a significant time effect for LH PC2 scores with a medium decrease in scores post-walk (partial η^2 = 0.077), meaning less prolonged activation post-walk (see Figure 5.12). There was a significant sex main effect (p<0.1) for VM and RF PC1 scores between sexes with medium to large sex differences (partial η^2: VM = 0.100, RF = 0.254) where females had higher scores than males, meaning higher overall activation magnitude (see Figure 5.13).

Table 5.3: Pre-post-walk knee extensor and knee flexor muscle activation principal component (PC) score responses to a 30-minute walk for males and females with radiographic knee OA presented as mean (standard deviation) and *p*-value

Pre-post-walk muscle activation response measure	Pre-Walk		Post-Walk		Sex Effect	Time Effect	Interaction Effect
	Male (n=22)	Female (n=22)	Male (n=22)	Female (n=22)	*P*-value	*P*-value	*P*-value
Knee extensors							
VLPC1	165.0 (89.6)	223.3 (141.2)	130.4 (71.0)	206.1 (132.9)	**0.052**	**<0.001**	**0.080**
VLPC2	-27.3 (31.9)	-22.3 (41.7)	-26.3 (30.1)	-19.1 (39.4)	0.568	0.340	0.595
VMPC1	146.3 (74.1)	222.1 (171.6)	121.5 (56.8)	209.1 (160.2)	**0.039**	**<0.001**	0.181
VMPC2	-30.7 (30.9)	-24.0 (52.6)	-29.3 (28.1)	-22.5 (43.5)	0.574	0.475	0.981
RFPC1	80.8 (58.0)	179.3 (112.5)	73.2 (43.9)	161.1 (96.7)	**<0.001**	**0.001**	0.154
RFPC2	13.3 (31.2)	19.7 (47.2)	9.6 (24.4)	15.3 (30.4)	0.547	0.174	0.903
Knee flexors							
LHPC1	168.3 (102.9)	232.5 (181.2)	148.1 (93.7)	221.4 (185.1)	0.125	**0.009**	0.443
LHPC2	-10.0 (67.2)	-13.5 (61.7)	-24.8 (52.5)	-15.4 (78.5)	0.878	**0.068**	0.154
MHPC1	162.8 (73.9)	207.5 (171.8)	138.2 (65.4)	190.7 (160.6)	0.210	**< 0.001**	0.423
MHPC2	-46.1 (54.2)	-54.7 (61.7)	-52.5 (52.9)	-50.4 (63.1)	0.852	0.732	**0.095**

Bold = Statistically significant difference ($p<0.1$)

PC1: A higher score indicates greater overall muscle activity magnitude

PC2: A more positive score indicates greater mid-stance compared to early-stance muscle activation amplitude (i.e., more prolonged activation)

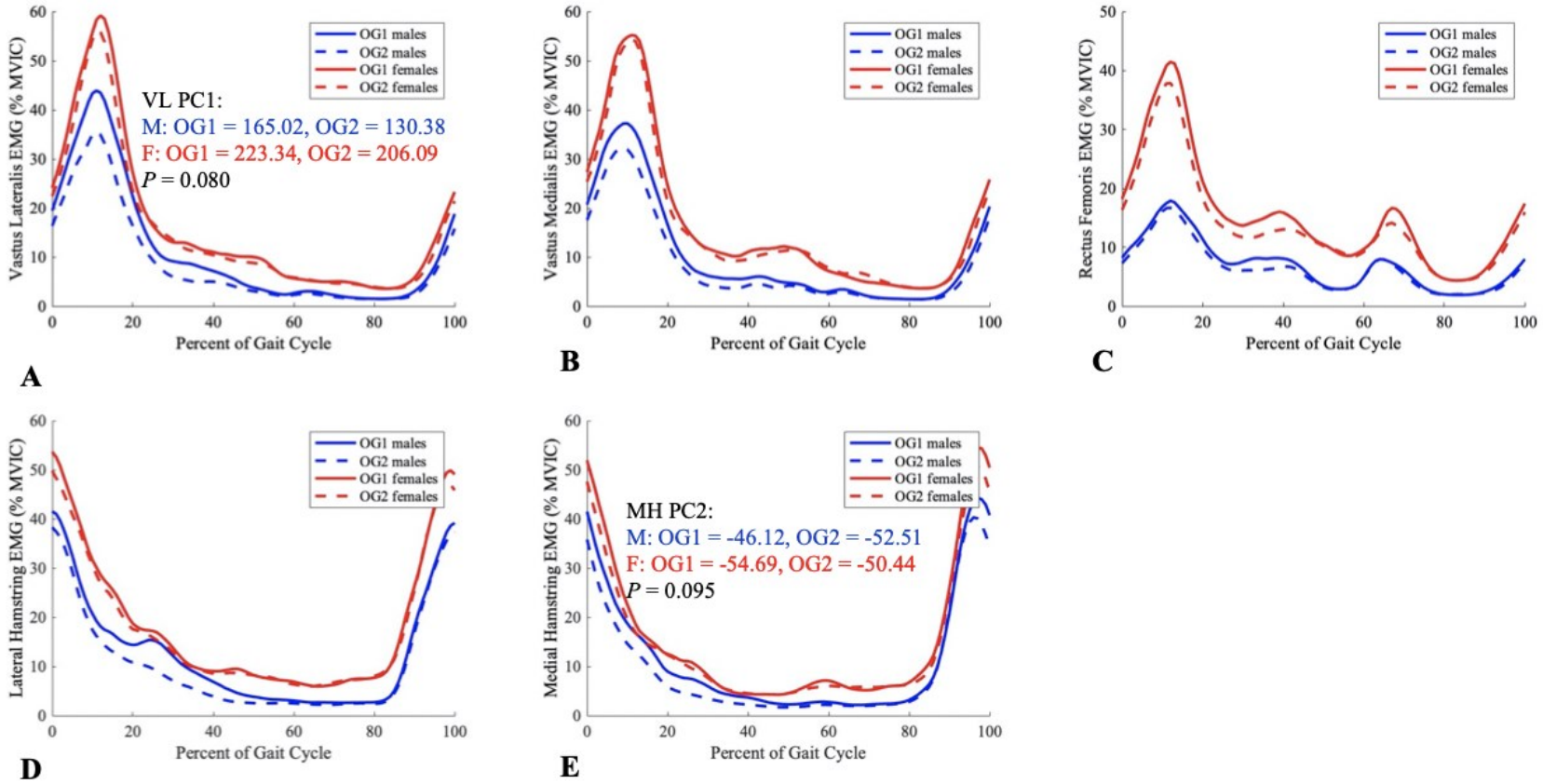

Figure 5.9. Ensemble average waveforms. Electromyography patterns (amplitude normalized to percent maximum voluntary isometric contraction) during gait for the knee extensor (A-C) and knee flexor (D, E) muscles by time (solid = over-ground walk 1, dashed = over-ground walk 2) and sex (red = females, blue = males). There was a significant sex by time interaction (p<0.1) for the vastus lateralis overall activation magnitude (A) and the medial hamstring prolonged activation (E). A higher PC1 score indicates greater overall muscle activity magnitude and a higher PC2 score indicates greater mid-stance compared to early-stance muscle activation amplitude (i.e., more prolonged activation).

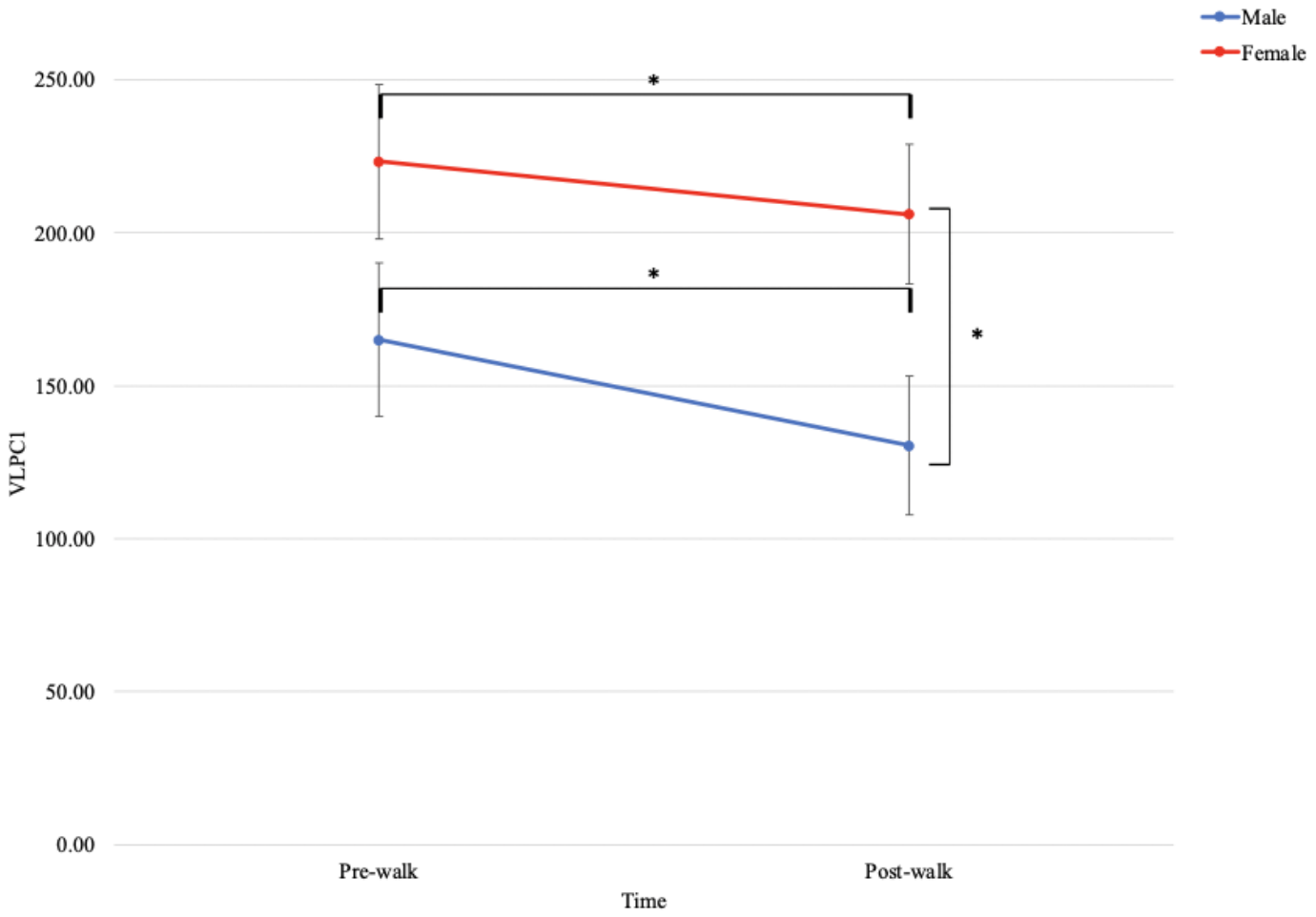

Figure 5.10. Interaction plot for the vastus lateralis overall activation magnitude scores (VL PC1) at two time points (pre-walk, post-walk) separated by sex (males = blue, females = red). * Post-walk males had a significantly lower VL overall activation magnitude than post-walk females, and both post-walk males and females had significantly lower VL overall activation magnitude than pre-walk males and females (p<0.05). Error bars indicate ± 1 standard error of the mean.

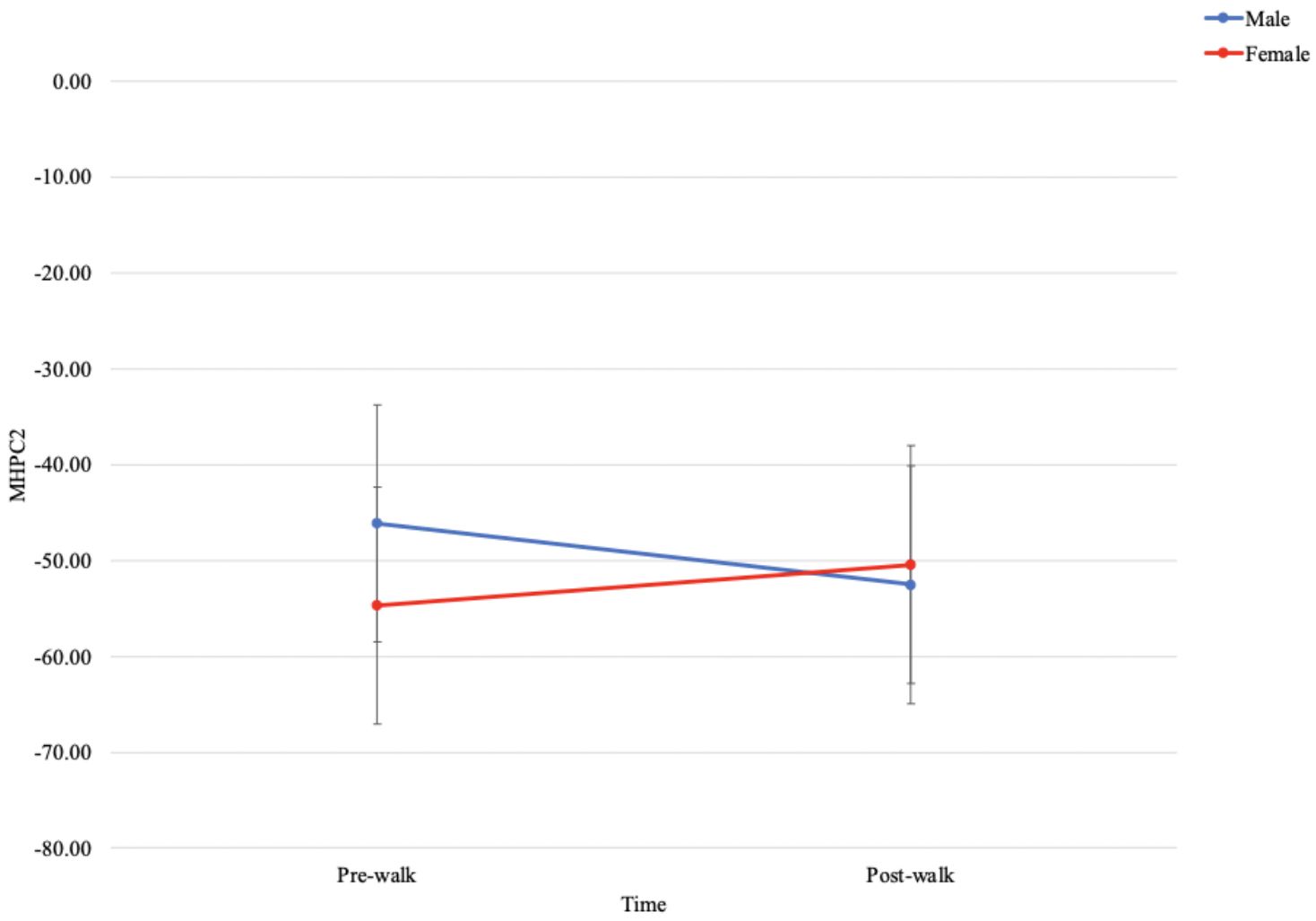

Figure 5.11. Interaction plot for the medial hamstring prolonged activation scores (MHPC2) at two time points (pre-walk, post-walk) separated by sex (males = blue, females = red). Error bars indicate ± 1 standard error of the mean.

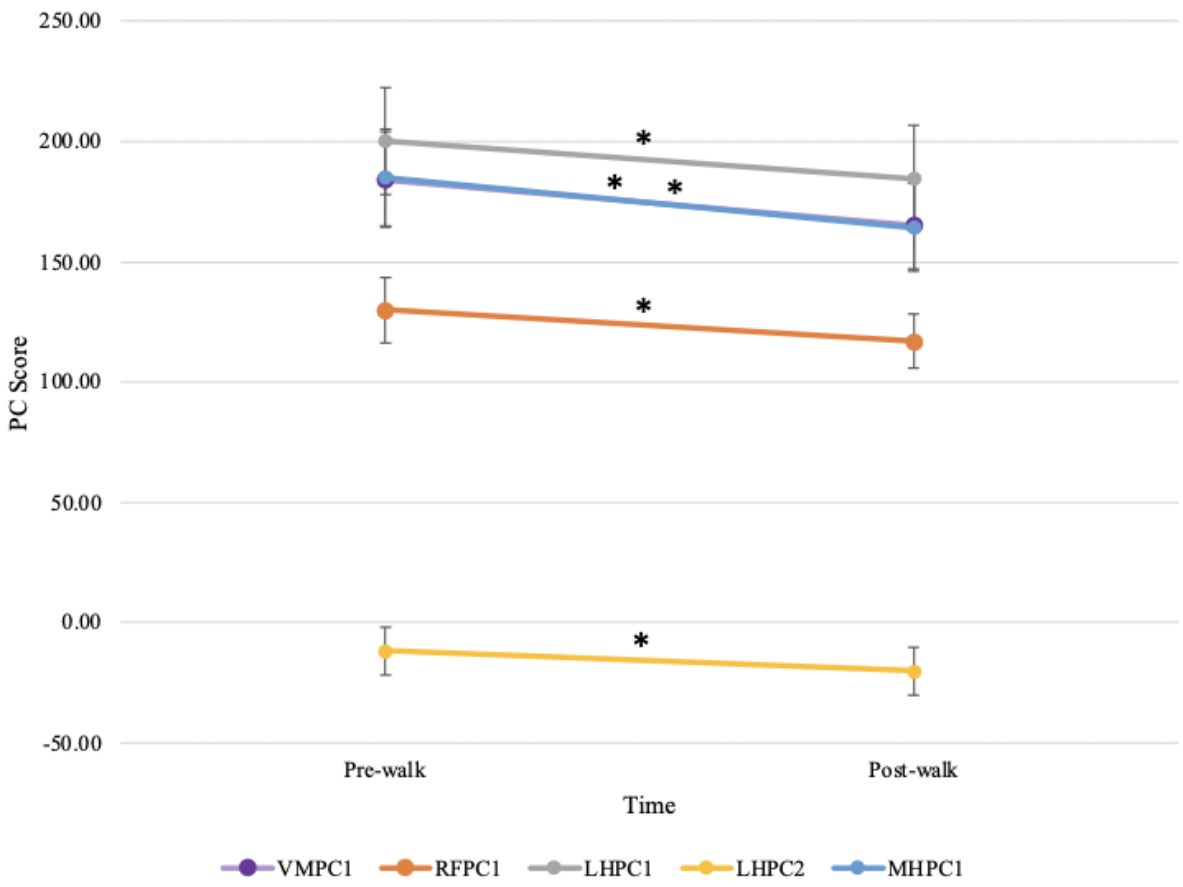

Figure 5.12. Time main effect plot for muscle activation scores with significant time main effects (p<0.1) including the vastus medialis, rectus femoris, lateral hamstring, and medial hamstring overall activation (VM PC1, RF PC1, LH PC1, MH PC1) and the lateral hamstring prolonged activation (LH PC2) scores at two time points (pre-walk, post-walk). * Overall activation magnitude and prolonged activation scores were higher pre-walk than post-walk for all muscles. Error bars indicate ± 1 standard error of the mean.

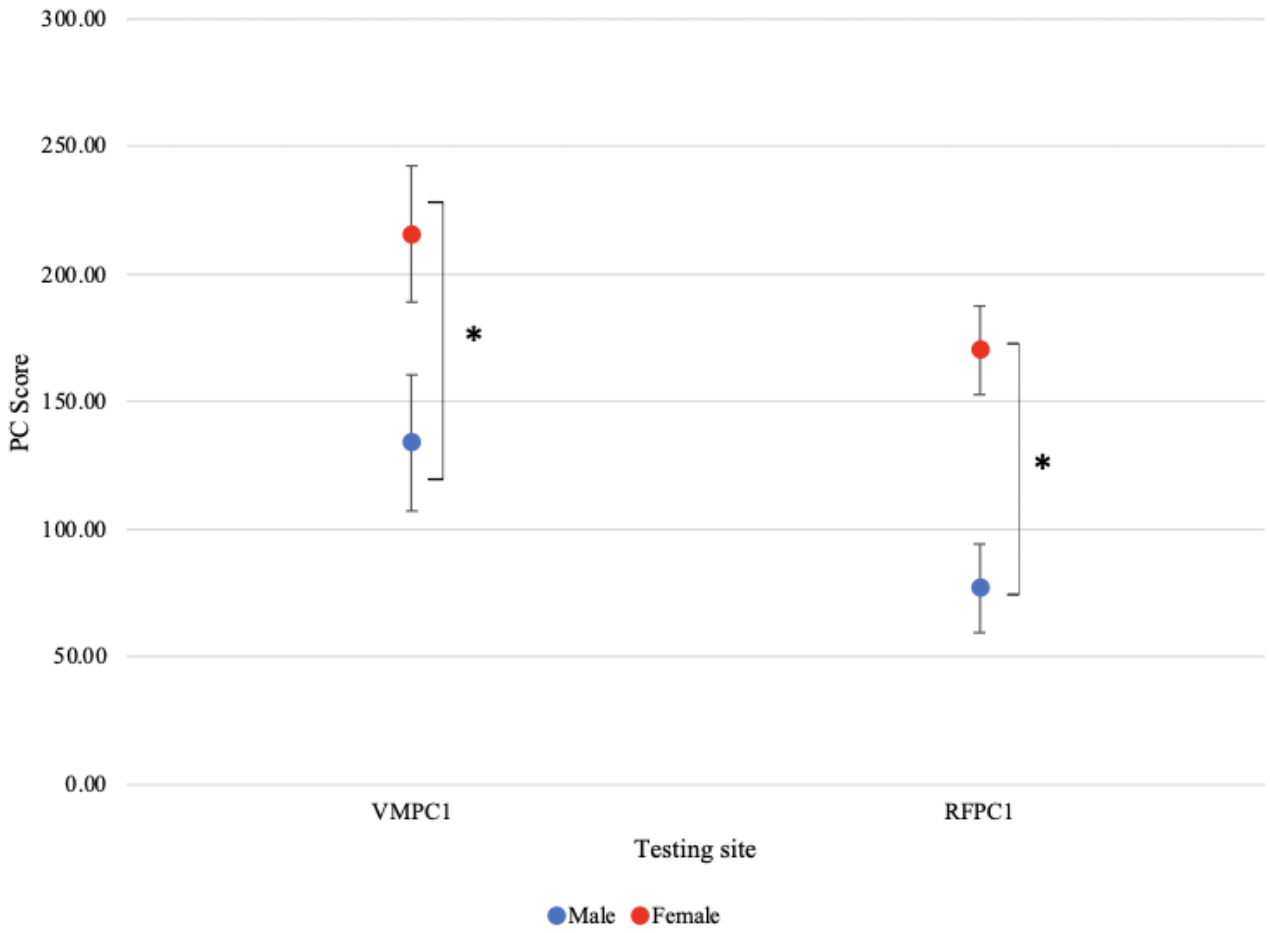

Figure 5.13. Sex main effect plot for muscle activation scores with significant sex main effects (p<0.1) including the vastus medialis and rectus femoris overall activation scores (VM PC1, RF PC1) separated by sex (males = blue, females = red). * Females had significantly higher VL and RF muscle activation magnitude scores than males. Error bars indicate ± 1 standard error of the mean.

Table 5.4: Post-hoc results for pre-post-walk response measures with significant sex by time interactions separated by sex (males, females) and time (pre-walk, post-walk) presented as *p*-value and partial η^2

Response measure	a) pre-walk: males versus females		b) post-walk: males versus females		c) males: pre- versus post-walk		d) females: pre- versus post-walk	
	P-value	Partial η^2	*P*-value	Partial η^2	*P*-value	Partial η^2	*P*-value	Partial η^2
Knee PPT (kg/cm^2)	**< 0.001**	0.233	**0.008**	0.152	0.217	0.072	**0.043**	0.173
KAM impulse (Nm/kg*s)	0.435	0.015	0.228	0.034	0.228	0.068	0.202	0.076
KAM 1st peak (Nm/kg)	0.994	< 0.001	0.408	0.016	**< 0.001**	0.438	0.622	0.012
KAM 1st peak to mid-stance minimum difference (Nm/kg)	0.775	0.002	0.352	0.021	**0.003**	0.351	0.593	0.014
VLPC1	0.109	0.06	**0.023**	0.117	**<0.001**	0.465	**0.004**	0.333
MHPC2	0.627	0.006	0.907	<0.001	0.127	0.108	0.383	0.036

Bold = Statistically significant difference ($p<0.05$)

Partial η^2 = Measure of effect size where 0.01 = small, 0.06 = medium, 0.12 = large

5.1.4 Linear Regression Models for Pre-Post-Walk Response Measures (Objective 4)

Correlations between body mass normalized KE and KF muscle strength and the pre-post-walk response score (i.e., post-walk score – pre-walk score) of the six response variables with a significant sex by time interaction (i.e., knee joint PPT, KAM impulse, KAM 1st peak, KAM 1st peak to mid-stance minimum difference, VL PC1 and MH PC2) are found in Table 5.5 for the total group and Tables 5.6 and 5.7 for the males and females separately. The linear regression models for these variables with significant correlations are presented in Table 5.8. Scatterplots and lines of best fit for each model are presented in Figures 5.14, 5.15, and 5.16.

For the total group, there was one significant correlation in Table 5.5. This was a significant positive correlation between normalized KF strength and KAM 1st peak pre-post-walk response score for the total group ($r = 0.34$). The scatterplot for this model is presented in Figure 5.14 and the linear regression analysis showed that while significant, normalized KF muscle strength explained 11% of the variance in the KAM 1st peak pre-post-walk response score (Table 5.8). There were no other models for the total group.

When males and females were analyzed separately, there were three significant correlations for males (Table 5.6) and one for females (Table 5.7) between normalized muscle strength and pre-post-walk response scores. For the males, there was a significant positive correlation between normalized KE muscle strength and MH PC2 pre-post-walk response scores ($r = 0.49$), normalized KF strength and MH PC2 pre-post-walk response scores ($r = 0.46$), and normalized KF strength and VL PC1 pre-post-walk response scores ($r = 0.61$). Scatterplots for these models are presented in Figure 5.15 and the linear regression analysis showed that normalized KE strength explained 24% of the variance in

the MH PC2 pre-post-walk response score and normalized KF muscle strength explained 21% and 38% of the variance in the MH PC2 and VL PC1 pre-post-walk response scores respectively.

For the females, there was a significant positive correlation between normalized KF strength and knee joint PPT pre-post-walk response scores (r = 0.66). The scatterplot for this model is presented in Figure 5.16 and the linear regression analysis showed that normalized KF strength explained 44% of the variance in the knee joint PPT pre-post-walk response score.

Table 5.5. Pearson's correlations between muscle strength and pre-post-walk response scores for the total group

	Knee joint PPT	**KAM impulse**	**KAM 1st peak**	**KAM 1st peak to mid-stance minimum difference**	**VLPC1**	**MHPC2**
KE strength normalized	0.02	0.22	0.13	0.02	0.19	-0.02
KF strength normalized	0.09	0.21	**0.34**	0.23	0.24	-0.03

Bold: Statistically significant correlation ($p<0.05$)

Table 5.6. Pearson's correlations between muscle strength and pre-post-walk response scores for males

	Knee joint PPT	KAM impulse	KAM 1st peak	KAM 1st peak to mid-stance minimum difference	VLPC1	MHPC2
KE strength normalized	0.08	0.36	-0.06	-0.30	0.40	**0.49**
KF strength normalized	0.22	0.30	0.23	-0.03	**0.61**	**0.46**

Bold: Statistically significant correlation ($p<0.05$)

Table 5.7. Pearson's correlations between muscle strength and pre-post-walk response scores for females

	Knee joint PPT	KAM impulse	KAM 1st peak	KAM 1st peak to mid-stance minimum difference	VLPC1	MHPC2
KE strength normalized	0.39	-0.31	-0.15	0.00	0.29	-0.27
KF strength normalized	**0.66**	-0.42	-0.16	0.10	0.28	-0.22

Bold: Statistically significant correlation ($p<0.05$)

Table 5.8 Linear regression models for pre-post-walk response scores with significant correlations with normalized muscle strength

Strength variable	Pre-post-walk response score variable	Sex Group	Constant	β coefficient	Standardized β coefficient	R^2	*p*-value
KF strength normalized	KAM 1st peak	Total	-0.01	0.05	0.34	0.11	0.027
KE strength normalized	MH PC2	Males	-36.85	22.79	0.49	0.24	0.021
KF strength normalized	VL PC1	Males	-105.54	93.77	0.61	0.38	0.002
KF strength normalized	MH PC2	Males	-32.94	35.11	0.46	0.21	0.030
KF strength normalized	Knee joint PPT	Females	-0.54	1.55	0.66	0.44	0.001

Constant: Value of outcome variable when predictor variable is zero
β coefficient: Regression coefficient (i.e., change in the outcome variable for a one-unit change in the predictor variable)
Standardized β coefficient: Standardized regression coefficient (i.e., change in the outcome variable for a one-standard deviation change in the predictor variable)
R^2: Coefficient of determination (i.e., percent of variance in outcome variable explained by predictor variable)

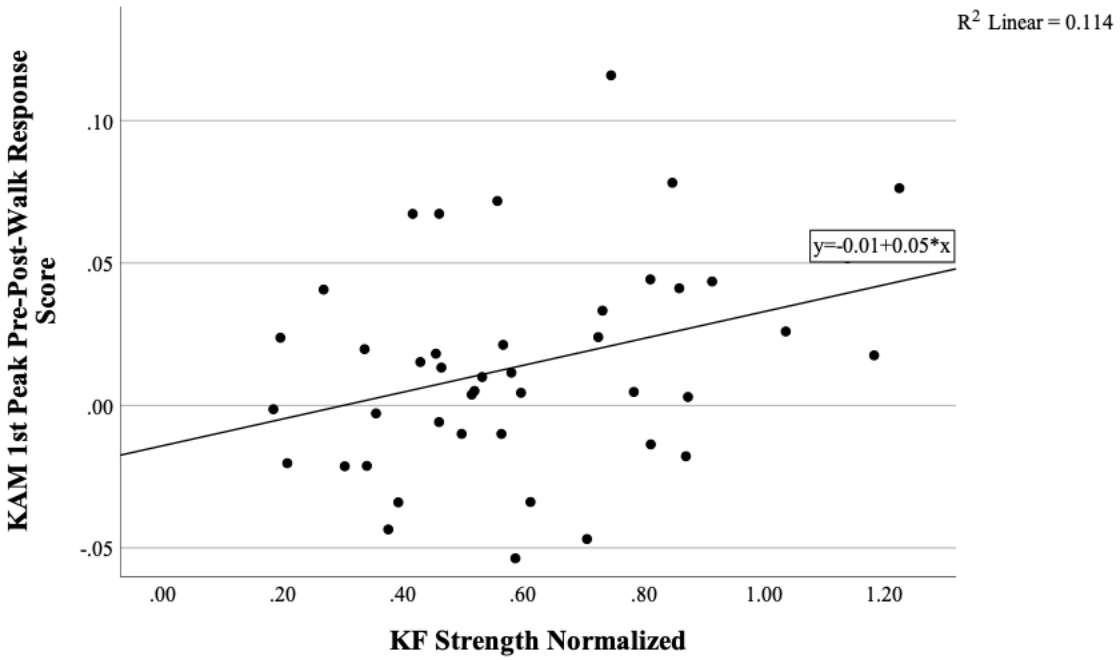

Figure 5.14. Scatterplot of the total group for the knee adduction moment (KAM) 1st peak pre-post-walk response score by normalized knee flexor (KF) strength.

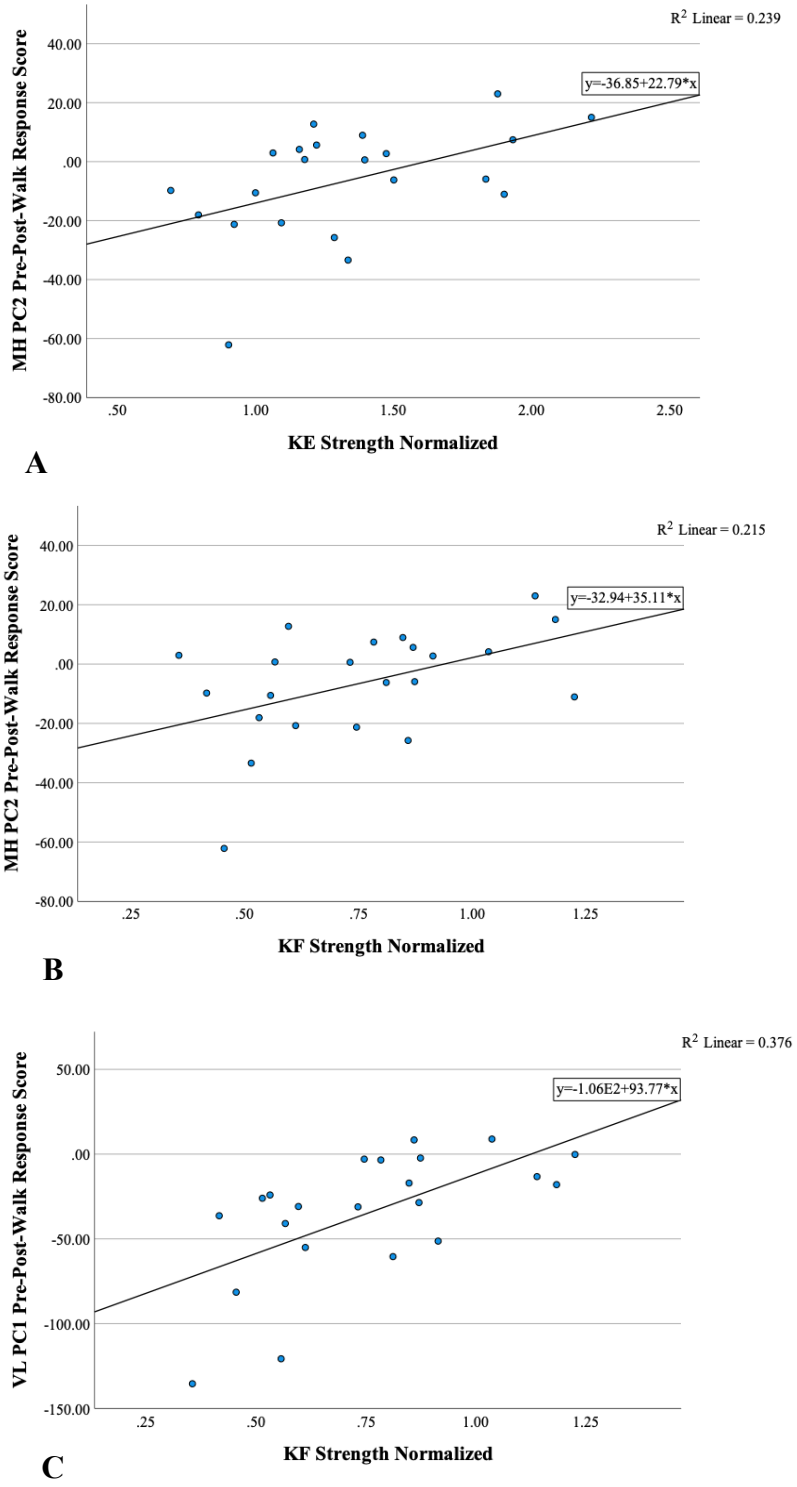

Figure 5.15. Scatterplots for the males for the medial hamstring prolonged activity (MH PC2) pre-post-walk response score by normalized knee extensor (KE) strength (A), MH PC2 pre-post-walk response score by normalized knee flexor (KF) strength (B), and vastus lateralis overall activation magnitude (VL PC1) pre-post-walk response score by normalized KF strength (C).

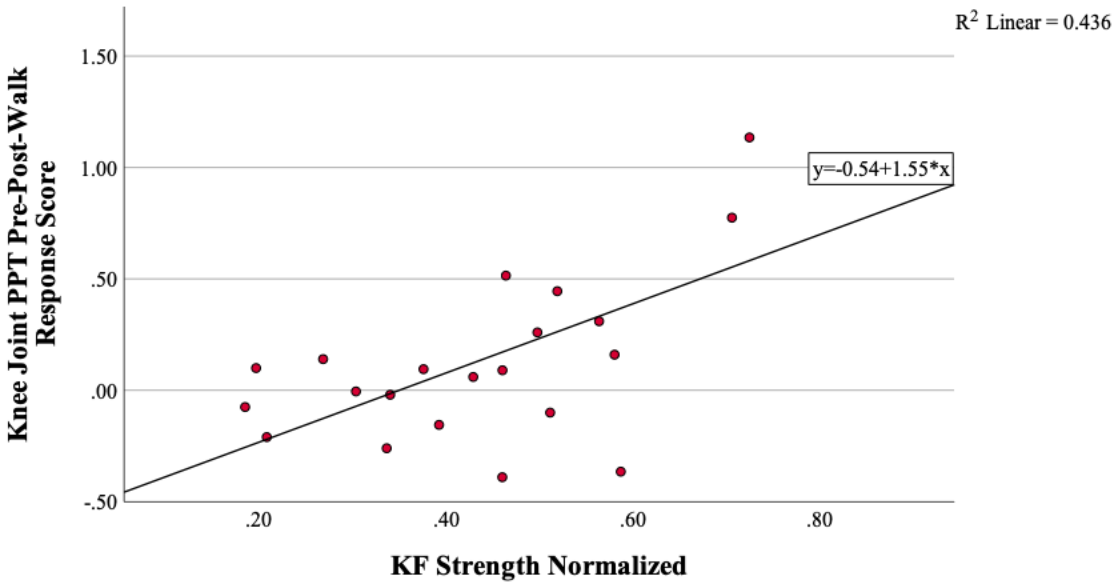

Figure 5.16. Scatterplot for the females for the knee joint pressure pain threshold (PPT) pre-post-walk response score by normalized knee flexor (KF) strength.

5.2 DISCUSSION

The objectives of this chapter were to determine in individuals with radiographic OA 1) if there were differences between sexes in responses to a standard 30-minute self-selected speed walk (Objective 3) and 2) how much variance muscle strength can explain in these pre-post-walk response scores (Objective 4). The key findings of this chapter are that males and females had different responses to the 30 minutes of continuous walking based on the significant sex by time interaction effect for the knee joint PPT, three frontal plane moment features (KAM), overall activation magnitude for the VL (PC1) and prolonged activity for the MH (PC2). There were changes after walking that were not different between sexes including the NPRS scores, KFM-KEM difference, KFM peak, overall muscle activation magnitude for the VM, RF, LH, and MH muscles, and prolonged activity of the LH. Muscle strength explained significant variance in only one pre-post-

walk response score variable for the total group. When separate models were created for each sex, muscle strength explained significant variance in two pre-post-walk response score variables for the males, and one pre-post-walk response score variable for the females.

All participants completed the 30-minute walk at their self-selected treadmill walking speed and the loading frequency between the two sexes was not different based on no difference in the number of steps taken between sexes. This confirms that both males and females experienced a similar loading frequency during the 30-minute walk.

5.2.1 Pre-Post-Walk Pain Intensity and Sensitization Response Measures

A self-reported measure of pain intensity (NPRS) and an objective measure of pain sensitization (PPT) were measured pre- and post-walk. These pain metrics were selected as the NPRS has been most often reported in the acute walking responses literature and it provides a unidimensional measure of pain intensity[157] whereas PPT testing is a common form of QST which is often used to study pain mechanisms given that it provides a physiological measure of pain sensitization[106]. While this study found that the pain intensity increase was statistically significant after walking, the mean NPRS score increase of 0.3 was less than a 2-point clinically meaningful difference[157,264].

Contrary to Hypothesis 3, pain intensity pre-post-walking responses were not different between males and females, where 26% of males and 27% of females experienced an increase in pain of at least 1 on a 10-point-NPRS and only 2/22 males and 3/23 females had a clinically meaningful increase of 2-points. These values are lower than previously reported in individuals with a knee OA diagnosis[46–48] where 40 to 50% of

individuals experienced pain increases on the NPRS of at least 1 after a 6-30 minute walking bout. This smaller increase in pain intensity may reflect the lower symptom severity as approximately 50% of this sample was asymptomatic. None of these studies examined sex and to the best of our knowledge, this is the first study to look at whether changes in pain intensity in response to walking differ between sexes and the results support minimal differences between sexes in those with radiographic knee OA and mild symptoms.

Contrary to Hypothesis 3, 30 minutes of walking led to decreased knee joint pain sensitivity in females, but increased pain sensitivity in males based on PPT testing. Despite these changes, females still had higher pain sensitization than males at both the pre-walk and post-walk time points based on the lower PPT measure. Consistent with the results from Chapter 4, females had higher pain sensitization than males at the VM and ECRL sites but neither site had a difference between pre- and post-walk time points.

Few studies have examined how exercise affects pain sensitization in individuals with OA, but in the general population, exercise is typically thought to have a hypoalgesic effect[139,140] whereas individuals with chronic pain have shown a dysfunctional analgesic response to exercise with generalized increases in pain sensitivity[141].

In general, there is evidence that PPTs at the local site increase in response to exercise, meaning decrease in local pain sensitization based on studies on older adults and adults with knee OA[275–279]. In this study, this effect was found in female participants only. Consistent with the literature[275–279], remote PPTs did not change after walking in either sex. Given the small number of studies and diversity in the exercise types included,

Hall et al. (2020)[151] concluded that there is very low-quality evidence supporting the relationship between pain sensitization and exercise (2020)[151]. None of the studies included in this review[151] separated their participants by sex and the current findings provide support for exercise-induced hypoalgesia in females at the local site, but not in males or at the remote sites, in individuals with radiographic knee OA and mild symptoms.

The two studies from the meta-analysis[151] that looked specifically at responses to aerobic exercise included participants diagnosed with knee OA and controls[275], and a severe pre-TKA and 6 months post-TKA group[279]. In the severe knee OA study, the pre-TKA group had an increase in PPTs after a 15-minute bike ride at 75% VO_{2max} at all test sites including the quadriceps, biceps, and trapezius with the greatest effects at the quadriceps. This differs from the current study, where PPTs increased only at the knee joint, and only in females. Thus, the current sample improved peripheral sensitization, whereas the pre-TKA cohort which showed improved central and peripheral sensitization potentially a result of worse radiographic and symptom severity.

In the current study, males had a mean decrease of 0.2 kgf/cm^2 and females an increase of 0.2 kgf/cm^2 after 30 minutes of continuous walking. The magnitudes of these responses are small, and less than the previously reported standard error of measurement and minimal detectable change for PPT testing in individuals with knee OA[234,272]. Thus, caution must be used when interpreting these findings, as it is plausible these differences are due to measurement error, despite being statistically significant. Nonetheless, this range is similar to the responses in individuals with diagnosed knee OA and a control group[275], where PPT responses ranged from decreases of 0.1 kgf/cm^2 in the OA group, to

increases of 0.4 kgf/cm^2 in the control group at the knee joint after 4-10 minutes of submaximal aerobic exercise using the Aerobic Power Index test[275]. The current sex-specific results are consistent with those from an athletic population that showed female, but not male, athletes decreased their pain sensitivity after treadmill running[280]. Overall, the data provides evidence to support the need to consider sex when evaluating pain responses to exercise, as males and females appear to have different pain sensitization responses to exercise.

Knee joint moment features have been previously related to changes in pain intensity after walking including greater decreases in peak KAM and KFM[46] and greater increases in knee contact forces after walking[49] in those who had increases in pain. To our knowledge, this is the first study to look at changes in knee joint moments and pain sensitization in response to walking. Interestingly, pain sensitization increased in males as did the KAM impulse, KAM 1st peak, and KAM 1st peak to mid-stance difference after walking. This increased magnitude of medial joint loading may help explain the increase in knee joint pain sensitization in males.

5.2.2 Pre-Post-Walk Knee Joint Moment Response Measures

Partially supporting Hypothesis 3, males increased all three frontal plane moment features whereas females had no change. Importantly, overground walking speed during the gait analysis test trials was not significantly different between sexes or between the pre-walk or post-walk overground walking trials and this is important given that slower walking speeds have been associated with lower knee joint moment peaks and difference measures[193,194,218,248,249].

Discrete KAM impulse measures showed no difference between the males pre- and post-walk values. However, based on examination of the KAM waveforms and the increase in KAM 1st peak, it appears that males had a small increase in KAM impulse, but that this increase was less than 0.1 Nm/kg. Given that females had a decrease in KAM impulse, it is likely that the interaction effect was a result of the different direction in responses between sexes, despite only small changes in magnitude. Males had a 7 and 15% increase in the KAM 1st peak and KAM difference measure respectively, with large effect sizes based on partial η^2 values. In contrast, females had a 2 and 5% decrease in KAM magnitude features (i.e., KAM 1st peak and KAM impulse) and no change in the KAM difference measure with small to medium effect sizes based on partial η^2 values. These findings capture a sex difference in the direction of responses, where males had an increase in medial compartment joint loading whereas females did not. This is indicative of a negative response for the males, as increases in KAM impulse and KAM peaks have been previously associated with knee OA progression[52–56,58].

The change in KAM features post-walk is an interesting finding, as walking velocity did not change between pre- and post-walk. The increase in KAM features post-walk in males is somewhat similar to the increase in knee contact forces predicted from muscle forces and joint reaction forces after 30 and 45-minutes of walking[49] but differs from decreases in peak KAM after a 20-minute walk[46] in individuals with diagnosed knee OA. However, neither of these studies separated their participants by sex or examined impulse or difference measures, and these divergent findings may be a result of the different responses between males and females in KAM features. The increase in KAM impulse and KAM 1st peak is a negative response given their association with

radiographic[52–56], symptomatic[120], and clinical[58] OA progression, whereas an increase in KAM 1st peak to mid-stance minimum difference is a positive response given that a decrease in this measure has been associated with clinical OA progression[58].

It was thought that females would have lower muscle strength and subsequently greater muscle fatigue after the 30-minute walk than males. Thus, it was hypothesized that females would have responses that more closely matched responses from a KE fatigue protocol in young adults[51], including an increase in KAM features. Surprisingly, males but not females experienced an increase in the KAM 1st peak and difference measure. The KE fatigue protocol was of much greater intensity than the 30-minute walk (approximately 40 to 50 maximum effort KE contractions) and it is likely that the 30-minute self-selected speed walking intervention was insufficient to fatigue the muscles to a similar extent. Thus, a mechanism other than fatigue likely contributed to the increase in KAM features in males.

The data did not support the hypothesis that females would develop a stiffer knee gait pattern than males, based on a greater decrease in their KFM-KEM difference. Again, this hypothesis was based on the expectation that females would experience greater KE muscle fatigue than males, resulting in knee joint moment responses similar to those reported in a KE fatigue protocol, including decreases in the KFM-KEM difference. Both males and females had a 3% increase in their KFM-KEM difference post-walk (partial $\eta^2 = 0.073$) consistent with a positive response towards a lower risk gait pattern post-walking. The KFM peak also increased after walking contributing to the increased KFM-KEM difference. These results support the increase in knee contact forces after

walking in a symptomatic knee OA group[49] and the small increase in peak KFM in a knee OA and control group[46].

Muscles are key contributors to joint stability and internal joint contact loads[70,198,199], and the decrease in overall muscle activation in all KE and KF muscles after walking may partially explain the more dynamic gait pattern after walking. Knee joint moments based on inverse dynamics have limitations in estimating internal contact forces[243] given that they are calculated from external forces and a number of inexact assumptions (e.g., generalized body segment parameters, assuming no co-activation of muscles). Thus, muscle activation patterns can help better interpret these knee joint moments, since muscles are key contributors to joint stability[70] and loading[178,179].

5.2.3 Pre-Post-Walk Muscle Activation Response Measures

Both males and females decreased their overall muscle activation magnitude post-walk in all muscles. Thus, the hypothesis that females would increase their overall muscle activity to a greater degree than males due to lower muscle strength was not supported. Overall increases in muscle activity magnitude post-walk were expected, based on an expectation of muscle fatigue and a need to increase muscle fibre recruitment to maintain the same forces during walking. It is plausible that the 30-minute self-selected speed walk was not long or vigorous enough to fatigue the muscles, and that it served more as a "warm-up" exercise. This lower activation magnitude has been previously shown following a 15-minute cycling warm-up exercise in healthy individuals[281].

The magnitude of this decrease was between 2-3% MVIC for the overall stance phase RMS for all muscles (see Appendix 9) when averaged across sexes but was

between 2-5% when sexes were analyzed separately. Though this magnitude of decrease is relatively small, even small levels of joint loading have been shown to influence chondrocyte cell death[182,183], and considering that this response may occur repeatedly while walking, the cumulative effect could still impact the overall loading exposure. To our knowledge, only one study has looked at muscle activation patterns in response to walking in knee OA pain, but the EMG values were normalized to the average stance phase activity during 10 walking strides, which prevents the direct comparison of these EMG results[46]. For their muscle activation measure, they reported KE-to-KF, and medial-to-lateral directed co-contraction ratios (DCCRs) and found overall decreases in KE-to-KF DCCRs for a no pain flare knee OA group, and overall increases in a pain flare knee OA group and a control group. However, these between group differences were not statistically significant, and no statistical test was performed to determine whether these responses were significantly different from baseline suggesting future research is needed to interpret these results.

Only two muscle activation measures had significantly different responses between sexes: the VL overall activation magnitude (PC1) and the MH prolonged muscle activity (PC2). These interaction effects are not explained by overground walking speed, as there were no differences in speed between sexes or between time points. Similar to all other KE and KF muscles, VL overall activation magnitude (VL PC1) decreased after walking. However, this decrease was of greater magnitude for males than females. Based on the overall stance phase RMS which is highly correlated with PC1 scores (see Appendix 5), males had a decrease of 5% MVIC whereas females had a decrease of 2% MVIC. MH prolonged muscle activity responses (MH PC2) occurred in different

directions where males moved towards less prolonged activity (lower PC2 scores) and females moved towards more prolonged activity (higher PC2 scores). However, caution should be used when interpreting this finding as the difference between sexes in early-stance and mid-stance RMS responses did not support differences in prolonged activity as both sexes had similar decreases (i.e., decreases of 2 and 3% MVIC).

The significant sex main effect for the VM and RF muscles overall activation magnitudes showed higher overall activation in the female compared to male participants. This finding supports the results from Chapter 4 that showed significant differences between sexes in RF overall activation, and differences of medium effect size (d = -0.57) between sexes in the VM that were not statistically significant. Given that Chapter 5 included measures at two time points (pre- and post-walk), the number of samples included in this analysis was two times the number of samples included in Chapter 4. This larger sample likely provided sufficient power to produce statistically significant results for the VM overall activation magnitude.

Given that muscle strength is associated with pain[90–92], knee joint moments and muscle activation patterns during walking[184,185], whether muscle strength could explain some of the variance in these responses was examined. Furthermore, the relationship between muscle strength and knee OA progression appears to differ between sexes[16,31,73,75,76,177], so males and females were additionally examined separately.

5.2.4 Strength as a Predictor of Pre-Post-Walk Response Measures

There was minimal support for Hypothesis 4, as body mass normalized KF muscle strength was significantly correlated with only one of the six pre-post-walk response

score variables, i.e., KAM 1st peak pre-post-walk response score, for the total sample. A higher normalized KF strength value was predictive of a greater increase in the KAM 1st peak post-walk but explained only 11% of the variance in the linear regression model. Thus, for the total sample, muscle strength was not a key contributor to the change in response variables after walking.

There is evidence that muscle strength affects knee OA progression differently between sexes where KE strength deficits seem to play a greater role in the rate of progression in females than males[16,31,73,75,76,177]. Therefore, the sex-specific correlations and linear regression model findings for normalized KE and KF muscle strength and the pre-post-walk response score variables are interesting because three models were developed for males and only one for females, and the latter was for KF and not KE strength.

All significant correlations for the males were positive in direction, meaning that a higher KF and KE strength was associated with a greater post-walk increase in the MH prolonged activity (PC2 score) and in VL muscle activation magnitude (PC1 score). The amount of variance explained by normalized KF or KF strength was much higher for the overall activation pre-post-walk response score at 38% compared to 24 and 21% for prolonged activity scores. However, given that prolonged muscle activity is associated with progression[60], a more prolonged muscle activation pattern after walking would typically be considered a negative response. Furthermore, higher muscle activation is also considered a more negative activation pattern as it is seen in higher knee OA severity groups[78,82,170,171,196,201–204] and higher KF muscle activity is associated with knee OA progression[60,61]. These findings suggest that higher strength may not be protective of

knee OA progression in males where higher strength has previously been linked to progression in maligned or unstable knees[282].

While only one correlation was significant for the female group, the regression model for normalized KF strength explained 44% of the variance in knee joint PPT. The positive relationship between variables indicates that a higher KF strength value is associated with a greater increase in PPT post-walk, meaning an improvement in pain sensitization at the local site. Decreasing local pain sensitization would be considered a positive response to walking in the females suggesting that muscle strengthening interventions and in particular improving KF strength may be most important to improve this pain response in females. This is consistent with the literature showing an association between lower muscle strength and worse pain[87–92]. This relationship between pain sensitization and strength, may partially explain the relationship between muscle strength and knee OA progression in females[16,31,73,75,76,177] and is consistent with a more systemic response in females.

Given that normalized KF and KE muscle strength were not significantly correlated with a number of pre-post-walk response score variables, future research is needed to determine which additional factors (e.g., pain[46], hormones[147], joint structure[130], biochemical biomarkers[26], innate immunity[283]) may be responsible for these differences between sexes in pre-post-walking responses.

Together, the findings of this chapter provide evidence that walking affects OA-specific responses differently between sexes and highlights the importance of conducting sex-specific analyses, specifically when examining responses to walking or other joint loading interventions. Males had increases in knee joint pain sensitization possibly a

result of the increases in medial joint loading as supported by the increase in all three KAM features, and together these features are associated with a greater risk of structural and symptom worsening. Within males, higher muscle strength was associated with increases in MH prolonged activity and VL activity magnitude, both suggesting that higher strength may not be protective against worse muscle activation responses in males.

Females increased their MH prolonged activity (PC2) which is associated with a greater risk of clinical OA progression but decreased their overall VL activity magnitude. The decrease in knee joint pain sensitization, KAM impulse and KAM 1st peak are all positive responses with respect to risk of knee OA progression. KF muscle strength explained 44% of the variance in knee joint PPT responses, highlighting the importance of strength training for adequate analgesic responses to walking in females. These findings provide evidence that sex-specific walking prescriptions are needed. Males may require gait re-training to reduce medial joint loading during walking, whereas strength training may provide limited benefit to improving responses to walking. Conversely, females may want to focus on strength training to ensure walking provides symptom relief and remains feasible.

5.3 CONCLUSION

The results of this chapter provide evidence that males and females with medial compartment radiographic knee OA have different responses to 30 minutes of walking in the knee joint PPT, KAM features, overall activation magnitude in the VL (PC1) and prolonged activation in the MH (PC2). Females had a small increase in knee joint PPT scores indicative of a decrease in local pain sensitization or a positive pain response to 30

minutes of walking. Males had a small decrease in knee joint PPT scores and an increase in KAM features indicative of an increase in local pain sensitization (i.e., negative local pain response) and a negative knee joint moment response as the KAM impulse and KAM 1st peak have previously been linked to radiographic and clinical progression. All participants increased their KFM-KEM difference measure and decreased their overall muscle activation magnitudes during walking, indicating improved knee joint moment and muscle activation patterns in terms of OA progression. Muscle strength explained significant variance in one of six pre-post-walking response measures for the total group, explaining 11% of the variance in the KAM 1st peak. When participants were separated by sex, muscle strength explained significant variance in VL and MH muscle activation responses for males and in the knee joint PPT response for females.

CHAPTER 6: CONCLUSION

Chapter 6 will provide a summary of key findings, the impact and clinical significance of these findings, and a final conclusion.

6.1 SUMMARY OF KEY FINDINGS

The overall goal of this study was to improve our understanding of whether differences in muscle strength between males and females with radiographic medial compartment knee OA can explain differences in acute pre-post-walking responses in pain intensity, pain sensitization, knee joint moments and muscle activation features during walking that have been associated with knee OA progression. To address this goal, the study objectives were to determine whether muscle strength, demographic, and clinical characteristics (Objective 1), pain intensity and sensitization, knee joint moments and muscle activation features (Objective 2), and pre-post-walk responses in pain intensity and sensitization, knee joint moment and muscle activation features (Objective 3) differed between sexes. Objective 4 determined whether muscle strength could explain significant variance in pre-post-response scores of responses that significantly differed between sexes. Summaries of the results for each objective are presented below.

6.1.1 Summary of Key Findings Chapter 4 (Objectives 1 & 2)

- Females had lower absolute and normalized KE and KF muscle strength than males.

- Females had higher pre-walk pain sensitization at the local and remote testing sites, which may indicate higher central sensitization and more neuropathic pain in females.
- Females had a lower pre-walk KFM-KEM difference measure indicative of a stiffer-knee gait pattern previously linked to OA progression.
- Females had a higher pre-walk RF overall activation magnitude.
- There were no significant differences between sexes in self-reported pain catastrophizing, OA-specific pain, symptoms, and physical function, and physical activity levels.

6.1.2 Summary of Key Findings Chapter 5 (Objectives 3 & 4)

- Males and females had different pre-post-walk responses in knee joint pain sensitization, where females decreased, and males increased their knee joint pain sensitization post-walk.
- Males and females had different pre-post-walk responses in the KAM impulse, which was a primary outcome, and in the KAM 1st peak and KAM 1st peak to mid-stance minimum difference. The increase in KAM impulse and 1st peak in males is a response consistent with an increased risk of OA progression.
- For the total group, there was a shift towards a more dynamic loading pattern post-walk (i.e., increased KFM-KEM difference measure primarily a result of the increase in KFM peak). This increase in KFM-KEM difference is a positive response as it is indicative of a decrease in the stiff-knee gait pattern previously associated with knee OA progression.

- Both sexes had a decrease in VM and RF overall muscle activation magnitude (PC1), a positive response given that higher VM and RF activation magnitudes have been previously associated with higher severity OA groups. Males and females had different pre-post-walk responses in VL overall activation magnitude (PC1) where both decreased their VL overall activation magnitude, but males did so to a greater degree.
- Both sexes had a decrease in LH and MH muscle activation magnitude (PC1), a positive response given that higher LH and MH activation magnitudes have been previously associated with knee OA progression. Males and females had different pre-post-walk responses in MH prolonged activity (PC2) where males decreased and females increased their MH prolonged activity, indicating a positive response for males and a negative response for females with respect to OA progression.
- For the total group, muscle strength explained significant variance in one pre-post-walk response score variable that differed between sexes. Normalized KF strength explained 11% of the variance in KAM 1st peak pre-post-walk response score where higher strength was associated with greater increases in KAM 1st peak.
- For the males, muscle strength explained significant variance in two pre-post-walk response score variables that differed between sexes. Normalized KE and KF strength explained 24% and 21% of the variance in MH prolonged activity and normalized KF strength explained 38% of the variance in VL overall

activation magnitude. Higher strength was associated with smaller decreases in muscle activity.

- For the females, muscle strength explained significant variance in one pre-post-walk response score variable that differed between sexes. Normalized KF strength explained 44% of the variance in the knee joint PPT pre-post-walk response score. Higher strength was associated with greater increases in knee joint PPTs, meaning greater improvements in pain sensitization

6.2 IMPLICATIONS

These findings provide a comprehensive analysis of how variables associated with OA processes differ between males and females with radiographic knee OA and how they change in response to a continuous walking protocol.

The higher pain sensitization in females at both the local and remote test sites provides evidence for greater overall pain sensitization and more neuropathic pain, which may be indicative of a more systemic disease compared to males. Worse pain, a stiffer-knee gait pattern, and higher muscle activation have been independently reported in individuals with more severe OA[173,174] and more importantly, these features have been shown to be predictive of clinical knee OA progression[60,58]. The current results suggest that in individuals with radiographic OA, and even before the presence of OA symptoms, females have pain and function features that are associated with a higher risk of clinical progression outcomes.

Identifying differences between males and females can help to create more personalized interventions that are sex-specific for potentially modifiable features. For

example, resistance strength training and neuromuscular training can improve muscle function through increased muscle strength and decreased muscle activation during walking, and this may be most important in females who have lower strength and higher muscle activation, both associated with knee OA progression. Furthermore, prescribing neuropathic pain treatments to females, who are more likely to have neuropathic pain based on higher local and remote PPTs, will likely improve female patient outcomes. Given the relationships among these features, an improvement in one feature has the potential to improve the others. For example, lower KE and KF muscle strength is correlated with a smaller KFM-KEM difference[72], therefore increasing muscle strength should improve the stiff-knee gait pattern. Evidence from a recent intervention study found that a yoga-based strengthening program improved KE and KF muscle strength, self-reported pain and symptoms, and the stiff-knee gait pattern, but did not result in changes in the frontal plane moments[206]. This supports the clinical translation of these findings in that strength training can not only improve strength, but also everyday knee pain and the stiff-knee gait pattern.

Decreasing pain is important, not only to improve patient well-being, but additionally because the presence of pain can impact muscle strength, knee joint moments, and muscle activity during walking. Understanding the type of pain can help inform pain management interventions given the differences in how nociceptive and neuropathic pain are managed. The results from this study and the literature suggest that females are more likely to have neuropathic pain, and this knowledge may help clinicians make decisions on whether neuropathic pain relief methods (e.g., tricyclics, serotonin and norepinephrine reuptake inhibitors) or nociceptive pain treatments (e.g., NSAIDs)[133]

should be first prescribed. Physical activity, such as walking, has been recommended as a method for pain relief in knee OA. However, some individuals with chronic pain do not experience the analgesic effects of exercise, and this may be potentially a result of neuropathic pain mechanisms[141]. Furthermore, there is a lack of direct evidence on the effects of continuous walking as recommended in knee OA management guidelines on OA processes including the multiple dimensions of pain, knee joint moments, and muscle activation patterns assessed in this study[37].

To better understand how pain intensity and sensitization, knee joint moments, and muscle activation patterns change in response to a 30-minute continuous walk, these responses were examined with a focus on whether responses differed between males and females with radiographic knee OA. The results suggest that a 30-minute level-ground walk at a self-selected walking speed is a feasible and beneficial intervention for individuals with radiographic knee OA of mild to moderate clinical severity. Evidence to support walking for general health benefits is widely reported[37] and our findings provide support for additional joint-specific improvements. Both sexes had increases in the KFM-KEM difference and decreases in the KE and KF muscles overall activity magnitude post-walk, and these are positive responses, as changes in the opposite direction have been previously linked to OA progression. Only small and non-clinically meaningful increases in pain intensity were found, which suggests this 30-minute walk is feasible for individuals with respect to pain perception.

The results support the need for sex-specific walking parameters. Both males and females experienced specific gait changes associated with OA progression, with males increasing their medial-to-lateral joint loading, and females increasing their MH

prolonged activity. Future studies should investigate how specific interventions (e.g., neuromuscular training) may be used to counteract or prevent these changes. Males experienced increases in knee joint pain sensitization, and while this did not result in changes in perceived pain intensity, it is plausible that longer duration walks may result in increased pain intensity and may not be feasible for males. Muscle strength alone was not responsible for the differences between sexes in most pre-post-walking responses and future research must examine additional factors to determine which factors influence these pre-post-walk responses. KF strength explained the most variance in knee joint PPT responses in females, highlighting the importance of strength training for adequate analgesic responses to walking in females.

6.3 LIMITATIONS AND CONSIDERATIONS

The results of this study need to be interpreted with an understanding of the study limitations. Knee joint moments based on inverse dynamics have limitations in estimating internal contact forces[243] given that they are calculated from external forces and a number of assumptions. For example, body segment mass and segment centre of mass are estimated from generalized body segment parameter equations based off the general population that may not be representative of the current sample. Furthermore, forces and moments calculated do not consider co-activation of muscles, and using joint moments alone often underestimates the calculated joint contact forces. The muscle activation measures included in this study can help to better interpret the external joint moments calculated.

Due to limitations imposed by the COVID-19 pandemic, additional participant recruitment was not feasible. For some EMG variables, a larger sample may have improved the power to detect sex differences such as the overall activation magnitude of the two vasti muscles in Chapter 4, where a power analysis indicated 36% and 46% power in the current sample. Of note is that there were sex differences in the overall activation magnitude for all muscles in Chapter 5, likely a result of the doubling of the number of samples from Chapter 4. Despite this limitation, the sample of 22 males and 23 females was sufficient to detect significant differences in key measures.

The overall data collection was approximately four hours long, and this could possibly lead to fatigue in participants by the time of strength testing. However, the length of collection was necessary to collect necessary biomarkers for future studies and given that this four-hour duration was consistent across all participants, it should not influence the differences between sexes. Additionally, the rest period between the final walks and muscle strength testing would have provided more than adequate time to recover based on previous literature[51]. The risk of doing maximal strength testing before the pain testing was a concern given that it could impact pain measures.

All participants had evidence of radiographic knee OA, but only half of participants had OA symptoms. Thus, only half of participants met a clinical diagnosis for knee OA. It is important to emphasize that findings from this study cannot be generalized to all individuals with a knee OA diagnosis, but rather represents a sample with radiographic evidence of knee OA (KL $\geq$ 1) both with and without OA symptoms.

6.4 FUTURE RESEARCH

This study identified specific pain, knee joint moment, and muscle activation responses that differed between sexes. Given the exploratory nature and small sample of this study, an alpha level of 0.1 was used for statistical hypothesis testing and future research is needed to confirm these results. A relatively small sample was analyzed, and future work should include a larger sample, which may cause additional sex differences to emerge. With a larger sample size, separate analyses could be conducted for asymptomatic and symptomatic individuals.

Furthermore, for Objective 4, Pearson's correlations and linear regression models were only created for response variables with significant sex by time interactions. Future work may want to include all pre-post-walk responses, regardless of whether or not these differed between sexes, to see whether variance in these responses can be partially explained by muscle strength. Furthermore, normalized KE and KF muscle strength were the only predictor variables included in the linear regression, but additional factors (e.g., self-reported pain and function scores, hormones, joint alignment) could also help explain variance in these responses. The findings of this thesis can help guide future research on knee OA responses to walking and support the need to consider sex in these analyses.

6.5 CONCLUSION

This study provides evidence that differences exist between sexes in individuals with radiographic medial compartment knee OA and mild symptoms in muscle strength, pain, joint moments and muscle activity during walking previously linked to risk of clinical OA progression. Specifically, females had lower absolute and normalized KE and KF muscle strength, higher local and remote pain sensitization, a stiffer-knee gait pattern

(i.e., lower KFM-KEM difference measure), and higher overall RF muscle activity which is a profile consistent with higher risk of clinical OA progression. Secondly, males and females had different responses to 30 minutes of walking where males had an increase in local pain sensitization (i.e., decrease in knee joint PPTs) and in KAM features, which can be considered negative responses as they are shifts towards features previously linked to OA progression. General responses for the entire sample included a less stiff-knee gait pattern (i.e., increase in KFM-KEM difference measure) and a decrease in muscle activation magnitude, which are positive responses as they shift away from features previously associated with OA progression. Finally, muscle strength explained 11% of the variance in the KAM 1st peak for the total group. When participants were separated by sex, muscle strength explained significant variance in VL and MH muscle activation responses for males and in the knee joint PPT response for females. The comprehensive examination of differences between sexes in pre-post-walking responses in pain, knee joint moment and muscle activation contributes to the much-needed evidence informing walking prescriptions for individuals with radiographic knee OA and mild symptoms, and supports the need for sex-specific walking guidelines.

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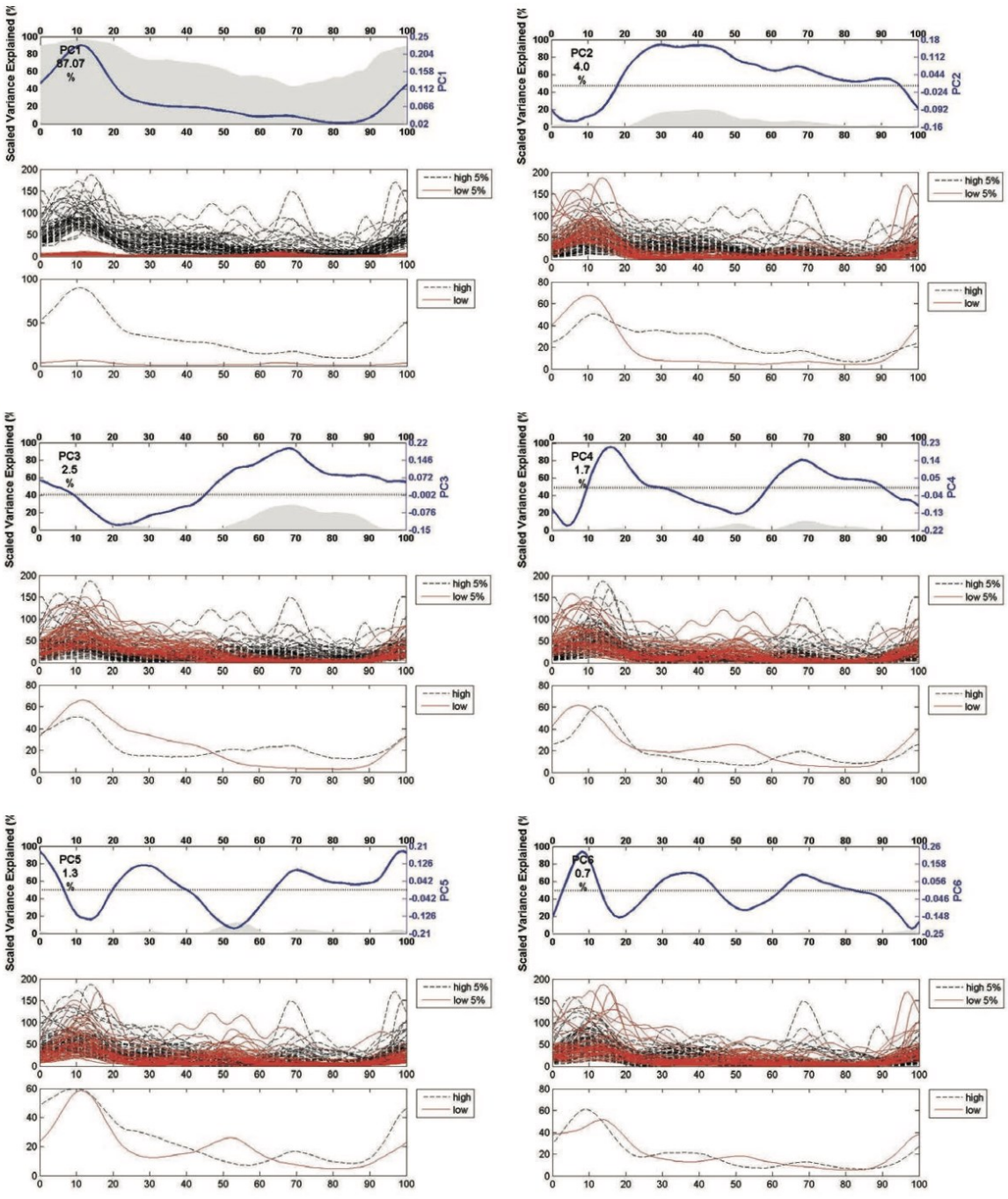
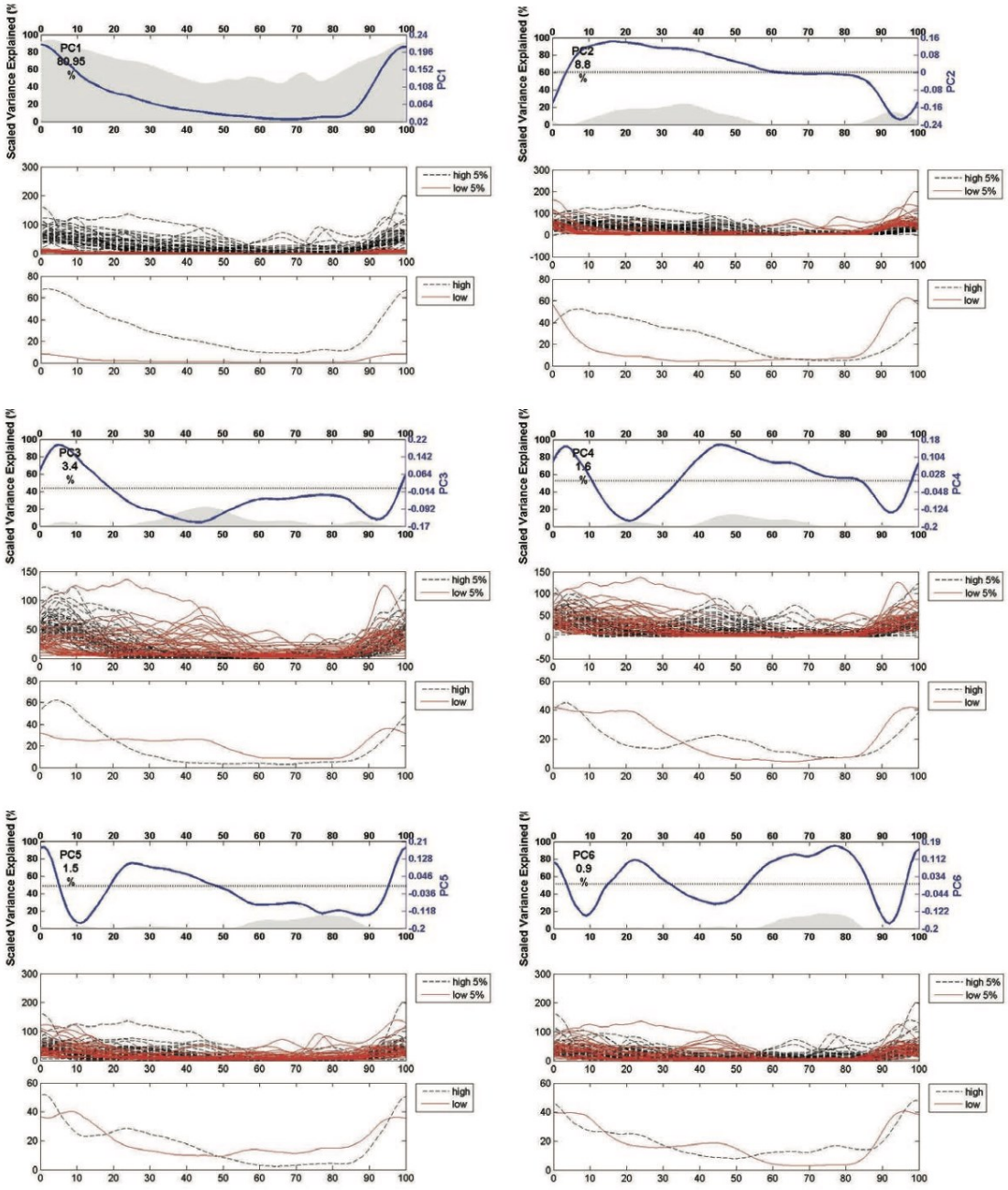
APPENDIX 1: PARTICIPANT INCLUSION AND EXCLUSION CRITERIA

Inclusion	Exclusion
• KL grade ≥ 1 > 45 years of age • Ability to walk 30 minutes consecutively • Medial compartment JSN ≥ lateral compartment JSN	• On waitlist for major lower-limb surgery (e.g., TKA) • Neurological, cardiovascular, or musculoskeletal condition that could alter gait or pose safety risk • Infection or inflammation not related to OA

APPENDIX 2: STANDARD PRINCIPAL COMPONENTS FOR THE KNEE EXTENSOR MUSCLES



APPENDIX 3: STANDARD PRINCIPAL COMPONENTS FOR THE KNEE FLEXOR MUSCLES



APPENDIX 4: CHAPTER 4 EMG RMS VALUES

	Males (n=22)	Females (n=22)	Mean Difference (M - F)
Stance-phase RMS			
VL	21.9	29.2	-7.3
VM	19.2	28.7	-9.5
RF	10.5	22.7	-12.2
LH	18.6	24.6	-6.1
MH	15.0	19.3	-4.3
Mid-stance RMS			
VL	11.1	15.1	-4.0
VM	7.8	13.7	-5.9
RF	7.8	13.7	-5.9
LH	8.2	13.6	-5.4
MH	6.0	8.2	-2.2
Early-stance RMS to mid-stance RMS difference			
VL	22.1	28.1	-6.0
VM	21.7	29.0	-7.3
RF	5.6	15.4	-9.8
LH	12.8	20.8	-8.0
MH	15.3	18.6	-3.3

APPENDIX 5: CHAPTER 4 EMG PC AND RMS CORRELATIONS

	VL PC1	VL PC2	VM PC1	VM PC2	RF PC1	RF PC2	LH PC1	LH PC2	MH PC1	MH PC2
Stance-phase RMS	**.998**	-0.266	**.999**	**-.333**	**.991**	**.630**	**.982**	**.527**	**.971**	**.399**
Mid-stance RMS	**.838**	0.257	**.807**	0.236	**.862**	**.859**	**.736**	**.747**	**.889**	**.551**
Early-stance RMS to mid-stance RMS difference	**.809**	**-.733**	**.876**	**-.706**	**.496**	**-.449**	**.774**	0.135	**.938**	**.358**

Bold = Statistically significant correlation ($p<0.05$)

To better interpret PC scores, discrete EMG measures were examined. These discrete measures have been previously correlated with EMG PC scores[60] and were the best match for this study's PCs descriptions.

In this study, PC1 scores were highly correlated with stance phase RMS amplitudes for all muscles with a range of r = 0.971 for the MH to r = 0.999 for the VM.

PC2 scores for the VM and VL had the highest correlations with the early-stance RMS to mid-stance RMS score (r = -0.706 and -0.733).

PC2 scores for the RF, LH and MH had the highest correlation with the mid stance RMS with a range of r = 0.551 for the MH to r = 0.859 for the RF.

APPENDIX 6: P-VALUES FROM ANOVAS ON ORIGINAL AND TRANSFORMED PRESSURE PAIN THRESHOLD (PPT) DATA FOR VARIABLES NOT MEETING THE ANOVA ASSUMPTIONS

Response measure	Transformation applied	Assumptions met?	Sex Effect *P*-value	Time Effect *P*-value	Interaction Effect *P*-value
Knee PPT	None	No	**0.002**	0.940	**0.030**
	Square root	Yes	**0.002**	0.649	**0.058**
VM PPT	None	No	**<0.001**	0.182	0.700
	Square root	Yes	**<0.001**	0.368	0.486
ECRL PPT	None	No	**0.039**	0.945	0.375
	Square root	Yes	**0.046**	0.783	0.393

Bold = Statistically significant difference ($p<0.1$)

APPENDIX 7: P-VALUES FROM ANOVAS ON ORIGINAL AND TRANSFORMED KNEE JOINT MOMENT DATA FOR VARIABLES NOT MEETING THE ANOVA ASSUMPTIONS

Response measure	Transformation applied or outliers removed	Assumptions met?	Sex Effect *P*-value	Time Effect *P*-value	Interaction Effect *P*-value
KAM impulse	None	No	0.316	0.744	**0.086**
	Log10	Yes	0.499	0.869	**0.095**
KFM late-stance peak extension	None	No	0.141	0.427	0.546
	Outliers removed	Yes	**0.018***	0.519	0.463

Bold = Statistically significant difference (p<0.1)
* Statistically significant difference found in transformed data that were not found in original data

APPENDIX 8: P-VALUES FROM ANOVAS ON ORIGINAL AND TRANSFORMED DATA FOR MUSCLE ACTIVATION VARIABLES NOT MEETING THE ANOVA ASSUMPTIONS

Response measure	Transformation applied or outliers removed	Assumptions met?	Sex Effect *P*-value	Time Effect *P*-value	Interaction Effect *P*-value
VLPC1	None	No	**0.052**	**<0.001**	**0.080**
	Log10	Yes	**0.019**	**<0.001**	**0.018**
	Outliers removed	Yes	**0.084**	**<0.001**	**0.061**
VMPC1	None	No	**0.039**	**<0.001**	0.181
	Log10	Yes	**0.043**	**<0.001**	**0.064***
VMPC2	None	No	0.574	0.475	0.981
	Outliers removed	Yes	0.195	0.533	0.850
RFPC1	None	No	**<0.001**	**0.001**	0.154
	Log10	Yes	**<0.001**	**0.001**	0.517
RFPC2¶	None	No	0.547	0.174	0.903
LHPC1	None	No	0.125	**0.009**	0.443
	Log10	Yes	0.101	**0.001**	0.181
LHPC2¶	None	No	0.878	**0.068**	0.154
MHPC1	None	No	0.210	**< 0.001**	0.423
	Outliers removed	Yes	0.445	**<0.001**	0.282
MHPC2	None	No	0.852	0.732	**0.095**
	Inverse reflect	Yes	0.183	0.910	**0.021**

Bold = Statistically significant difference ($p<0.1$)

¶ Transformed and outlier removed data did not result in assumptions being met

* Statistically significant difference found in transformed data that was not found in original data

APPENDIX 9: CHAPTER 5 EMG RMS VALUES

	Pre-Walk		Post-Walk	
	Males (n=22)	Females (n=22)	Males (n=22)	Females (n=22)
Stance RMS				
VL	21.9	29.2	17.3	27.3
VM	19.2	28.7	16.1	26.5
RF	10.5	22.7	9.4	20.5
LH	18.6	24.6	15.5	22.9
MH	15.0	19.3	12.0	16.8
Mid stance RMS				
VL	11.1	15.1	7.7	14.4
VM	7.8	13.7	5.8	11.7
RF	7.8	13.7	6.8	12.9
LH	8.2	13.6	7.4	13.1
MH	6.0	8.2	3.6	7.8
Early-stance RMS to mid-stance RMS difference				
VL	22.1	28.1	18.7	25.7
VM	21.7	29.0	18.9	27.7
RF	5.6	15.4	5.8	15.7
LH	12.8	20.8	13.9	19.4
MH	15.3	18.6	13.3	15.9