THE IMPLICATIONS OF MEDIAL AND LATERAL CO-CONTRACTIONS OF

KNEE MUSCLE PAIRS ON CARTILAGE HEALTH AFTER ANTERIOR

CRUCIATE LIGAMENT RECONSTRUCTION

by

Abdulmajeed Alfayyadh

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fulfillment of the requirements for the degree of Doctor of Philosophy in Biomechanics
and Movement Science

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After finishing my high school, I got accepted into the Medical school of Jouf University, Saudi Arabia. I began the program with great passion and enthusiasm, which made me finish the first year being one of the best two students in the Med school. By that time, however, I was thinking deeply about my future profession, and I ended up with a shocking decision for all people around me. Yes, I decided to transfer to the school of Physical Therapy and Rehabilitation Sciences. Since I made that decision, I have had a dream of earning a Ph.D. (Doctor of Philosophy) degree in Movement Sciences. Fortunately, dreams come true after a few years in one of the top-ranking schools in Physical Therapy and Movement Sciences in the US, the University of Delaware. Thus, I am so thankful to Allah for giving me strength and knowledge to complete my Ph.D. satisfactorily. Also, I am so grateful to the individuals who have helped me unconditionally and to the funding sources which have supported me financially to be successful during my Ph.D. journey.

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ABSTRACT

Anterior cruciate ligament (ACL) tears are very common, with over 120,000 individuals suffering these injuries every year in the United States. As ACL injuries often occur among active young adults, ACL reconstruction (ACLR) is recommended to restore knee stability and help patients return to pre-injury levels of activity. However, ACLR does not seem very effective in protecting knee cartilage from osteoarthritis (OA) development. Particularly, 50 – 70% of the individuals 5 – 15 years following ACLR develop knee OA. Thus, it is significant to assess the knee cartilage early after ACLR and investigate the potential mechanisms raising the risk of post-traumatic knee OA onset.

Knee gait mechanics (kinetics and kinematics) have been investigated excessively after ACLR. Several studies have reported that altered knee loading early after ACLR is one of the primary mechanisms leading to knee OA. While the co-contraction indices (CCIs), indicative of co-contraction magnitude, of medial/lateral knee muscle pairs influence tibiofemoral loading, little is known about these indices early after ACLR and to what extent they impact cartilage integrity. Thus, the overall objective of this work is to understand the influences of medial/lateral knee muscle pairs co-contractions on the tibiofemoral cartilage health after ACLR.

This dissertation aimed to compare the CCIs of medial and lateral knee muscle pairs in the involved and uninvolved limbs between sexes during gait 3 months after ACLR (Aim 1). Also, this work aimed to evaluate the association between medial compartment loading and the CCIs of knee muscle pairs early after ACLR (Aim 2), and to examine the relationship between knee muscle pairs CCIs and the biochemical

structure of medial tibiofemoral cartilage (via assessing T_2 relaxation time, indicative of collagen matrix degeneration) within the involved limb 3 and 24 months after ACLR (Aim 3). All aims were derived from a longitudinal cohort study, which had a gait biomechanical modeling and quantitative magnetic resonance imaging (qMRI). All participants had unilateral ACLR and underwent biomechanical gait analysis and sagittal MRI scan at 3, 6, and 24 months after ACLR.

The key findings of Aim 1 suggest that individuals walk with higher CCIs of the lateral knee musculature in the involved limb (vs. uninvolved) 3 months after ACLR, and women (vs. men) walk with different neuromuscular adaptation by producing higher CCIs of muscle pairs incorporating the lateral gastrocnemius. Aim 2 indicates that individuals 3 months after ACLR exhibit higher CCIs of lateral knee musculature (vs. medial) during walking. This imbalance between the CCIs of medial and lateral knee muscle pairs in the involved limb is associated with medial compartment underloading of the tibiofemoral joint. Finally, Aim 3 reports that the alterations in CCIs of medial/lateral knee muscle pairs are not associated with the T_2 relaxation times of any region in the medial tibiofemoral cartilage. This may suggest that walking with altered co-contractions of knee muscles might not be harmful to medial cartilage following ACLR.

In summary, individuals early after ACLR walk with higher co-contraction of the lateral knee musculature (vs. medial) in the involved limb (vs. uninvolved). The same neuromuscular strategy has been seen among women only in the muscle pairs containing the lateral gastrocnemius. These early neuromuscular alterations after ACLR might contribute to aberrant medial tibiofemoral loading. Although no association has been

found between these alterations and cartilage health, addressing the altered CCIs early

after ACLR using neuromuscular intervention protocols might help to restore

symmetrical knee loading. Thus, future work should investigate this question.

Chapter 1

THE IMPLICATIONS OF MEDIAL AND LATERAL CO-CONTRACTIONS OF KNEE MUSCLE PAIRS ON CARTILAGE HEALTH AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION: INTRODUCTION, RATIONALE, AND SPECIFIC AIMS

1.1. Introduction

In the United States, an anterior cruciate ligament ACL injury is the most common ligamentous tear, from which approximately 200,000 – 250,000 individuals suffer every year [1,2]. The young population between 15 and 25 years old are commonly exposed to this injury [1,3,4]. While the main function of the ACL ligament is to resist forces leading to anterior tibial translation and work with other knee soft tissue to maintain knee functional stability [5,6], many individuals having a torn ACL choose to undergo a surgical intervention, anterior cruciate ligament reconstruction (ACLR), instead of just undergoing rehabilitation therapy [1,4,7,8]. As this procedure is recommended to enable the individuals after ACL injury to restore their knee function, stability, and pre-injury level of activity [1,3,9], both men and women display a high ACLR prevalence rate [4,7], approximately 130,000 - 200,000 surgeries every year. However, ACLR does not seem very effective in protecting knee cartilage from degeneration [10–15].

In general, knee osteoarthritis (OA) is the most common arthritis disease that affects the synovial joint tissues, including the articular cartilage that covers the bone end of the femur and tibia, which more likely results in pain, stiffness, and functional disability [15–17]. Also, the pathological changes that occur in the knee joint affected by OA include cartilage loss, narrowing joint space, subchondral remodeling, and osteophytes

development [16,18]. These symptoms can be detected in the late stages of OA pathology using traditional techniques, such as X-ray imaging and arthroscopy. However, such methods cannot assess the cartilage in the early stages of OA progression [18,19], especially after knee injuries as they contribute to the onset of knee OA [17,20,21]. Following ACLR, Medial tibiofemoral OA develops in 25% to 70% of the individuals undergoing ACLR 5 to 15 years later [10–15]. Since a high percentage of individuals who undergo ACLR are younger than 25 years old [1,3,4], they might develop a post-traumatic knee OA in very young ages, which might impact them negatively in terms of their level of activity, social/psychological state, and quality of life [10,14,15,22]. Hence, it is significantly important to assess the knee cartilage early after ACLR, and understand what are the potential mechanisms raising the risk of knee OA progression after surgery.

While the articular cartilage of knee joint is a mechanosensitive tissue, mechanical cartilage homeostasis is required to maintain cartilage health (**Figure 1.1**) [20,23]. Several studies have reported that individuals after ACLR walk with biomechanical [24–31] and neuromuscular alterations [32–34], some of which are sex-dependent [35–40]. Knee biomechanical alterations associated with OA onset are detected during walking as early as 3 months after ACLR [41,42]. Additionally, a recent study found that those who walk with medial tibiofemoral underloading in the involved limb 6 months after ACLR develop radiographic knee OA 5 years later [28]. As gait mechanics have been investigated excessively after ACLR, investigating neuromuscular control deficits early after ACLR is warranted as these deficits are associated with altered medial knee loading and cartilage degeneration [43–45].
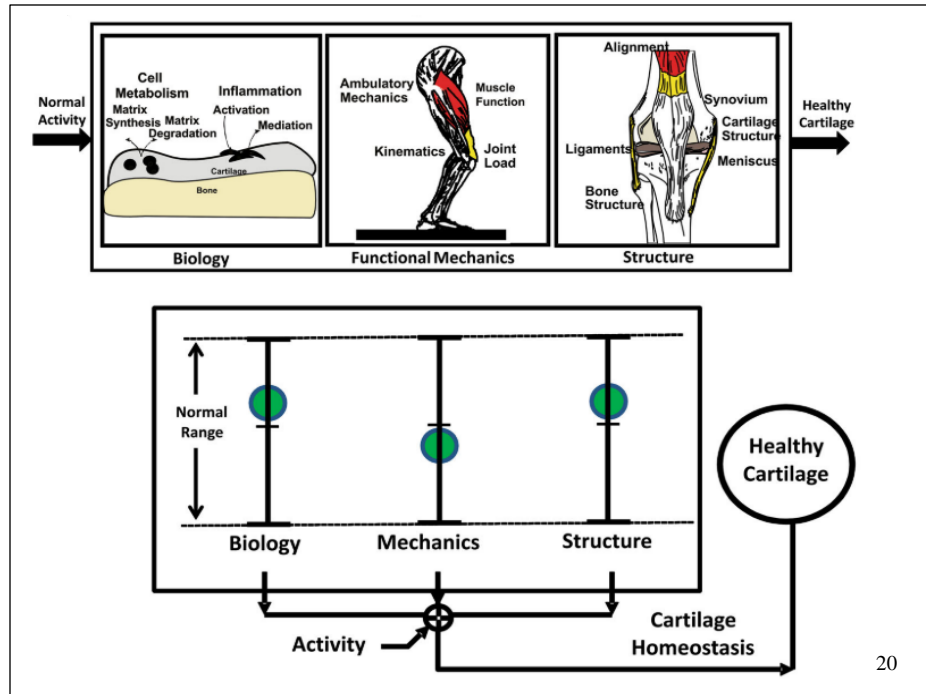
Figure 1.1 The osteoarthritis OA model showing how knee OA develops. The components of the OA model (biological, mechanical, and structural) must be within normal ranges to maintain healthy cartilage.

Altered co-contraction of knee antagonistic muscles are commonly seen after ACLR [44,46–48]. These alterations can be evaluated using co-contraction index (CCI), with a higher CCI indicative of more co-contraction [49]. Sex-based differences in knee muscle activations and CCIs after ACLR exist during sport tasks [50,51]. Although CCIs of medial and lateral knee muscles influence tibiofemoral loading [52,53] (**Figure 1.2**), little is known about these CCIs in both limbs and sexes during gait early after ACLR, and whether or not these CCIs are associated with medial tibiofemoral loading. Also, a limited

knowledge has been observed regarding the association between medial/lateral CCIs of knee muscles and the biochemical composition of cartilage following ACLR.
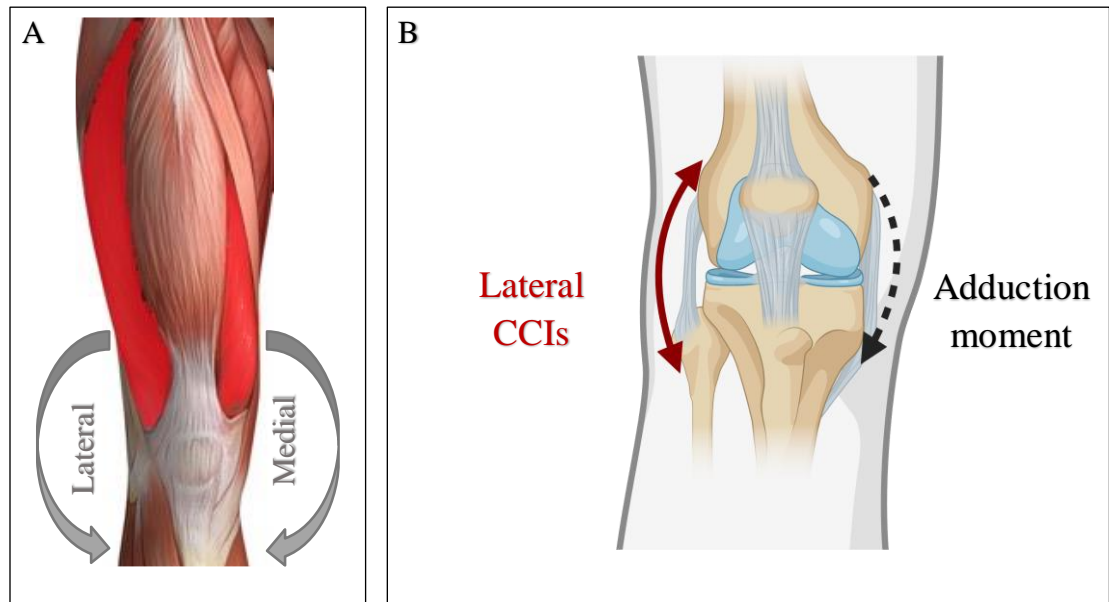
Figure 1.2 The co-contraction indices (CCIs) of medial and lateral knee muscle pairs vs. knee loading. A) shows the CCIs of medial (vastus medialis, semitendinosus, and medial gastrocnemius) and lateral (vastus lateralis, the long head of biceps femoris, and lateral gastrocnemius) muscle pairs. B) shows that external adduction moment is resisted by the co-contraction of lateral knee musculature, and vice versa.

The overall goal of this project is to understand the implications of medial/lateral knee muscles co-contractions on tibiofemoral cartilage health after ACLR. In particular, this dissertation aims to investigate medial/lateral CCIs of knee muscle pairs in both limbs and sexes (Aim 1) and to what extent these indices are associated with medial tibiofemoral loading (Aim 2) early after ACLR; also, this dissertation aims to examine the association between the co-contraction of medial/lateral knee muscle pairs and cartilage health, through assessing the biochemical structure of the cartilage (Aim 3)

after ACLR. Conducting this project will reduce the knowledge gap in literature regarding neuromuscular control alterations during gait early after ACLR. Also, it will help us gain more insights and a better understanding about the neuromuscular control impairments, specifically the co-contraction of knee muscles, and their potential relationship to early medial tibiofemoral OA progression after surgery. In general, the findings of the present work may provide valuable insights to predict post-traumatic knee OA and help in designing preventive rehabilitation protocol that could prevent or delay the progression of knee OA after ACLR.

1.2.   Scientific Rationale

The overall rationale of this project is that gait alterations in neuromuscular strategies, specifically CCIs of medial and lateral knee muscle pairs, may exist early following ACLR. These alterations may contribute into altered medial tibiofemoral loading after surgery, which may then lead to deteriorations in the biochemical composition of cartilage.

1.2.1.   Scientific Rationale for Aim 1: Sex and Limb-related CCIs after ACLR

Both men and women display a high ACLR prevalence rate [4,7], approximately 130,000 - 200,000 surgeries every year, with an annual increase in the rate of ACLR among women compared to men [1,4,7,8]. However, the degenerative changes in tibiofemoral cartilage are commonly seen a few years after ACLR [10–15], especially within the medial compartment of tibiofemoral joint [5,10,13,14]. Several studies reported altered knee gait mechanics, some of which are sex-dependent [27,36,38], in the involved limb after ACLR [24–31]. Some of these alterations, such as medial tibiofemoral loading and external

knee adduction moments, are observed as early as 3 months after surgery and associated with poor cartilage health [41,42]. Although medial and lateral CCIs of knee muscles influence frontal knee loading [52–54] (**Figure 1.2**), little is known about these indices during gait early after ACLR. Thus, Aim 1 will compare the CCIs of medial and lateral knee muscles between limbs in both sexes 3 months after ACLR. Aim 1 is significant because it will identify neuromuscular strategies that might be used differently between limbs and sexes during walking early after ACLR.

1.2.2.   Scientific Rationale for Aim 2: Knee Loading vs. CCIs after ACLR

Our lab recently found a negative association between all knee muscle co-contractions and medial compartment forces of the tibiofemoral joint at 6 months after ACLR [44]. While this suggests a potential contribution of CCIs in altered knee loading after surgery, medial and lateral CCIs of knee muscles were not investigated separately. Several studies on healthy people have shown that the co-contraction of medial and lateral knee muscles has the potential to mitigate lateral and medial tibiofemoral loading, respectively [52–54] (**Figure 1.2**). Additionally, healthy individuals exhibit a balance between medial and lateral knee muscle CCIs during walking [46]. While elevated co-contraction of medial and/or lateral knee musculature are commonly observed in the involved limb (vs. the uninvolved limb) after ACLR in various tasks [47,55,56], these alterations may contribute to asymmetric knee loading between limbs during gait. Although lower medial compartment forces were detected in the involved limb (vs. the uninvolved limb) during walking at 3 months following ACLR [42], the association between altered CCIs of knee muscle pairs and medial knee loading early after ACLR has

not been thoroughly investigated. Aim 2 will examine the association between medial/lateral CCIs of knee muscle pairs and medial tibiofemoral loading at 3 months after ACLR. Given that the magnitude of muscle CCIs can be modified by implementing neuromuscular interventions [57], Aim 2 will provide valuable knowledge which may help design rehabilitation protocols aimed at restoring asymmetric knee loading, and then delaying the onset of post-traumatic knee OA.

1.2.3.   Scientific Rationale for Aim 3: Cartilage Health vs. CCIs after ACLR

The late stage of knee OA causes morphological and irreversible changes in the cartilage (**Figure 1.3**) [20,58]. Thus, it's significant to discover and diagnose the early indicatives of knee OA to reduce its progression and symptoms. The early signs of knee cartilage degeneration after ACLR can be detected by using more advanced quantitative magnetic resonance imaging (qMRI) techniques [19,59,60]. One of the qMRI techniques frequently used is T_2 mapping technique, which has the potential to detect subtle biochemical alterations within knee cartilage, such as breaking down the collagen matrix and losing proteoglycans (**Figure 1.3**), using T_2 relaxation time [59,60]. An elevated T_2 relaxation time is indicative of higher water content and collagen matrix deterioration [59,60]. Many have shown biochemical changes in knee cartilage of the involved limb early after ACLR [41,61,62]. Additionally, multiple studies have reported associations between the T_2 relaxation time of medial tibiofemoral cartilage of the involved limb and external knee adduction moments, a surrogate measure of medial compartment loading [44], during walking at 3 and 6 months following ACLR [41,62]. Individuals at 3 to 6 months after ACLR walk with a reduced medial tibiofemoral loading in the involved limb (vs. the

uninvolved limb) [28,42], which is considered one of the responsible mechanisms for post-traumatic knee OA onset 5 years after ACLR [28]. While frontal knee loading is influenced by medial and lateral CCIs of knee muscles [52–54], it's unknown if the alterations in these indices during walking early after ACLR influence the integrity of cartilage. Aim 3 will investigate the relationship between medial/lateral knee muscle CCIs during walking and the biochemical structure of medial tibiofemoral cartilage (through evaluating T_2 relaxation times) following ACLR. This aim will identify the early gait neuromuscular strategies after ACLR that might predict long term cartilage health.
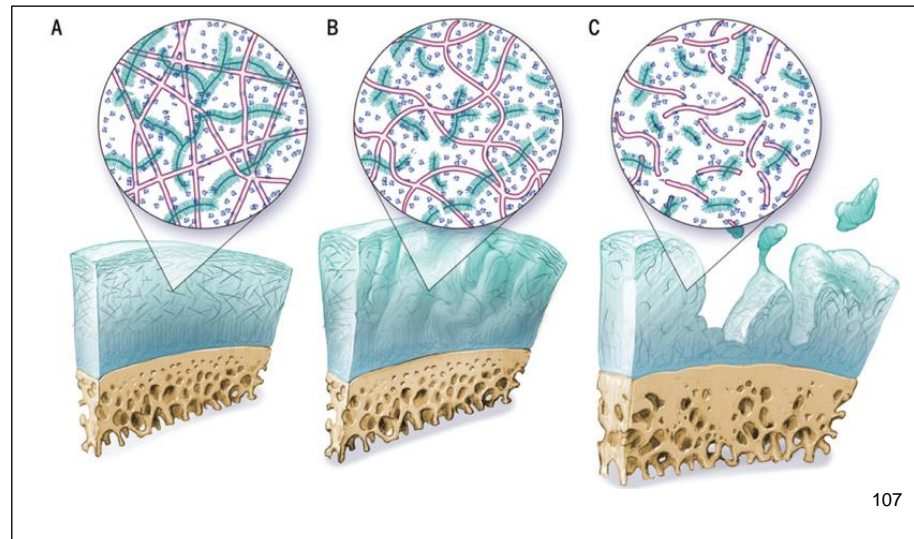
Figure 1.3 The compositional changes in a healthy articular cartilage after osteoarthritis (OA) development. The blue layer refers to cartilage, and yellow layer refers to subchondral bone. The enlarged circles show collagen matrix with pink color, and proteoglycan molecules with blue color. A) Healthy cartilage, B) early signs of cartilage degeneration, which characterized with some proteoglycans loss and collagen matrix disruption; C) Late stage of cartilage degeneration, which characterized with deformed cartilage, excessive proteoglycans loss, and excessive collagen matrix disruption.

1.3.   Significance

Successful completion of this project will reduce the gap observed in literature regarding the early neuromuscular alterations that occur after ACLR and might contribute into altered knee gait mechanics, and eventually lead to tibiofemoral OA progression. While aberrant knee loading is considered one of the primary mechanisms of the onset of knee OA after ACLR [28], It's challenging and impractical to estimate this loading using neuromusculoskeletal modeling or surrogate measures (i.e. external knee adduction moments [44]) in clinical settings. In contrast, measuring the co-contraction of knee muscles is much easier and more affordable using EMG device. Thus, the knowledge of this project will help predict post-traumatic knee OA after ACLR using the CCIs of medial and lateral knee musculatures. Also, given that the magnitude of muscle CCIs can be modified by implementing neuromuscular training protocols [57], the findings of this dissertation will inform clinicians to help design comprehensive rehabilitation protocols that address neuromuscular alterations in addition to biomechanical alterations following ACLR, which may delay the development of knee OA.

1.4.   Innovation

This dissertation work will add new information to the current literature regarding the early potential neuromuscular mechanisms that might lead to tibiofemoral cartilage degradation after ACLR. Given that alterations in knee gait mechanics following ACLR and their associations with structural [28–30] and biochemical [41,63,64] changes in cartilage have been excessively examined, our knowledge is lacking in regard with neuromuscular control alterations during walking after ACLR. One of these

common alterations that need more investigation is to what extent individuals early after ACLR walk with altered CCIs of medial and lateral knee muscles as these indices influence frontal tibiofemoral loading among healthy people [52–54]. To our knowledge, only one gait study has examined medial and lateral CCIs of knee muscles after ACLR [47]; however, the time from surgery was neither early after surgery nor in one specific time frame. Also, sex-related alterations in CCIs were not covered although several studies have displayed that knee muscles co-contractions and activations vary based on sex [50,52,65].

This work will investigate the CCIs of medial and lateral knee muscles between limbs for both sexes during gait 3 months after ACL reconstruction (Aim 1). Also, as no previous work has investigated the association between altered CCIs of medial and lateral knee muscles and tibiofemoral loading during gait early after ACLR (Aim 2), and no work has longitudinally examined the association between altered medial and lateral CCIs of knee muscles and the biochemical composition of knee cartilage after ACLR (Aim 3), these questions will be addressed in the preset project. Thus, this work will enhance our understanding on the potential neuromuscular mechanisms that might contribute to OA development after ACLR. Furthermore, this knowledge will help significantly in assessing long term cartilage health after ACLR, and in creating effective rehabilitation interventions to prevent or delay the progression of knee OA.

1.5. Specific Aims

The overall goal of this project is to identify potential neuromuscular alterations, occurring early after ACLR during gait, which might influence medial

tibiofemoral loading and subsequently increase the susceptibility of cartilage to the development of post-traumatic knee OA.

Aim 1. *Compare the CCIs of medial and lateral knee muscle pairs in the involved and uninvolved limbs between sexes during gait 3 months after ACLR.*

> *Hypothesis 1.1:* The involved limb would display higher CCIs of medial and lateral knee muscle pairs compared to the uninvolved limb during gait 3 months after ACLR.

> *Hypothesis 1.2:* Women would display higher CCIs of medial and lateral knee muscle pairs compared to men.

Aim 2. *Evaluate the association between the CCIs of medial and lateral knee muscle pairs and peak medial compartment force (pMCF) during gait 3 months after ACLR.*

> *Hypothesis 2.1:* Medial compartment underloaders would show greater CCIs of lateral knee muscle pairs compared to symmetric loaders and overloaders in the involved limb during gait 3 months after ACLR.

> *Hypothesis 2.2:* The involved limb would show a more lateral-dominated imbalance in medial-to-lateral [M:L] CCI ratios compared to the uninvolved limb during gait 3 months after ACLR.

> *Hypothesis 2.3:* Lower M:L CCIs ratios would be associated with lower pMCFs in the involved limb during gait 3 months after ACLR.

Aim 3. *Examine the relationship between medial/lateral knee muscle CCIs during gait 3 months after ACLR and the biochemical structure of medial tibiofemoral cartilage (via assessing T_2 relaxation times) within the involved limb 3 and 24 months after surgery.*

Hypothesis 3.1: Higher CCIs of medial and lateral knee muscle pairs in the involved limb 3 months after ACLR would be associated with prolonged medial tibiofemoral T_2 relaxation times 3 and 24 months after ACLR.

Hypothesis 3.2: Lower medial-to-lateral (M:L) CCIs ratios (medial CCIs < lateral CCIs) of the involved limb 3 months after ACLR would be associated with greater medial tibiofemoral T_2 relaxation times at 3 and 24 months following ACLR.

**LIMB AND SEX-RELATED DIFFERENCES IN KNEE MUSCLE CO-
CONTRACTION EXIST 3 MONTHS AFTER ANTERIOR CRUCIATE
LIGAMENT RECONSTRUCTION**

2.1   Abstract

Interlimb and sex-based differences in gait mechanics and neuromuscular

control are common after anterior cruciate ligament reconstruction (ACLR). Following

ACLR, individuals typically exhibit elevated co-contraction of knee muscles, which may

accelerate knee osteoarthritis (OA) onset. While directed (medial/lateral) co-contractions

influence tibiofemoral loading in healthy people, it is unknown if directed co-contractions

are present early after ACLR and if they differ across limbs and sexes. The purpose of

this study was to compare directed co-contraction indices (CCIs) of knee muscles in both

limbs between men and women after ACLR. Forty-five participants (27 men) completed

overground walking at a self-selected speed 3 months after ACLR during which

quadriceps, hamstrings, and gastrocnemii muscle activities were collected bilaterally

using surface electromyography. CCIs of six muscle pairs were calculated during the

weight acceptance interval. The CCIs of the vastus lateralis/biceps femoris muscle pair

(lateral musculature) was greater in the involved limb (vs uninvolved; $p = 0.02$).

Compared to men, women exhibited greater CCIs in the vastus medialis/lateral gastrocnemius and vastus lateralis/lateral gastrocnemius muscle pairs ($p < 0.01$ and $p = 0.01$, respectively). Limb- and sex-based differences in knee muscle co-contractions are detectable 3 months after ACLR and may be responsible for altered gait mechanics.

2.2 Introduction

Anterior cruciate ligament (ACL) tears are common, with over than 120,000 individuals suffering this injury every year in the United States [66]. After ACL injury, anterior cruciate ligament reconstruction (ACLR) is often recommended to restore knee stability and help patients return to pre-injury levels of activity [9]. However, individuals who undergo ACLR often display alterations in gait kinematics and kinetics, such as reduced peak knee flexion angles (pKFA), peak knee flexion moments (pKFM), and peak knee adduction moments (pKAM) [67]; which are associated with the development of post-traumatic knee osteoarthritis (OA) [28,30].

Co-contraction of antagonistic knee muscles (quadriceps, hamstrings, and gastrocnemii) is a common neuromuscular strategy in the presence of knee joint injuries and disorders, such as ACL injury/surgery, articular cartilage defects, and OA [46,47,68,69]. Greater medial and lateral knee muscle co-contractions have been reported in the involved limb of individuals with an ACL injury during gait (vs. the uninvolved limb and healthy controls); these may be attributed to higher hamstring muscle activity which is often used in an attempt to maintain knee joint stability after injury [46,70]. Following ACLR, Blackburn et al., (2019) found that individuals walk with greater lateral knee muscle co-contraction in the involved limb (vs. healthy controls). To the best of our

knowledge, this is the only study to investigate the co-contraction of medial and lateral knee muscles separately during gait after ACLR. However, it should be noted that this study did not assess the activity of the gastrocnemii despite their influence on tibiofemoral loading [71].

Previous work from our lab has shown a negative correlation between knee muscles co-contraction and medial tibiofemoral contact forces in both limbs after ACLR [44]. We have also observed a link between medial tibiofemoral underloading 6 months after ACLR and the development of knee OA 5 years after ACLR [28]. Thus, the findings of the aforementioned studies could suggest a potential relation between knee muscle co-contraction and knee OA after ACLR. Given that the magnitude of muscle co-contraction can be modified by implementing neuromuscular training protocols [57], an enhanced understanding of knee neuromuscular activity after ACLR may provide valuable insights that may help inform rehabilitation strategies aimed at delaying the development of knee OA.

Differences in gait mechanics have been reported between men and women with ACL injury and following ACLR [36,38,40]. Six-to-twelve months after ACLR, women demonstrate greater quadriceps strength deficits (vs. men) [35]. Additionally, knee function, time to return to sport, and patient-reported and objectively-measured clinical outcomes after ACLR are all influenced by sex [72]. Investigating sex-based neuromuscular adaptations after ACLR may add to the current knowledge regarding gait differences between men and women and may help explain differences in outcomes after surgery.

Altered gait mechanics after ACLR have been extensively investigated. The magnitude and duration of muscular co-contraction may also impact knee loading and cause detrimental effects in the cartilage [52,73]; however, little is known about medial and lateral knee muscles co-contraction during gait early after ACLR. Also, the studies that have investigated co-contraction indices (CCIs) in individuals with ACL injury and ACLR [44,46,47,70] have not taken sex-specific differences into consideration. Thus, the purpose of this study was to compare CCIs in the involved and uninvolved limbs between sexes 3 months after ACLR. We hypothesized that the involved limb would display higher CCIs compared to the uninvolved limb, and that women would display higher CCIs compared to men.

2.3   Methods

2.3.1   Participants

45 participants (27 men, 18 women) were included in this study, which is part of a cohort study (NIH: R01-HD087459). Participants were included in this study if they met the following criteria: age between 16 and 45 years, primary unilateral ACLR, no history of other injuries or surgeries in either limb, no concurrent grade III ligament injury, and no repairable meniscus injury. This study was approved by the Institutional Review Board of the University of Delaware. Informed consent was granted by all participants; both parental consent and minor assent were obtained if participants were under 18 years.

2.3.2   Gait Analysis

All participants completed motion analysis during overground walking 3

months after ACLR. Prior to data collection, surface electromyography (EMG) electrodes were placed bilaterally over the muscle bellies of vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF), semimembranosus (MH), biceps femoris (LH), medial gastrocnemius (MG), and lateral gastrocnemius (LG) based on SENIAM recommendations [74]. Prior to electrode placement, the areas over the muscle bellies were shaved and cleaned. All participants then were instructed to perform maximal voluntary isometric contractions (MVICs) for each group of muscles (quadriceps, hamstrings, and gastrocnemii) [75]. MVICs were used for EMG normalization unless the EMG values exceeded the MVICs during a walking trial, in which case these values were used for normalization. After completing MVICs, anatomical retroreflective markers were placed bilaterally on bony landmarks (iliac crests of the pelvis, greater trochanters of the hips, femoral condyles, malleoli, and $1^{st}/5^{th}$ metatarsal heads) to compute subject-specific segment lengths and joint centers [76]. For segment tracking, rigid shell clusters containing four markers were attached to the posterior aspect of the pelvis and distal/lateral aspects of both thighs and shanks; additionally, 2 separate markers were placed on the top/bottom of both heels [76].

Participants were instructed to walk down a 6-meter pathway at self-selected speed that was maintained within ±5% throughout the experiment. Participants performed eight walking trials for each leg from which five were used for data analysis after inspecting the quality and consistency of EMG signals and marker data. Any trials showing data in which the curves contained artifacts and/or discontinuities (gaps between heel strike to toe off) were not included in analyses. All EMG signals were sampled at

17

1080 Hz via a MA-300 EMG system (Motion Lab Systems, Baton Rouge, LA). For each muscle, EMG signals were high-pass filtered (30 Hz) using a second-order Butterworth filter, rectified, low-pass filtered (6 Hz) to create linear envelopes, and normalized to MVICs.

EMG data were processed in Visual3D (C-Motion, Germantown, MD). As the activation of quadriceps, hamstrings, and gastrocnemii muscles contribute to medial and lateral tibiofemoral loading [71,77], CCIs were computed bilaterally for the following muscle pairs: VM-MH, VM-MG, VM-LG, VL-LH, VL-LG, and VL-MG. CCIs were defined as the lowest EMG signal divided by the highest EMG signal, multiplied by the sum of these values [49].

$$CCI = \frac{Lowest\ EMG\ value}{Highest\ EMG\ value} \times (Lowest\ EMG\ value + Highest\ EMG\ value)$$

In each walking trial, the average CCI during the weight acceptance interval (100 ms before heel strike to pKFA) of stance phase was calculated for each muscle pair in each limb, then the average CCIs of five walking trials were calculated [49]. The weight acceptance interval included two intervals: the preparatory interval (100 ms before heel strike to heel strike) and the load acceptance interval (heel strike to peak knee flexion angle). This was done because high knee muscle activations occur prior to heel strike in order to help maintain knee stability [49]. Also, CCIs of knee muscles prior to heel strike have been linked to pKFM and pKAM [47], which, in turn, are associated with biochemical signs of knee OA early after ACLR [41].

2.3.3 Statistical Analyses

All statistical tests were performed using SPSS software (version 26.0, SPSS Inc., Chicago, IL). Normality, skewness, and kurtosis of the variables of interest were assessed using Shapiro-Wilk tests. Independent t-test and chi-squared test were used to compare the demographic characteristics between sexes. A two-way repeated measures ANOVA (limb [involved and uninvolved] $\times$ sex [men and women]) was performed to assess the effects of limb, sex, and their interaction. Partial eta squared values were calculated to assess the effect size of the outcomes (small size = 0.01, medium size = 0.06, and large size = 0.14) [78]. As this is an explorative study, no adjustments of multiple comparisons were implemented [79]. A p-value less than 0.05 was considered statistically significant for all analyses.

2.4 Results

There were no significant differences of age, graft type, and body mass index (BMI) between men and women. Unsurprisingly, men had significantly greater mass and height compared to women (**Table 2.1**).

A significant main effect of limb was present in VL-LH muscle pair ($p = 0.02$) with medium effect size ($\eta p^2 = 0.12$; **Table 2.2**). For the VL-LH muscle pair, the involved limb (vs. uninvolved) demonstrated significantly higher CCIs in both the men and women (**Figure 2.1**). For the VL-LG muscle pair, the main effect of limb approached significance ($p = 0.07$). No other muscle pairs displayed a main effect of limb (**Table 2.2**).

Table 2.1 Demographic characteristics of men (n = 27) and women (n = 18) participants.

Variables	Men	Women	*p*
Graft type	12 BPTP, 8 hamstring, 7 allograft	11 BPTP, 5 hamstring, 2 allograft	0.41
Age (years)	24 (7)	22 (7)	0.26
Mass (kg)	82.57 (11.04)	63.80 (9.21)	**<0.01**
Height (m)	1.79 (0.05)	1.64 (0.07)	**<0.01**
BMI (kg/m^2)	25.69 (3.66)	23.87 (3.08)	0.09
Walking speed (m/s)	1.57 (0.16)	1.55 (0.17)	0.62

Mean (± standard deviation). Bold values are significant (p < 0.05).
BPTP = bone-patellar tendon-bone graft; kg = kilogram; m = meter; s = seconds.

There were significant main effects of sex in the VM-LG ($p < 0.01$) and VL-LG ($p = 0.01$) muscle pairs with large effect sizes ($\eta p^2 = 0.24$ and $\eta p^2 = 0.15$, respectively; **Table 2.2**). Compared to men, women demonstrated significantly higher CCIs in the VM-LG and VL-LG muscle pairs regardless of limb (**Figure 2.1**). No other muscle pairs displayed a main effect of sex (**Table 2.2**). There was no interaction between limb and sex for any of the muscle pairs assessed.

Table 2.2 Knee muscle co-contraction indices of the involved and uninvolved limbs in men and women 3 months after ACLR.

Variables	Men		Women		Limb		Sex	
	IN	UN	IN	UN	p	ηp^2	p	ηp^2
VM-MH	0.12 (0.08)	0.11 (0.07)	0.13 (0.08)	0.15 (0.09)	0.53	0.01	0.32	0.02
VM-MG	0.07 (0.04)	0.06 (0.03)	0.07 (0.04)	0.08 (0.06)	0.99	< 0.01	0.34	0.02
VM-LG	0.09 (0.06)	0.08 (0.03)	0.13 (0.07)	0.14 (0.05)	0.58	< 0.01	**< 0.01**	**0.24**
VL-LH	0.19 (0.13)	0.15 (0.06)	0.16 (0.09)	0.12 (0.06)	**0.02**	0.12	0.15	0.05
VL-LG	0.12 (0.07)	0.08 (0.04)	0.14 (0.08)	0.13 (0.05)	0.07	0.07	**0.01**	**0.15**
VL-MG	0.09 (0.06)	0.06 (0.03)	0.09 (0.06)	0.08 (0.07)	0.15	0.05	0.47	0.01

Mean (± standard deviation).
Bold values are significant (p < 0.05) and large effect size ηp^2 > 0.14
IN = involved limb; UN = uninvolved limb; VM-MH = vastus medialis/semimembranosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius.

3 Months Co-contraction Indices
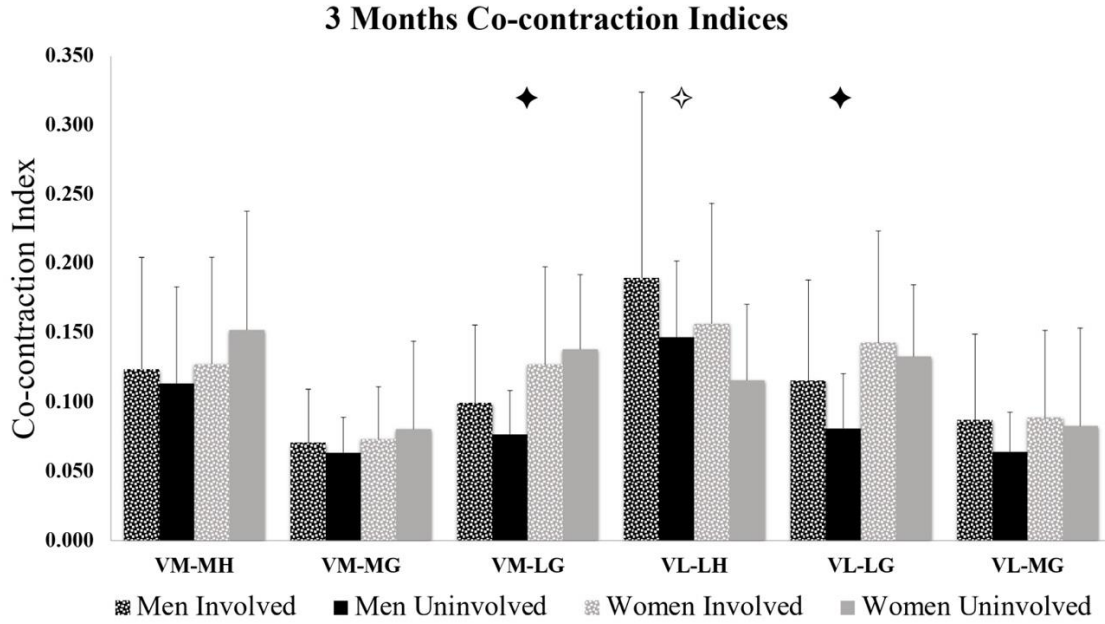
Figure 2.1 Knee muscle co-contraction indices of involved and uninvolved limbs in men and women 3 months after ACLR (Mean [± standard deviation]). VM-MH = vastus medialis/semimembranosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius. ✦ main effect of sex ($p < 0.05$); ✧ main effect of limb ($p < 0.05$)

2.5   Discussion

Our first hypothesis that knee muscle CCIs would be greater in the involved limb (vs. uninvolved) was partially supported. Compared to the uninvolved limb, the involved limb exhibited significantly higher (24% higher) CCIs in the VL-LH muscle pair with a medium effect size ($\eta p^2 = 0.12$) during the weight acceptance interval of stance phase. The VL-LG CCIs also tended to be higher (20% higher) in the involved limb with a medium effect size ($\eta p^2 = 0.07$), but statistical significance was not reached. This suggests that individuals early after ACLR may be more likely to walk with elevated CCIs of knee lateral musculature in the involved limb. In contrast, Blackburn et al., (2019) did not report any significant difference between limbs after ACLR in lateral knee muscle CCIs. Rather, they saw higher CCIs in medial knee muscles of the involved limb (vs. controls) at heel strike. However, that study investigated the CCIs of medial and lateral quadriceps/hamstrings at 3 separate intervals during gait: preparatory, heel strike, and load acceptance, whereas our study examined CCIs over one interval (weight acceptance), which encompassed all 3 of their intervals. Also, the sample used in Blackburn's study included individuals 6 months to 5 years after ACLR, rather than at one specific time frame. These factors may explain the differences in our findings. It also may be that higher co-contractions occur on the lateral side early after ACLR and more medially later on. Future work needs to examine how co-contraction indices evolve with time after ACLR.

The results of our study align well with reports that those with knee OA displayed higher magnitudes of CCIs in lateral knee muscle pairs throughout the stance

phase during gait [80]. Additionally, lateral knee muscle pair CCIs increase while knee OA progresses from asymptomatic to severe OA [81]. This suggests that the increased CCIs of lateral knee musculature may be a protective strategy to provide more knee stability and avoid pain. Further studies are warranted to examine whether or not lateral CCIs of knee muscles 3 months after ACLR can predict long term cartilage health.

Walking with elevated knee muscle CCIs is a prevalent neuromuscular strategy among individuals who have knee OA, or have had an ACL tear/surgery [46,47,70]. Knee proprioception deficits are present in individuals with knee OA [82]. Evidence suggests that knee muscle CCIs are reduced after the proprioception sensation of the involved knee improves [82]. Similar proprioception deficits are present pre/post ACLR [83], which may explain why CCIs are elevated after surgery. However, the reasons and consequences of the development of this strategy is not adequately understood. Wellsandt et al., (2016) found that individuals who developed radiographic knee OA 5 years after ACLR walk with lower medial tibiofemoral compartment loading in the involved limb 6 months after surgery, compared to those who did not develop knee OA. Knee muscle CCIs and medial tibiofemoral contact forces are inversely correlated in both limbs 6 months after ACLR [44]. Among healthy individuals, tibiofemoral loading is influenced by the magnitude of medial and lateral knee muscle CCIs [52,53]. Future studies should investigate the association between medial tibiofemoral contact forces and lateral CCIs of knee muscles early after ACLR as this may provide insights into the mechanism leading to altered tibiofemoral loading.

Altered knee gait mechanics early (6-12 months) after ACLR are linked to the development of post-traumatic knee OA [28,67]. Several studies found that peak external knee adduction/flexion moments have been associated with the severity of knee OA and alterations in knee cartilage thickness [29,84,85]. Similarly, our lab found associations between peak external knee adduction/flexion moments and biochemical signs of knee cartilage degeneration in the involved limb 3 months after ACLR [41]. The VL-LH CCIs are associated with knee flexion excursion and external abduction/flexion moments in the involved limb after ACLR [47]. This suggests that the increased CCIs of the VL-LH muscle pair during walking occur in response to reduced external knee adduction moments, which are commonly reported after ACLR [44]; although it is also possible that the elevated VL-LH CCIs could be the cause of this reduction in moments. Future studies should examine the association between lateral CCIs of knee muscles and gait mechanics early after ACLR.

Our second hypothesis that women would display greater knee muscle CCIs (vs. men) 3 months after ACLR was also partially supported. The VL-LG and VM-LG CCIs were significantly higher (26% and 37%, respectively) in women (vs. men) regardless of limb, with large effect sizes ($\eta p^2 = 0.15$ and 0.24, respectively). While this suggests that certain CCIs after ACLR are sex-dependent, it should be noted that it is unknown if these findings were the result of the ACLR or just reflective of typical sex-based differences, as this is the first study to assess medial and lateral CCIs between men and women. Interestingly, our findings showed that differences in CCIs between men and women occurred only in the muscle pairs containing the lateral gastrocnemius (LG). Two

potential explanations can be provided; first, women after ACLR might walk with decreased knee range of motion as a stabilizing strategy by co-contracting the LG and knee extensors. The second potential explanation is that the LG might be used differently between sexes. This is supported by Di Nardo et al., (2015) who found that healthy women tend to activate their LG, tibialis anterior, and VL more during walking (vs. healthy men). Future studies need to examine differences in lateral gastrocnemius strength after ACLR between sexes and investigate LG activation patterns between healthy individuals and those with ACLR for both men and women.

This study has several limitations that need to be considered. It should be mentioned that there was a difference in sample size between men and women. However, this difference is small, and the sphericity assumptions were not violated for any of the variables of interest [87]. Secondly, we have limited information regarding rehabilitation protocols used among our participants, which might lead to discrepancies in muscular strength and neuromuscular ability. However, as our data was collected early (3 months) after reconstruction, only small differences in muscular strength and neuromuscular activity are expected. As we used surface electrodes, the magnitudes of EMG signals may be influenced by skin artifacts (motion) [88] and the placement of electrodes over muscles. Additionally, inconsistencies in the quality of the MVICs between participants might have occurred, which may influence the calculation of CCIs. We attempted to mitigate this possibility by encouraging all participants to contract their muscle maximally during MVIC collection. If the EMG magnitude of any muscle during walking was higher than the corresponding MVIC value, the higher value was used to normalize the EMG data of

this muscle. As we only analyzed CCIs in the first 50% of stance phase, future work is needed to investigate the entirety of stance which might reveal more neuromuscular alterations. Finally, some of the participants in this study underwent meniscectomies in addition to ACLR; while it is possible that this may have influenced the results, previous work suggests that there are no differences in directed co-contraction (medial and lateral) of knee muscles between healthy individuals and those with partial meniscectomies [89].

In summary, our findings indicate that individuals display higher CCIs of the VL-LH muscle pair (lateral knee musculature) in the involved limb (vs. uninvolved) 3 months after ACLR. Also, women (vs. men) exhibit different neuromuscular adaptation by producing higher CCIs of muscle pairs incorporating the lateral gastrocnemius 3 months after ACLR. These discrepancies in neuromuscular adaptations between limbs and sexes may lead to altered gait mechanics; thus, this study reiterates the importance of early neuromuscular interventions to address potential gait alterations following ACLR. Future studies should investigate the association between knee muscle CCIs and medial tibiofemoral loading after ACLR, and whether or not CCIs early after reconstruction can provide insights into the long-term health of knee cartilage.

2.6   Acknowledgments

Chapter 3

UNBALANCED MEDIAL-TO-LATERAL KNEE MUSCLE CO-CONTRACTIONS ARE ASSOCIATED WITH MEDIAL TIBIOFEMORAL UNDERLOADING DURING GAIT THREE MONTHS AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

NOTE: This is the submitted version to the Journal of Biomechanics.

3.1   Abstract

Background: Altered medial/lateral knee muscle co-contraction (measure by co-contraction indices, CCI) occurs during gait early after anterior cruciate ligament reconstruction (ACLR). Changes in peak medial compartment forces (pMCF) are also observed early after ACLR and are linked to the development of knee osteoarthritis. We do not know if imbalanced co-contraction is associated with these alterations in knee load.

Research question: Is there an association between pMCF and the CCIs of medial/lateral knee muscle pairs during free speed walking three months after ACLR?

Methods: Cross-sectional study. Bilateral knee gait mechanics and electromyography (EMG) data were collected from 44 participants 3 months following surgery. For each limb, CCIs of six muscle pairs and medial-to-lateral (M:L) CCIs ratios were calculated during the first 50% of the stance phase of gait. Bilateral pMCFs were calculated using a subject-based neuromusculoskeletal model. Based on interlimb pMCF symmetry, participants were divided into three groups: symmetric loaders, underloaders, and overloaders. A 2×3 (limb $\times$ group) ANOVA was used to compare CCIs between limbs

in all groups. A Wilcoxon test was used to compare CCIs ratios between limbs. A partial Spearman's test was performed to examine the association between CCIs ratios and pMCF.

Results: The CCIs of the vastus lateralis-lateral gastrocnemius muscle pair was higher in the involved limb of underloaders (vs. the uninvolved limb and vs. the involved limb of symmetric loaders). The ratio of M:L CCIs was significantly lower (more lateral CCIs) in the involved limb, which was associated with lower pMCF.

Significance: Individuals early after ACLR who walk with higher CCIs of lateral knee musculature (vs. medial), have medial tibiofemoral underloading. Future research should examine whether unbalanced CCIs of knee musculature early after ACLR are related to long-term cartilage degradation.

3.2   Introduction

Anterior cruciate ligament (ACL) tears are prevalent, particularly among young adults [66]. These injuries result in knee instability, leading to reduced levels of activity and lower functional confidence [1,4]. Thus, many choose to undergo ACL reconstruction (ACLR) to restore functional knee stability and to return to their pre-injury level of activity [7,8]. Despite these beneficial outcomes, up to 70% of individuals develop radiographic medial tibiofemoral osteoarthritis (OA) within 10-15 years of surgery [10,11,15].

One mechanism contributing to the development of post-traumatic knee OA after ACLR is alterations in medial tibiofemoral loading [28]. These alterations are attributed to altered knee mechanical and neuromuscular strategies. Using a validated EMG-based neuromusculoskeletal model [71], we found that those who walk with medial

tibiofemoral underloading 6 months after ACLR develop radiographic knee OA 5 years post-surgery [28]. Additionally, we have observed asymmetric peak medial compartment forces (pMCFs, involved less than uninvolved) as early as 3 months after ACLR [42]. These asymmetrical loading patterns are associated with early, pre-osteoarthritic, alterations in the biochemical composition of knee cartilage [41]. Thus, there is a critical need to investigate the potential mechanisms leading to altered knee loading in the early months after ACLR.

While altered knee gait mechanics and their influence on knee load following ACLR have been extensively studied [13,28,44], little is known regarding the association between neuromuscular alterations and knee loading after surgery. Altered co-contraction of the muscles crossing the knee joint (quadriceps, hamstrings, and gastrocnemii) [32,47,90] are common following ACLR. Elevated lateral knee muscle co-contractions (quadriceps and hamstrings) have been reported in the involved limb (vs. uninvolved) during gait 3 months after surgery [90] and are found to shift more medially with time [47]. These alterations in knee muscle co-contraction indices (CCIs) [49], may contribute to asymmetric knee loading between limbs during walking following ACLR.

Altered co-contraction of knee musculature after ACLR is associated with surrogate measures of knee load such as frontal plane moments [54] and pMCFs [44]. Few, however, have examined the association between CCIs of medial/lateral knee muscle pairs and measures of knee load. Prior work among healthy individuals suggests that medial and lateral knee musculature CCIs provide resistance against knee abduction and adduction loading, respectively [52,53]. Thus, alterations to the normal balance of medial-to-

lateral co-contraction may be related to abnormal loading following ACLR. Acquiring insights into the relationships between CCIs of medial/lateral knee muscle pairs and pMCF early after ACLR may provide valuable knowledge concerning the underlying mechanisms leading to the development of post-traumatic OA.

The purpose of this study was to evaluate the association between the CCIs of medial and lateral knee muscle pairs and peak medial compartment force (pMCF) during gait 3 months after ACLR. We hypothesized that (1) medial compartment underloaders would show greater CCIs of lateral knee muscle pairs compared to symmetric loaders and overloaders in the involved limb, (2) the involved limb would show a more lateral-dominated imbalance in medial-to-lateral [M:L] CCI ratios compared to the uninvolved limb, and (3) lower M:L CCIs ratios would be associated with lower pMCFs in the involved limb during gait 3 months after ACLR.

3.3   Methods

3.3.1   Participants

Forty-four participants, from a larger longitudinal cohort study, were included in this study following approval by the Institutional Review Board of the University of Delaware (**Table 3.1**). Criteria for participant enrollment included: 16 to 45 years old for age, primary unilateral ACLR, no prior lower-limb injuries or surgeries, no concurrent grade III ligament tears, and no repairable meniscal tears. Prior to participating, all participants signed a written informed consent. For individuals under the age of 18, both minor assessment and parental consent were obtained before enrollment.

3.3.2   Gait Analysis

All participants underwent gait analysis during overground walking 3 months

(3.1 ± 0.5) following ACLR using previously described methodologies [71,91]. Briefly, prior

to walking, surface electromyography (EMG) electrodes were placed bilaterally over

knee extensors [vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF)] and

flexors [semitendinosus (MH), long head of the biceps femoris (LH), medial

gastrocnemius (MG), and lateral gastrocnemius (LG)] after shaving and cleaning the

regions over each respective muscle belly. All electrode placements followed SENIAM

guidelines [92]. Following EMG electrode placement, all participants performed bilateral

maximal voluntary isometric contractions (MVICs) for each of the assessed muscle

groups (quadriceps, hamstrings, and gastrocnemii) [91]. After MVIC testing, each

participant was outfitted with retro-reflective markers which were placed bilaterally on

bony landmarks of interest (iliac crests of the pelvis, greater trochanters of the hips,

femoral condyles, malleoli, and $1^{st}/5^{th}$ metatarsal heads) [91]. For motion tracking, rigid

shell clusters with four markers were placed on the pelvis, thighs, and shanks.

Additionally, two separate markers were placed on the top and bottom of each heel [91].

Participants completed 16 walking trials (8 for each leg) on a 6-meter walkway

at self-selected speed which was maintained throughout the experiment (± 5 %). During

walking, kinematic data were collected using an 8-camera Vicon system (Oxford Metrics

Limited, London, England) with a sampling rate of 120 Hz and kinetic data were

collected at a sampling rate of 1080 Hz using a force platform (Bertec Corp.,

Worthington, OH, USA). Simultaneously, EMG data were recorded at 1080 Hz using a

MA-300 EMG system (Motion Lab Systems, Baton Rouge, LA).

Kinematic, kinetic, and EMG data were processed in Visual3D (C-Motion, Germantown, MD) and used as inputs into a validated EMG-based neuromusculoskeletal model to calculate joint contact forces [71,93]. Prior to analysis, EMG data were high-pass filtered (cutoff 30 Hz), rectified, low-pass filtered (cutoff 6 Hz), and then normalized to MVICs to create linear envelopes. First, a SIMM (SIMM 4.0.2, Musculographics, Inc., Chicago, IL) musculoskeletal model was scaled for each participant based on their anthropometric measurements. This model has four segments (foot, shank, thigh, and pelvis) driven by the action of 10 muscles crossing the knee joint. The EMG signals of the mentioned seven muscles, in addition to the vastus intermedius (estimated as the average of vastus medialis and lateralis), the semimembranosus (set equivalent to the semitendinosus) and the short head of the biceps femoris (set equivalent to long head of the biceps femoris,), were transformed into muscle activations [71,93]. Model parameters involved in muscle force estimation were then tuned so that the internal frontal plane knee moments were in close agreement with external moments computed via inverse dynamics [71,93]. Finally MCFs were calculated using the estimated muscle forces via a previously described frontal plane moment balance approach [71,77]. From these, we found the pMCF in each limb during stance phase and subsequently normalized these to an individual's body weight (BW).

Bilateral co-contraction indices (CCIs) for six muscle pairs (VM-MH, VM-MG, VM-LG, VL-LH, VL-LG, and VL-MG) were calculated as the lowest EMG signal divided by the highest EMG signal and multiplied by the sum of these signals [49].

$$CCI = \frac{Lowest\ EMG\ value}{Highest\ EMG\ value} \times (Lowest\ EMG\ value + Highest\ EMG\ value)$$

The CCIs for each muscle pair was calculated for each walking trial then averaged across all walking trials for that limb. The average CCIs were calculated during the weight acceptance interval (100 ms before heel strike to maximal knee flexion angle) of stance phase [49]. Additionally, M:L CCIs ratios were calculated by dividing the medial muscle pair index by the lateral muscle pair index for quadriceps, hamstrings, and gastrocnemii muscles, resulting in the following ratios: VM-MH / VL-LH and VM-MG / VL-LG [52].

Participants were divided into three groups based on interlimb medial compartment loading symmetry (involved limb – uninvolved limb): symmetric loaders, underloaders, and overloaders. To do this, we used a previously determined meaningful interlimb difference (MILD) for pMCF (0.4 BW) [13]. Specifically, those who had a pMCF interlimb difference (ILD) between ± 0.4 BW were considered symmetric loaders, while those with pMCF ILD greater than 0.4 BW or smaller than - 0.4 BW were considered overloaders and underloaders, respectively.

3.3.3   Statistical Analyses

SPSS software (version 26.0, SPSS Inc., Chicago, IL) was utilized to perform all statistical analyses. Shapiro-Wilk tests were performed to check data normality, skewness, and kurtosis. Demographic characteristics were compared between groups (symmetric loaders, underloaders, and overloaders) using one-way ANOVAs and chi-

squared tests. A 2x3 [Limb x Loading Group] repeated measures ANOVA was performed to examine the effects of limb and loading classification, and their interactions, on muscle CCIs while controlling for sex, walking speed, body mass index (BMI), and age. If significant effects were observed, two-tailed post hoc Wilcoxon test and Kruskal-Wallis test were used to compare CCIs between limbs and between groups, respectively. Due to the exploratory nature of the current study, adjustments for multiple comparisons were not performed [79]. The effect size of the findings were evaluated using Partial eta squared values (small size = 0.01, medium size = 0.06, and large size = 0.14) [78], and Cohen's D values (small size = 0.2, medium size = 0.5, and large size = 0.8) [94]. M:L CCIs rations were compared between limbs using a Wilcoxon test. The associations between the M:L CCIs ratios and pMCFs were evaluated using a two-tailed partial Spearman's correlation, while using gender, walking speed, BMI, and age as covariates. Alpha was set to 0.05 for all analyses.

3.4   Results

There were no significant differences in demographic characteristics between the loading groups (symmetric loaders, underloaders, and overloaders; **Table 3.1**).

The ANOVA analysis for the VL-LG muscle pair displayed a significant limb × loading group interaction ($p = 0.02$) with large effect size ($\eta p^2 = 0.17$; **Table 3.2**). For VM-LG muscle pair, the interaction between limb and loading group approached significance ($p = 0.07$) with medium effect size ($\eta p^2 = 0.13$; **Table 3.2**). No other interactions or main effects were present for the other muscle pairs (**Table 3.2**).

Post hoc investigations revealed that underloaders walked with significantly

higher CCIs in the VL-LG muscle pair of the involved limb (vs. uninvolved) ($p < 0.01$, d = 0.89). Additionally, underloaders displayed significantly higher involved limb CCIs in the VL-LG muscle pair compared to symmetric loaders ($p = 0.02$, $\eta p^2 = 0.18$).

The M:L CCIs ratio of the VM-MH / VL-LH was significantly lower in the involved limb compared to the uninvolved limb ($p < 0.01$, $d = 0.98$; **Figure 3.1**) indicating more co-contraction in the lateral muscle pair than medial within the involved limb. There was a significant positive association between the M:L CCIs ratio of VM-MH / VL-LH and pMCF of the involved limb ($p = 0.02$, $r = 0.40$; **Figure 3.2**), indicating that more laterally dominated co-contraction (vs. medial) was associated with lower medial compartment loading. No other associations were present between the ratio of CCIs and pMCF (**Table 3.3**).

Table 3.1 Demographic characteristics for each loading group (n = 44).

Variables	Symmetric Loaders (n = 15)	Underloaders (n = 22)	Overloaders (n = 7)	*p*
Sex	6 women, 9 men	9 women, 13 men	3 women, 4 men	0.99
Graft type	6 BPTP, 7 hamstring, 2 allograft	9 BPTP, 6 hamstring, 6 allograft, 1 hybrid	5 BPTP, 1 hamstring, 1 allograft	0.53
Age (years)	23 (6)	23 (7)	23 (10)	0.94
Mass (kg)	77.05 (12.76)	75.52 (14.96)	71.34 (13.42)	0.67
Height (m)	1.74 (0.13)	1.72 (0.08)	1.74 (0.09)	0.69
BMI (kg/m^2)	25.20 (1.94)	25.54 (4.35)	23.35 (2.71)	0.35
Walking speed (m/s)	1.51 (0.17)	1.59 (0.17)	1.59 (0.13)	0.37

Mean (± standard deviation). BMI = body mass index;
BPTP = bone-patellar tendon-bone graft; kg = kilogram; m = meter; s = seconds.

Table 3.2 Knee muscle co-contraction indices of the involved and uninvolved limbs during gait 3 months after ACLR.

Variables	Symmetric Loaders		Underloaders		Overloaders		Limb		Loading		Interaction	
	IN	UN	IN	UN	IN	UN	p	ηp^2	p	ηp^2	p	ηp^2
VM-MH	0.13 (0.11)	0.13 (0.08)	0.12 (0.07)	0.14 (0.09)	0.12 (0.04)	0.11 (0.05)	0.52	0.01	0.77	0.01	0.55	0.03
VM-MG	0.07 (0.04)	0.07 (0.05)	0.07 (0.03)	0.07 (0.04)	0.08 (0.05)	0.07 (0.06)	0.78	< 0.01	0.83	0.01	0.94	< 0.01
VM-LG	0.08 (0.04)	0.11 (0.04)	0.12 (0.07)	0.09 (0.06)	0.13 (0.06)	0.09 (0.06)	0.09	0.07	0.49	0.04	0.07	0.13
VL-LH	0.17 (0.16)	0.12 (0.05)	0.19 (0.09)	0.14 (0.06)	0.19 (0.11)	0.13 (0.07)	0.82	< 0.01	0.99	< 0.01	0.96	< 0.01
VL-LG	0.09 (0.05)	0.11 (0.04)	0.15 (0.09)	0.09 (0.05)	0.14 (0.06)	0.10 (0.06)	0.55	0.01	0.41	0.05	**0.03**	**0.17**
VL-MG	0.08 (0.04)	0.07 (0.05)	0.08 (0.05)	0.07 (0.04)	0.10 (0.09)	0.07 (0.08)	0.45	0.02	0.62	0.03	0.77	0.01

Mean (± standard deviation). Statistically significance ($p < 0.05$) and large effect size $\eta p^2 > 0.14$ are indicated by bold values. IN = involved limb; UN = uninvolved limb; VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius.
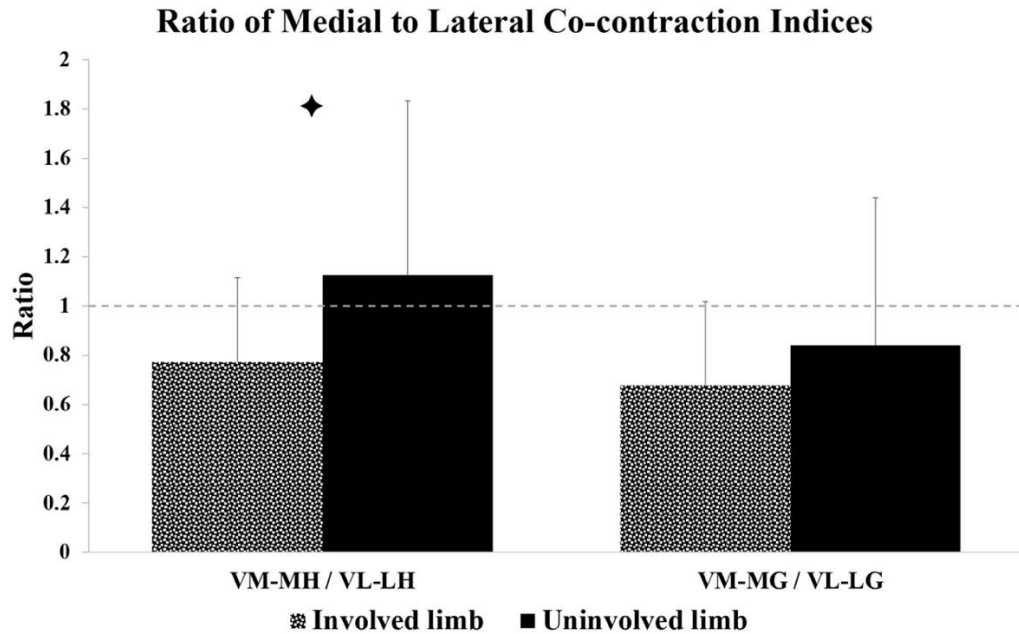
Figure 3.1 Ratio of medial-to-lateral knee muscle pairs co-contraction indices for
participants 3 months after ACLR during walking (Mean [± standard
deviation]). The ratio of medial-to-lateral quadriceps/hamstrings CCIs was
lower in the involved limb than the uninvolved limb. VM-MH = vastus
medialis/semitendinosus; VL-LH = vastus lateralis/long head of the biceps
femoris; VM-MG = vastus medialis/medial gastrocnemius; VL-LG = vastus
lateralis/lateral gastrocnemius. ✦ significant difference ($p < 0.05$).
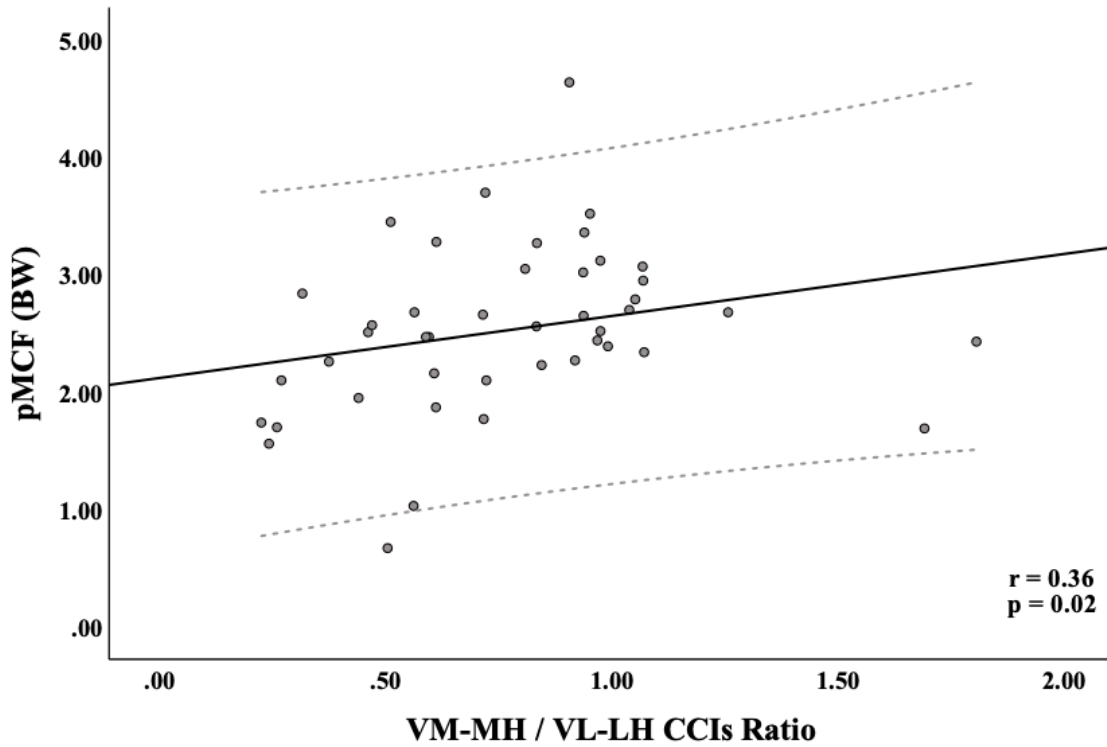
Figure 3.2 A positive association between the ratio of medial-to-lateral quadriceps/hamstrings CCIs and pMCF in the involved limb. VM-MH = vastus medialis/semitendinosus; VL-LH = vastus lateralis/long head of the biceps femoris; pMCF = peak medial compartment force; BW = body weight.

Table 3.3 pMCF versus the ratio of Medial-to-lateral CCIs of knee muscle pairs.

Limb	Knee loading	Ratio of Medial-to-Lateral CCIs	*r*	*p*
IN limb	pMCF	VM-MH / VL-LH	**0.36**	**0.02**
		VM-MG / VL-LG	0.17	0.28
UN limb	pMCF	VM-MH / VL-LH	0.18	0.28
		VM-MG / VL-LG	0.08	0.65

Statistically significance association is indicated by bold values (p < 0.05).
IN = involved limb; UN = uninvolved limb; CCIs = co-contraction indices.

3.5   Discussion

To our knowledge, this is the first study to investigate the association between medial compartment force and the co-contraction of medial and lateral knee muscle pairs following ACLR. Our findings partially supported our hypotheses. We found that that underloaders walked with greater lateral CCIs in the involved limb compared to symmetric loaders for the VL-LG muscle pair. Additionally, we found that individuals walk with more lateral-dominant CCIs (vs. medial) in the involved limb following ACLR, and that this imbalance is associated with lower medial compartment forces. These neuromuscular strategies may contribute to altered knee loading early after ACLR, which, in turn, may negatively impact long-term knee cartilage health.

Regarding the first hypothesis, only the CCIs of VL-LG muscle pair exhibited significant differences between loading groups. Specifically, underloaders walked with higher VL-LG CCIs in the involved limb (vs. the uninvolved limb and vs. the involved limb of symmetric loaders). This suggests that individuals with lower pMCF in the involved limb (vs. uninvolved) early after ACLR may be more likely to walk with elevated co-activation of lateral quadriceps and gastrocnemius. This contradicts the work of Blackburn et al. (2019) which reported higher CCIs of medial knee muscle pairs in the involved limb (vs. healthy control), rather than lateral knee muscle pairs [47]. That study, however, did not include the activity of gastrocnemii in CCIs analysis despite their impact of tibiofemoral loading [71]. Additionally, their participants were from a later and broader time range following ACLR (6 months to 5 years after surgery). This broader time range may have obscured important time-dependent alterations. Future studies need

to investigate these indices across multiple later time points, such as 6 and 24 months after surgery, and examine how these indices evolve with time.

A recent study found that individuals walking with medial tibiofemoral underloading in the involved limb 6 months after ACLR develop radiographic knee OA 5 years following surgery [28]. Those individuals showed an inverse association between medial compartment loading and the CCIs of all knee muscle pairs (quadriceps, hamstrings, and gastrocnemii together) in both limbs [44]. This association, however, could be representative of a normal behavior as it was seen in both limbs. Regarding medial/lateral CCIs of knee muscles, however, our previous work reported that individuals walk with higher CCIs of knee lateral musculature in the involved limb (vs. uninvolved) 3 months after ACLR [90]. Similarly, several OA studies demonstrated that OA patients walk with elevated CCIs in lateral knee muscle pairs during the stance phase, and the co-contraction of lateral muscle pairs increases while OA severity increases [80,81,95]. This may suggest that individuals early after ACLR walk with increased lateral CCIs due to pain and/or neuromuscular deficits such as impaired proprioception and excitability of the nervous system, while OA patients use the same technique as protective strategy to reduce compression and pain on the medial compartment. Future work is needed to examine the potential impact of elevated lateral CCIs on knee cartilage early after ACLR, and whether these indices can be altered by rehabilitation interventions after surgery.

Our second and third hypotheses were also partially supported. We found a significantly lower M:L CCI ratio in the involved limb (vs. uninvolved) for the VL-LH /

VM-MH pairs. We also saw a trend towards a lower M:L CCI ratio for the quadriceps and gastrocnemii pairs within the involved limb, but statistical significance was not achieved. This suggests that individuals tend to co-contract their involved limb's knee muscle pairs with a lateral-leaning imbalance during walking early after ACLR. Although it is not fully understood how these neuromuscular patterns develop and how these can impact knee cartilage, it is possible that they could cause alterations in tibiofemoral loading. A recent study showed that healthy people walk with balanced co-contraction between medial and lateral knee muscle pairs [46]. Thus, walking with unbalanced M:L CCIs of knee musculature following ACLR might induce aberrant tibiofemoral loading, which may eventually lead to detrimental effects to cartilage.

When examining the influence of unbalanced M:L CCIs on medial knee load, we found a positive association between the M:L CCIs ratio of quadriceps/hamstrings and pMCF in the involved limb. This suggests that walking with lower CCIs ratios (lateral CCIs > medial CCIs) is associated with lower medial tibiofemoral loading in the involved limb 3 months following ACLR. This is not necessarily surprising as medial and lateral knee muscle pairs have varus/valgus moment arms that work oppositely in terms of frontal knee loading resistance [52,53]. Thus, altered medial-to-lateral co-contraction patterns may alter load magnitude and distribution, which may ultimately be harmful to cartilage [20,21,28,52,53,96]. If load is being shifted, the newly loaded region of cartilage may not have the capacity to withstand the applied loading, which may result in the breakdown of cartilage components [20,21]. If load remains in the same location but changes magnitude, it could be that, given cartilage's mechanosensitive nature, the cartilage may

adapt to the new amount of loading [97,98]. This, in turn, could be problematic when knee loading returns to its pre-ACLR level, as it's possible that the cartilage may be incapable of withstanding such loading (i.e., it's now a relative overloading), which might cause degenerative changes in cartilage [99]. Future research should examine whether or not unbalanced CCIs of medial and lateral knee muscle pairs early after ACLR contribute to long-term cartilage degradation.

Estimating tibiofemoral loading using neuromusculoskeletal modeling or via surrogate measures (i.e. external knee adduction moment [44]) is difficult and unfeasible for a clinical environment. Given that the magnitude of muscle co-contraction can be measured easily using an EMG device and can be modified by implementing neuromuscular training protocols [57], the findings of the current study might be important for clinicians to consider early after ACLR. More research, however, is warranted to understand why these differences in medial and lateral CCIs of knee muscle pairs exist early after ACLR, and whether symmetric knee loading between limbs after surgery can be restored through treating the alterations in the CCIs of medial and lateral knee musculature.

Several limitations should be considered in this study. First, the sample sizes of the three groups (symmetric loaders, overloaders, and underloaders) were relatively small which might limit the generalization of our findings to the entire ACLR population. Secondly, we had limited knowledge about the rehabilitation protocols applied to our participants, so discrepancies in neuromuscular strategies may exist because of differing strategies. Additionally, the use of surface EMG electrodes could potentially affect the

magnitudes of EMG signals due to skin artifacts (motion). Each electrode was secured to the participant using both tape and wraps to minimize these potential motion artifacts. Finally, as the data of the current study is part of a larger cohort study, we did not conduct a power analysis for the specific questions asked in this study as doing so is considered an inappropriate practice [100]. Thus, there is a chance that other variables may have been deemed not significant due to lack of statistical power rather than true statistical non-significance.

3.6   Conclusion

Early after ACLR, individuals exhibited higher CCIs of lateral knee musculature during walking. This imbalance between the CCIs of medial and lateral knee muscle pairs in the involved limb was associated with medial compartment underloading of the tibiofemoral joint. These gait neuromuscular strategies early after surgery may contribute to aberrant knee loading in this population and may be a mechanism for eventual post-traumatic OA development.

3.7   Acknowledgments

Chapter 4

MEDIAL AND LATERAL KNEE MUSCLE CO-CONTRACTIONS DURING GAIT 3 MONTHS AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION ARE NOT ASSOCIATED WITH TIBIOFEMORAL CARTILAGE T2 RELAXATION TIMES

4.1. Abstract

Background: Alterations in the co-contraction indices (CCIs), indicative of muscular co-contraction magnitude, of medial and lateral knee muscle pairs during gait are detected early after anterior cruciate ligament reconstruction (ACLR). Aberrant medial tibiofemoral loading occurring early after ACLR is one of the responsible mechanisms of the onset of knee osteoarthritis (OA). Although medial/lateral knee muscle CCIs influence tibiofemoral loading, it's unknown if altered co-contraction is associated with poor cartilage health, assessed by quantitative MRI (qMRI).

Research question: Is there an association between the CCIs of medial/lateral knee muscle pairs 3 months after ACLR and the T_2 relaxation times of medial tibiofemoral cartilage 3 and 24 months after surgery?

Methods: A prospective cohort study. Knee gait electromyography (EMG) data of the involved limb were collected from 28 participants 3 months after ACLR. CCIs of four muscle pairs and medial-to-lateral (M:L) CCIs ratios were quantified during the first half of the stance phase of gait. All participants underwent a supine MRI using a sagittal T_2 mapping sequence at 3 and 24 months after surgery. The T_2 relaxation times of 6 tibial and 6 femoral regions in the medial compartment of the involved limb were calculated. A

partial Spearman's correlation test was used to assess the associations between CCIs and T$_2$ relaxation times.

Results: No statistically significant associations were detected between the CCIs of the involved limb and the T$_2$ relaxation times of any region of interest in the medial compartment neither at 3 months nor 24 months after ACLR.

Significance: Altered co-contraction of medial/lateral knee muscle pairs of the involved limb during gait early after ACLR are not associated with the deteriorations occurring in the biochemical structure of tibiofemoral cartilage. Future research with a larger cohort should reproduce the present study and examine the biochemical alterations in the medial cartilage in later than 2 years after surgery.

4.2.   Introduction

An anterior cruciate ligament (ACL) injury is one of the most prevalent and devastating knee injuries among the young population in the United States [66]. To overcome the functional consequences induced by ACL injury, ACL reconstruction (ACLR) becomes the prioritized option with most of those patients [7,8]. However, it's well reported in the literature that knee cartilage is commonly impacted by ACLR, resulting in osteoarthritis (OA) development 5-15 years following sugary [10,11,15]. Having knee OA in young individuals is a serious issue as it causes pain and reduced level of activity, which in turn influence their quality of life [15,101]. This also adds to the annual cost of OA health care in the United States, which is around $450 billion [11]. Therefore, an effort needs to be exerted to understand the underlying mechanisms associated with the onset of knee OA

following ACLR so that modifiable biomechanical factors might be identified and used in preventative rehabilitation protocols.

The biochemical structure of tibiofemoral cartilage is composed of a dense matrix that has collagen, proteoglycans, chondrocytes, and mainly fluid [102,103]. These components enable the cartilage to handle the applied loading in different directions. The initial biochemical alterations in knee cartilage composition might result in structural degeneration [20,58], a late stage of OA process, which is conventionally diagnosed using methods such as X-ray imaging and arthroscopy [18,19]. In this stage, yet, the morphological changes occurring in the cartilage are significant and irreversible. Thus, discovering and diagnosing the early signs of knee OA may help avoid its progression and/or delay its associated symptoms.

Recently, the early signs of knee cartilage degeneration after ACLR can be detected by using more advanced magnetic resonance imaging (MRI) techniques, such as transverse T_2 relaxation time (indicative of collagen matrix and water content in cartilage) [19,59]. This technique is a quantitative MRI (qMRI) method that evaluates the biochemical composition of cartilage via assessing T_2 relaxation time [19,59,60]. It has been used commonly in research aiming to evaluate knee cartilage health after ACLR [41,62,63], which suggested that biochemical changes in knee cartilage of the involved limb often occur in the early months (3-6 months) following ACLR. Also, several studies have reported associations between elevated T_2 relaxation time of medial tibiofemoral cartilage of the involved limb and elevated external knee adduction moments 3 and 6 months

following ACLR [41,62]. This may suggest that altered tibiofemoral loading following ACLR plays an important role in post-traumatic OA onset and progression.

Recent studies found that individuals early after ACLR (3-6 months) tend to walk with reduced medial tibiofemoral loading in the involved limb (vs. uninvolved) [28,42], which is considered one of the responsible mechanisms for post-traumatic OA development five years later [28]. The co-contraction indices (CCIs), indicative of co-contraction magnitude [49], of medial and lateral knee muscle pairs influence the magnitude of frontal tibiofemoral loading [52–54]. We found recently that individuals at 3 months after ACLR walk with higher lateral CCIs of knee muscles in the involved limb [90]. As lateral and medial knee muscle pairs have valgus and varus moment arms, respectively [52–54], walking with higher lateral CCIs of knee muscles may reduce the loading applied on the medial compartment. Additionally, healthy individuals walk with a balance between medial and lateral knee muscle CCIs [46]. This suggests that walking with unbalanced medio-lateral CCIs of knee muscles following ACLR may alter medial tibiofemoral loading, and may then impact cartilage health. To our knowledge, however, no study has investigated the role of altered CCIs of medial/lateral knee muscle pairs in the development of OA following ACLR, using T_2 relaxation times (a measure for early signs of OA).

The purpose of this study was to examine the relationship between medial/lateral knee muscle CCIs during gait 3 months after ACLR and the biochemical structure of medial tibiofemoral cartilage (via assessing T_2 relaxation times) within the involved limb 3 and 24 months after surgery. It was hypothesized that 1) higher CCIs of

medial and lateral knee muscle pairs in the involved limb 3 months after ACLR would be associated with prolonged medial tibiofemoral T_2 relaxation times 3 and 24 months after ACLR, and 2) lower medial-to-lateral (M:L) CCIs ratios (medial CCIs < lateral CCIs) of the involved limb 3 months after ACLR would be associated with greater medial tibiofemoral T_2 relaxation times at 3 and 24 months following ACLR.

4.3. Methods

4.3.1. Participants

Twenty-eight participants were recruited in this study, which is a part of a prospective longitudinal study that was granted the Institutional Review Board IRB approval at the University of Delaware (**Table 4.1**). All participants provided written informed consents prior participation. All participants were unilateral ACLR with age ranged from 16 to 45 years old. The exclusion criteria were: 1) previous other major injuries or surgeries in either leg, 2) concurrent grade III ligamentous tears, 3) repaired meniscal tear, and 4) MRI contraindications. Testing included gait analysis at 3 months and MRI analysis at 3 and 24 months following ACLR.

4.3.2. Gait Analysis

All participants completed gait analysis during overground walking 3 months after ACLR using an 8-camera Vicon system (Oxford Metrics Limited, London, England) sampled at 120 Hz and a force platform (Bertec Corp., Worthington, OH, USA) sampled at 1080 Hz. Prior walking trials, retroreflective markers were placed on bony landmarks of the involved limb, while rigid shell clusters that have 3-4 markers were placed at the pelvis, thighs, and shanks; 2 separate markers also were placed on each heel

[91]. All participants completed 8 walking trials for 6 meters at a self-selected speed, which was maintained during the experiment (± 5 %).

A MA-300 EMG system (Motion Lab Systems, Baton Rouge, LA) was used to collect surface electromyography (EMG) signals from the involved limb at 1080 Hz using for the following muscles based on SENIAM guidelines [92]: vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF), semitendinosus (MH), long head of the biceps femoris (LH), medial gastrocnemius (MG), and lateral gastrocnemius (LG). Maximal voluntary isometric contractions (MVICs) were collected for each musculature group (quadriceps, hamstrings, and gastrocnemii) to normalize raw EMG data collected during walking trials [91]. Raw EMG signals were also high-pass filtered (30 Hz), rectified, and low-pass filtered (6 Hz), and eventually converted to linear envelops. All biomechanical variables (kinematic, kinetic, and EMG) were processed via Visual3D (C-Motion, Germantown, MD).

Variables of interest included co-contraction indices (CCIs) for four muscle pairs (VM-MH, VM-MG, VL-LH, and VL-LG) within the involved limb. These indices were calculated as the smallest EMG magnitude was divided by the largest EMG magnitude and then the result was multiplied by the sum of these magnitudes [49].

$$CCI = \frac{Smallest\ EMG\ value}{Largest\ EMG\ value} \times (Smallest\ EMG\ value + Largest\ EMG\ value)$$

The average CCIs of each muscle pair were calculated during the weight acceptance interval (100 ms before heel strike to peak knee flexion angle) of stance phase

in each walking trial, and then the CCIs of five walking trials were averaged [49].

Additionally, M:L CCIs ratios were calculated by dividing the index of medial muscle

pair by the index of lateral muscle pair for quadriceps, hamstrings, and gastrocnemii

muscles, which resulted in 2 CCIs ratios: VM-MH / VL-LH and VM-MG / VL-LG [52].

Table 4.1 Demographic characteristics for participants (n = 28).

Variable	Participants
Sex	13 women, 15 men
Graft type	13 BPTB, 9 hamstring, 6 allograft
Age (years)	23 (6)
Mass (kg)	73.61 (15.06)
Height (m)	1.72 (0.09)
BMI (kg/m^2)	24.68 (3.76)
Walking speed (m/s)	1.54 (0.16)

Mean (± standard deviation).
BMI = body mass index; BPTB = bone-patellar tendon-bone graft; kg = kilogram; m = meter; s = seconds.

4.3.3. MRI Acquisition and Analysis

The involved knee of all participants got scanned at 3 and 24 months after

unilateral ACLR via a supine knee MRI using a 3 Tesla magnet Siemens scanner

(Washington D.C.) with a 15-channel transmit-receive knee coil (Siemens) to evaluate

medial tibiofemoral cartilage. A sagittal T_2 mapping sequence was performed during all

MRI scans; the parameters used in T_2 mapping sequence for all participants is listed in

Table 4.2. The analysis of MRI images was proceeded using 3D Slicer

(https://www.slicer.org/) [104]. To reduce the potential systematic errors during analysis, T_2

maps were calculated using a mono-exponential fit with a two-parameter (pixel-by-pixel)

model while discarding the first echo in each sequence [105,106]. During images analysis,

three slices corresponding to the medial compartment center, which is the region with

maximal distributed force, were each segmented into 6 regions of interest (ROI). These

regions are anterior, central, and posterior of the tibial and femoral cartilage (**Figure 4.1**).

These regions were also divided into deep and superficial layers to account for laminar

differences seen in the structure of cartilage's collagen matrix [59], resulting in 12 ROI

total. The average T_2 relaxation time in each ROI was calculated by averaging the

magnitudes of T_2 relaxation time across all three slices.
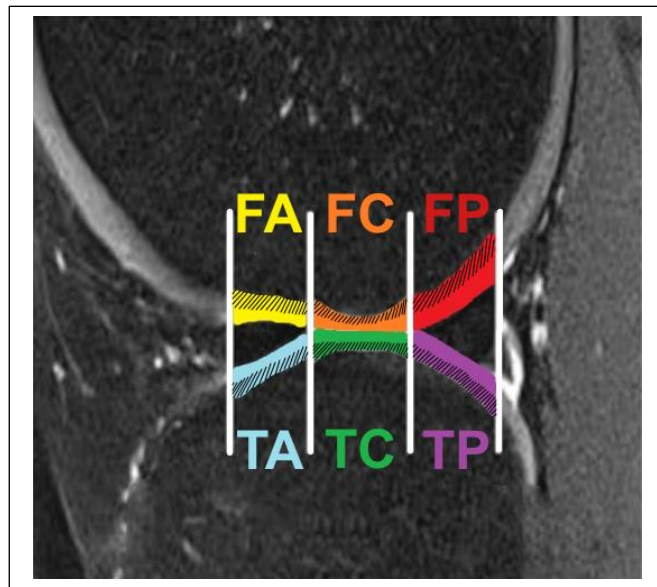
Figure 4.1 MRI sagittal knee scan showing the regions of interest of the medial compartment of tibiofemoral cartilage. Femoral regions include: femoral anterior (FA), femoral central (FC), and femoral posterior (FP). Tibial regions include: tibial anterior (TA), tibial central (TC), and tibial posterior (TP). All regions of interest are subdivided into deep (black striped) and superficial (solid) regions.

4.3.4.   Statistical Analyses

All statistical analyses were executed using SPSS software (version 26.0, SPSS Inc., Chicago, IL). The normality of all variables was checked by performing Shapiro-Wilk tests. The associations between the CCIs of knee muscle pairs and the T_2 relaxation time of each ROI (deep and superficial layers) in the medial compartment of the involved limb was evaluated using a two-tailed partial Spearman's correlation, while using gender, walking speed, BMI, and age as covariates. The same statistical model was also used to examine the association between each CCIs ratio and the T_2 relaxation time of each ROI (deep and superficial layers). The p-value for statistical significance of all analyses was set at 0.05.

Table 4.2 T_2 mapping sequence parameters.

Parameter	3 months after ACLR				24 months after ACLR
	Participants 1-6	Participants 7 and 9	Participant 8	Participants 10-28	Participants 1-28
Field of view	160 mm	140 mm		150 mm	150 mm
Slice thickness	2 mm	3 mm	2 mm	3 mm	3 mm
Repetition time	4480 ms	3090 ms	2590 ms	3090 ms	3090 ms
Echo times	12.5, 25, 37.5, 50, 62.5, 75 ms	13.8, 27.6, 41.4, 55.2, 69, 82.8, 96.6 ms		10, 20, 30, 40, 50, 60, 70 ms	10, 20, 30, 40, 50, 60, 70 ms

ACLR = anterior cruciate ligament reconstruction.

4.4.   Results

The data of twenty-eight participants (**Table 4.1**) were included in partial Spearman's correlation analysis to examine the association between the CCIs of knee muscles 3 months after ACLR and femoral and tibial regions' T_2 relaxation time 3 and 24 months after ACLR in the involved limb.

4.4.1.   CCIs vs. T_2 relaxation times - 3 months after ACLR

No statistically significant associations were seen between the CCIs of the involved limb and the T_2 relaxation times of any region of interest neither superficial nor deep layer. The association between the VM-MH CCIs and the deep layer of femoral anterior region approached significance with a moderate effect ($p = 0.056$, $r = 0.396$; **Table 4.4**). Similarly, a positive association between the VM-MG CCIs and the superficial layer of tibial central region approached significance with a moderate effect ($p = 0.062$, $r = 0.387$; **Table 4.5**). Regarding the CCIs ratio and T_2 relaxation times, a negative association between VM-MG / VL-LG CCIs ratio and the deep layer of tibial central region approached significance with a moderate effect ($p = 0.083$, $r = -0.361$; **Table 4.6**).

4.4.2.   CCIs - 3 months after ACLR vs. T_2 relaxation times - 24 months after ACLR

No statistically significant associations were seen between the CCIs of the involved limb and the T_2 relaxation times of any region of interest neither superficial nor deep layer. Yet, positive associations between the VM-MG CCIs and the superficial and

deep layers of tibial central region showed moderate effects ($p = 0.110$, $r = 0.335$; **Table 4.9**; $p = 0.090$, $r = 0.354$; **Table 4.10**; respectively).

Table 4.3 T$_2$ relaxation times of femoral regions (superficial layer) versus knee muscle co-contraction indices and ratios of the involved limb – 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
FA						
r	-.016	.111	-.009	.218	-.068	-.229
p	.941	.606	.966	.306	.751	.282
FC						
r	-.130	.059	-.314	-.084	.168	.059
p	.544	.786	.135	.697	.432	.785
FP						
r	.053	.071	.207	.052	-.073	.009
p	.807	.740	.331	.808	.733	.968

Statistically significance association is indicated by bold values ($p < 0.05$).
VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; FA = femoral anterior; FC = femoral central; FP = femoral posterior.

Table 4.4 T2 relaxation times of femoral regions (deep layer) versus knee muscle co-contraction indices and ratios of the involved limb – 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
FA						
r	.396	.169	.239	.202	.076	-.081
p	.056	.429	.261	.345	.723	.706
FC						
r	-.145	.135	-.175	.199	-.110	-.142
p	.498	.528	.413	.351	.608	.509
FP						
r	-.042	.084	.020	.317	-.232	-.334
p	.845	.695	.928	.131	.274	.110

Statistically significance association is indicated by bold values ($p < 0.05$).
VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; FA = femoral anterior; FC = femoral central; FP = femoral posterior.

Table 4.5 T_2 relaxation times of tibial regions (superficial layer) versus knee muscle co-contraction indices and ratios of the involved limb – 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
TA						
r	.038	-.223	-.134	-.162	.242	-.074
p	.859	.295	.531	.450	.255	.731
TC						
r	.083	.387	.181	.135	-.085	.091
p	.700	.062	.397	.531	.692	.674
TP						
r	-.169	-.041	-.073	-.202	-.005	.167
p	.428	.850	.733	.344	.983	.437

Statistically significance association is indicated by bold values ($p < 0.05$).
VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; TA = tibial anterior; TC = tibial central; TP = tibial posterior.

Table 4.6 T2 relaxation times of tibial regions (deep layer) versus knee muscle co-contraction indices and ratios of the involved limb – 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
TA						
r	-.142	.114	-.207	.157	-.014	-.137
p	.508	.595	.332	.462	.949	.523
TC						
r	-.138	.003	-.075	.248	-.093	-.361
p	.520	.988	.729	.242	.666	.083
TP						
r	.160	.069	-.093	.019	.200	-.023
p	.455	.750	.666	.931	.349	.915

Statistically significance association is indicated by bold values ($p < 0.05$).

VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; TA = tibial anterior; TC = tibial central; TP = tibial posterior.

Table 4.7 T$_2$ relaxation times of femoral regions (superficial layer) 24 months after ACLR versus knee muscle co-contraction indices and ratios of the involved limb 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
FA						
r	.088	.006	.188	-.074	.003	.124
p	.682	.977	.379	.733	.990	.563
FC						
r	-.218	.034	-.224	-.031	.113	.037
p	.307	.875	.292	.887	.600	.862
FP						
r	-.158	-.132	.001	.120	-.149	-.233
p	.461	.540	.997	.577	.488	.273

Statistically significance association is indicated by bold values (p < 0.05).
VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; FA = femoral anterior; FC = femoral central; FP = femoral posterior.

Table 4.8 T2 relaxation times of femoral regions (deep layer) 24 months after ACLR versus knee muscle co-contraction indices and ratios of the involved limb 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
FA						
r	.015	.007	.214	.065	-.263	-.045
p	.946	.973	.316	.764	.214	.836
FC						
r	-.007	.097	.083	.239	-.206	-.169
p	.975	.651	.702	.262	.334	.429
FP						
r	-.314	.053	-.149	.148	-.216	-.116
p	.135	.805	.487	.489	.311	.588

Statistically significance association is indicated by bold values (p < 0.05).
VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; FA = femoral anterior; FC = femoral central; FP = femoral posterior.

Table 4.9 T$_2$ relaxation times of tibial regions (superficial layer) 24 months after ACLR versus knee muscle co-contraction indices and ratios of the involved limb 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
TA						
r	.265	.225	.109	-.057	.061	.247
p	.211	.290	.622	.793	.776	.244
TC						
r	-.065	.335	-.104	.023	-.042	.250
p	.764	.110	.629	.913	.844	.240
TP						
r	.130	-.006	.081	-.147	-.059	.306
p	.546	.978	.708	.493	.783	.146

Statistically significance association is indicated by bold values ($p < 0.05$).
VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; TA = tibial anterior; TC = tibial central; TP = tibial posterior.

Table 4.10 T2 relaxation times of tibial regions (deep layer) 24 months after ACLR versus knee muscle co-contraction indices and ratios of the involved limb 3 months after ACLR.

	VM-MH	VM-MG	VL-LH	VL-LG	VM-MH / VL-LH	VM-MG / VL-LG
TA						
r	-.235	-.097	-.282	-.126	-.028	.070
p	.269	.652	.181	.559	.897	.744
TC						
r	-.097	.354	.006	.038	-.173	.282
p	.651	.090	.980	.859	.419	.182
TP						
r	.223	.095	.043	.082	-.019	.115
p	.295	.658	.840	.703	.932	.594

Statistically significance association is indicated by bold values ($p < 0.05$).
VM-MH = vastus medialis/semitendinosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/the long head of biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius; TA = tibial anterior; TC = tibial central; TP = tibial posterior.

4.5. Discussion

To our knowledge, this study was the first study exploring the relationship between medial and lateral knee muscle CCIs during gait and the biochemical structure of medial tibiofemoral cartilage (via assessing T_2 relaxation times) within the involved limb after ACLR. Our findings contradict our hypotheses and indicate that walking with altered co-contractions of medial and lateral knee muscle pairs 3 months after surgery were not associated with the biochemical markers (T_2 relaxation times) reflective of cartilage health neither at 3 months nor 2 years after ACLR. This may suggest that walking with altered co-contraction of knee muscles early after ACLR may not have a harmful impact on the biochemical composition of cartilage later on.

One of the mechanisms leading to OA development after ACLR is underloading the medial compartment of the knee joint [28]. Particularly, our lab found that those who walk with medial tibiofemoral underloading 6 months after ACLR develop radiographic OA 5 years later [28]. As medial tibiofemoral underloading is detected at 3 months after ACLR during gait [42], the impact of medial compartment underloading on cartilage health may begin as early as 3 months after surgery. Several studies reported that tibiofemoral loading is influenced by the co-contraction of medial and lateral knee musculature [52–54]. As medial and lateral knee muscle pairs have varus and valgus moment arms, respectively, they work oppositely to provide resistance against alterations in frontal knee loading. In previous research, we found that higher lateral knee muscle CCIs were detected at 3 months after ACLR in the involved limb during gait [90]; thus, it's thought that these alterations may impact knee loading, and then predispose cartilage's

components to degradation. However, our findings in the current study displayed no significant associations between medial/lateral CCIs of knee muscle pairs and the T_2 relaxation times of any ROIs neither at 3 nor 24 months after ACLR.

Three months following ACLR, our findings did not show any associations between the CCIs of knee muscles and the T2 relaxation times of tibiofemoral cartilage. In contrast, Pietrosimone et al. (2019) found that quadriceps weakness is associated with MRI biochemical markers indicative of early cartilage degeneration (6 months after ACLR) in the medial compartment of the involved limb [45]. This association was seen in the entire femoral regions, yet it was more significant in the femoral anterior region compared to the other regions. Although that study did not investigate the activation and/or strength of medial and lateral knee extensors separately, it suggests that neuromuscular alterations and deficits early after ACLR may result in deteriorations in the biochemical elements that make up the articular cartilage. Although not statistically significant, similarly, our findings displayed a positive moderate association ($p = 0.056$, $r = 0.396$) between the VM-MH CCIs and the T_2 relaxation times of the deep layer of femoral anterior region 3 months after ACLR. Additionally, our lab, in a recent work, investigated the association between gait knee mechanics and cartilage health (via assessing T_2 relaxation times) at 3 months after ACLR [41]. That study found a positive association between external knee adduction moments and T_2 relaxation times of the deep layer of femoral central and posterior regions. As higher external knee adduction moments produce greater loading on the medial compartment [44,54], it's not surprising to have an association with the T2 relaxation times of femoral regions in the medial

compartment. This implies that walking with higher medial CCIs of knee muscles may not induce an elevated loading applied on the medial compartment as greater external knee adduction moments do. Otherwise, elevated CCIs of knee muscles may cause changes in cartilage biochemistry that can't be detected as early as 3 months after ACLR. Future studies should use more advanced techniques to examine the influence of altered knee muscle co-contractions on tibiofemoral cartilage following ACLR.

Regarding superficial layers, our findings displayed a positive moderate association (not statistically significant) ($p = 0.062$, $r = 0.387$) between the VM-MG CCIs and the T_2 relaxation times of tibial central region 3 months after ACLR. This may suggest that the superficial layer of tibial regions (vs. femoral regions) may be more susceptible to altered co-contraction of medial knee musculature. However, these findings are inconclusive since the statistical significance of the association was not achieved, and because there are no other studies examining the influence of medial CCIs of knee muscles and/or external adduction moments on the superficial layer of tibial regions during gait early after ACLR. Future research with a larger sample size should examine the influence of altered CCIs of knee muscles on the superficial layer of tibiofemoral cartilage early after ACLR.

In terms of the longitudinal influence of altered knee muscle CCIs on cartilage health, the present study did not show any significant associations. Contrary to our findings, Titchenal et al. (2018) investigated the association between the T_2 relaxation times of femoral and tibial regions (deep layer) and external knee adduction moments 24 months after ACLR [64]. That study demonstrated significant associations between higher

external knee adduction moments and prolonged T_2 relaxation times of femoral and tibial central regions. However, the previous study did not investigate the longitudinal influence of altered external knee adduction moment on tibiofemoral cartilage health. Instead, the association was assessed at a specific time point, which was 24 months after ACLR; also, the changes in superficial layers of tibiofemoral cartilage were not addressed. Regardless of the statistical significance of our findings, the present study displayed positive moderate associations between the VM-MG CCIs 3 months after ACLR and the T_2 relaxation times of the superficial and deep layers ($p = 0.110$, $r = 0.335$; $p = 0.090$, $r = 0.354$; respectively) of tibial central region 24 months later. Although the CCIs of knee muscles and external knee moments influence knee frontal loading [44,52–54], little is known regarding the longitudinal effect of altered knee muscle co-contractions and frontal knee moments on the biochemical composition of tibiofemoral cartilage after ACLR. Thus, this question should be addressed in future research.

A previous study showed that healthy people walk with a balance between medial and lateral CCIs of knee muscle pairs [46]. This is not surprising as several studies reported that alterations in frontal knee loading are resisted by the co-contraction of medial and lateral knee muscles [52–54]. However, our findings did not show any significant associations between the ratios of M:L CCIs and the T_2 relaxation times of any ROIs in tibiofemoral cartilage. In contrast, multiple studies reported that the balance between medial and lateral quadriceps cross-sectional area (CSA) may be a contributing factor in medial tibiofemoral OA development [107,108]. Although these studies demonstrated the balance between knee extensors' CSA (not CCIs), they support the fact that the balance

between medial and lateral knee muscles might be essential for cartilage health. While the CCIs of medial and lateral knee muscle pairs influence frontal knee loading [52–54], it's possible that our cohort walked without a severe imbalance between medial and lateral CCIs. Another explanation might be that the influence of unbalanced CCIs existed, but it did not achieve the statistical significance due to our small cohort. The second explanation might be more accurate as our findings showed moderate associations between M:L ratios of CCIs and the T_2 of some ROIs. Particularly, the present study displayed negative moderate associations (not statistically significant) between the M:L CCIs ratio of VM-MG / VL-LG and the T_2 relaxation times of the deep layer of tibial central and femoral posterior regions ($p = 0.083$, $r = -0.361$; $p = 0.110$, $r = -0.334$; respectively) 3 months after ACLR, indicating that more laterally dominated co-contraction (vs. medial) might be associated with prolonged T_2 relaxation times (signs of biochemical deterioration of cartilage) in these regions. During rehabilitation after ACLR, although quadriceps strength is considered one of the primary components of the protocol [109–111], a recent study found that restoring quadriceps strength symmetry early after ACLR was not enough to restore symmetrical medial tibiofemoral loading [112]. It's unknown whether or not the separate evaluation and intervention of medial and lateral knee muscles is more effective after ACLR to reduce the potential abnormal knee loading after surgery, and then reduce the risk of knee OA development. Thus, further research is needed to examine whether or not rehabilitation protocols targeting medial and lateral knee muscles' activation and strength separately help restore knee gait biomechanics, and then maintain healthy cartilage.

Although the biochemical structure of medial femoral central and posterior regions are commonly affected by altered knee gait mechanics and neuromuscular deficits after ACLR [41,45,64], our findings surprisingly did not show any association (even if not statistically significant) between knee muscle CCIs and the T_2 relaxation times of these regions. This might be because not all individuals after ACLR develop knee OA as most of the epidemiological studies investigating OA prevalence after ACLR reported that degenerative changes in cartilage are detected from 5 years and later after surgery [10,11,15]. Thus, it might be kind of early to detect signs of poor cartilage health at 2 years after ACLR. Also, we believe that the present study might have been underpowered to detect significant associations between knee muscle CCIs and the T_2 relaxation times of any ROI.

Several limitations need to be considered in this study. First, having a small sample size was one of the limitations that might impact our findings; thus, future studies with longitudinal larger cohorts are warranted to provide sufficient power. Secondly, our knowledge about the rehabilitation interventions utilized with our participants was limited, which may have resulted in variations in neuromuscular strategies. Also, the use of surface EMG electrodes might potentially influence the EMG values due to skin motion; thus every electrode was affixed to the participant using a combination of tape and wraps. Additionally, it should be mentioned that our findings regarding knee muscles CCIs represented the initial half of the stance phase during gait. Therefore, future investigations should encompass the entirety of the stance phase to capture a more comprehensive range of neuromuscular alterations that may be observed and associated

with cartilage health. In terms of MRI scan parameters, they were different between the participants at 3 months after ACLR (**Table 4.2**). However, these differences were made purposefully to produce images with high quality and resolution, and they had minimal impact on T_2 relaxation time calculations [113].

4.6.   Conclusion

This study did not demonstrate any significant association between the co-contraction of medial/lateral knee muscle pairs and the T_2 relaxation times of any region in the medial tibiofemoral cartilage. The findings of the present study suggest that walking with altered co-contractions of medial and/or lateral knee muscles might not be harmful to medial cartilage following ACLR. However, further research with a larger sample size is warranted to examine to what extent early altered knee neuromuscular strategies after ACLR may influence cartilage composition in later than 2 years after surgery.

4.7.   Acknowledgments

Chapter 5

CONCLUSIONS

5.1.   Overall Purpose

The overall goal of this work is to identify early neuromuscular alterations after ACLR during gait, which might influence medial tibiofemoral loading and subsequently increase the susceptibility of cartilage to the development of post-traumatic knee OA. This dissertation particularly aimed to evaluate the co-contraction of medial and lateral knee muscle pairs (Aim 1) and to what extent altered co-contractions of knee muscle influence medial tibiofemoral loading (Aim 2) during gait early following ACLR. This dissertation also aimed to examine the association between altered knee muscle co-contractions and the biochemical structure of medial knee cartilage after ACLR (Aim 3). This work provides new insights into the potential neuromuscular mechanisms leading to altered tibiofemoral loading after ACLR. Also, this information might be beneficial for clinicians to design effective rehabilitative protocols for individuals after ACLR.

5.2.   Aim 1 Summary

5.2.1.   Aim 1 Purpose

Compare the CCIs of medial and lateral knee muscle pairs in the involved and uninvolved limbs between sexes during gait 3 months after ACLR.

5.2.2. Aim 1 Hypotheses

Hypothesis 1.1: The involved limb would display higher CCIs of medial and lateral knee muscle pairs compared to the uninvolved limb during gait 3 months after ACLR.

Hypothesis 1.2: Women would display higher CCIs of medial and lateral knee muscle pairs compared to men.

5.2.3. Aim 1 Findings

Our findings support Hypothesis 1.1 partially, as individuals early after ACLR walk with higher CCIs of the VL-LH muscle pair (lateral knee musculature) in the involved limb (vs. uninvolved). Also, Hypothesis 1.2 was supported partially by our findings that showed women (vs. men) exhibit different neuromuscular adaptation by producing higher CCIs of muscle pairs incorporating the lateral gastrocnemius 3 months after ACLR. Thus, these discrepancies in neuromuscular adaptations between limbs and sexes may lead to altered gait mechanics after ACLR.

5.3. Aim 2 Summary

5.3.1. Aim 2 Purpose

Evaluate the association between the CCIs of medial and lateral knee muscle pairs and peak medial compartment force (pMCF) during gait 3 months after ACLR.

5.3.2. Aim 2 Hypotheses

Hypothesis 2.1: Medial compartment underloaders would show greater CCIs of lateral knee muscle pairs compared to symmetric loaders and overloaders in the involved limb during gait 3 months after ACLR.

Hypothesis 2.2: The involved limb would show a more lateral-dominated imbalance in medial-to-lateral [M:L] CCI ratios compared to the uninvolved limb during gait 3 months after ACLR.

Hypothesis 2.3: Lower M:L CCIs ratios would be associated with lower pMCFs in the involved limb during gait 3 months after ACLR.

5.3.3. Aim 2 Findings

Our results displayed that underloaders (involved loading less than uninvolved loading) walk with greater lateral CCIs in the involved limb compared to symmetric loaders (involved and uninvolved limb loads equivalent) for the VL-LG muscle pair 3 months after ACLR. Additionally, we found that individuals walk with more lateral-dominant CCIs (vs. medial) in the involved limb following surgery, and that this imbalance is associated with lower medial compartment forces. Our findings suggest that altered knee muscle co-contractions patterns may contribute to altered knee loading early after ACLR, which, in turn, may negatively impact long-term knee cartilage health.

5.4. Aim 3 Summary

5.4.1. Aim 3 Purpose

Examine the relationship between medial/lateral knee muscle CCIs during gait 3 months after ACLR and the biochemical structure of medial tibiofemoral

cartilage (via assessing T_2 relaxation times) within the involved limb 3 and 24

months after surgery.

5.4.2. Aim 3 Hypotheses

Hypothesis 3.1: Higher CCIs of medial and lateral knee muscle pairs in the involved limb 3 months after ACLR would be associated with prolonged medial tibiofemoral T_2 relaxation times 3 and 24 months after ACLR.

Hypothesis 3.2: Lower medial-to-lateral (M:L) CCIs ratios (medial CCIs < lateral CCIs) of the involved limb 3 months after ACLR would be associated with greater medial tibiofemoral T_2 relaxation times at 3 and 24 months following ACLR.

5.4.3. Aim 3 Findings

Our findings contradict our hypotheses and indicate that walking with altered co-contractions of medial and lateral knee muscle pairs 3 months after ACLR are not associated with the biochemical markers (T_2 relaxation times) reflective of cartilage health neither at 3 months nor 24 months after ACLR. This may suggest that walking with altered co-contraction of knee muscles early after ACLR may not have a harmful impact on the biochemical composition of cartilage during the first 2 years after surgery.

5.5. Clinical Implications and Future Research

This dissertation has answered some important questions related to the neuromuscular mechanisms that might be associated with the development of knee OA after ACLR. The findings of this dissertation may help clinicians and rehabilitation specialists to design effective rehabilitation strategies for individuals following ACLR

aimed at addressing early altered co-contraction of medial and lateral knee muscle pairs. Future work is warranted to examine if addressing these alterations early after ACLR would help restore knee loading symmetry after surgery and subsequently protect knee cartilage from degeneration. Also, future research should investigate if the alterations in knee muscle co-contractions after ACLR are caused by deficits in central nervous system, structure/function of knee muscles, or both. It's also recommended to investigate the neural characteristics (spinal reflexes, corticospinal excitability, and cortical activation) of knee muscles after ACLR, and whether alterations in these characteristics are associated with the onset of post-traumatic knee OA.

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Appendix A

AIM2: KNEE MUSCLE CO-CONTRACTION INDICES DURING GAIT 3 MONTHS AFTER ACLR VS. HEALTHY SUBJECTS

This figure shows knee muscle co-contraction indices of the involved and uninvolved limbs 3 months after ACLR (Mean [± standard deviation]) in symmetric loaders, underloaders, overloaders, and healthy subjects (involved and uninvolved CCIs were averaged). IN = involved limb/ UN = uninvolved limb; VM-MH = vastus medialis/semimembranosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius.

3 Months Co-contraction Indices

Figure legend: IN Symmetric loaders, UN Symmetric loaders, IN Underloaders, UN Underloaders, IN Overloaders, UN Overloaders, Control

Categories (x-axis): VM-MH, VM-MG, VM-LG, VL-LH, VL-LG, VL-MG

Y-axis: Co-contraction Index
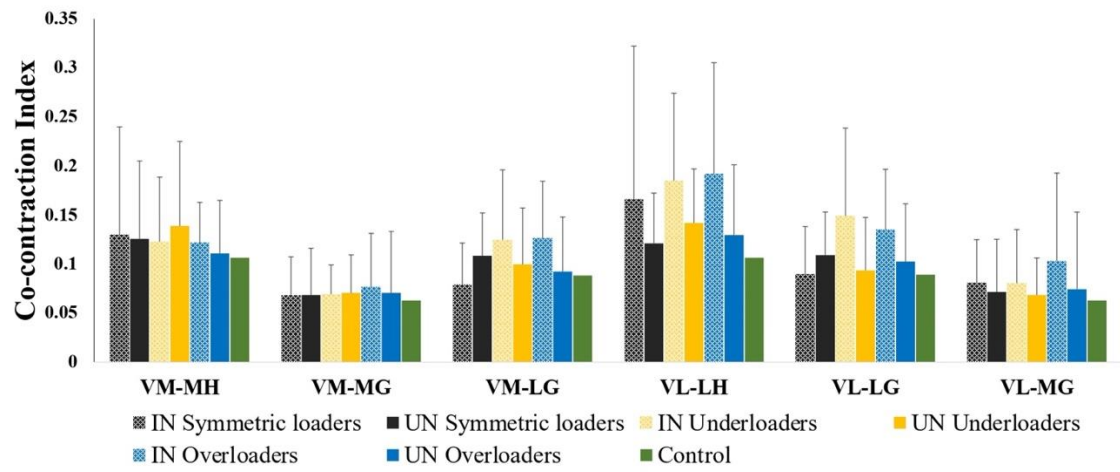
Appendix B

AIM3: SCATTERPLOTS FOR THE ASSOCIATIONS SHOWING MODERATE EFFECT SIZE

These figures show the association between knee muscle co-contraction indices/ratio of the involved limb at 3 months after ACLR and the T2 relaxation values of some regions of medial tibiofemoral cartilage at 3 and 24 months after ACLR. CCI = co-contraction index; VM-MH = vastus medialis/semimembranosus; VM-MG = vastus medialis/medial gastrocnemius; VM-LG = vastus medialis/lateral gastrocnemius; VL-LH = vastus lateralis/biceps femoris; VL-LG = vastus lateralis/lateral gastrocnemius; VL-MG = vastus lateralis/medial gastrocnemius.

Femoral Anterior Region (Deep Layer)

Tibial Central Region (Superficial Layer)

Tibial Central Region (Deep Layer)

Tibial Central Region (Superficial Layer)

R² Linear = 0.153

Tibial Central Region (Deep Layer)

R² Linear = 0.113
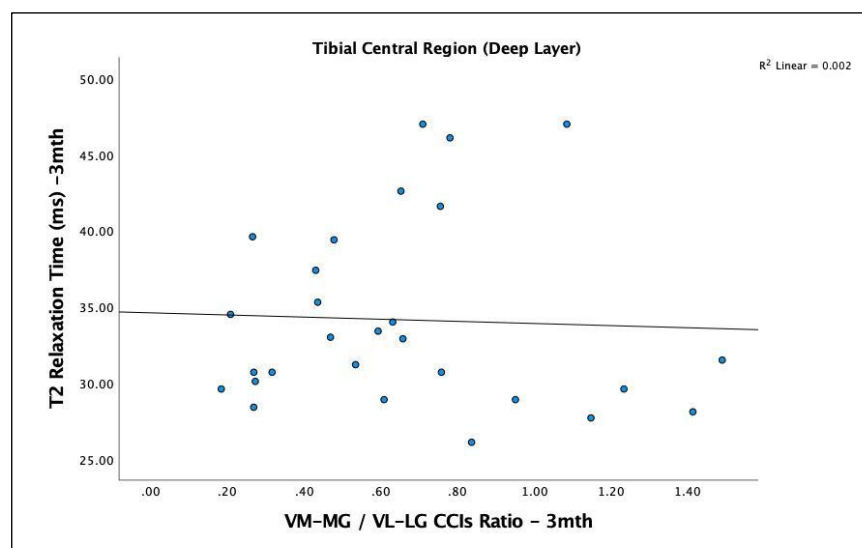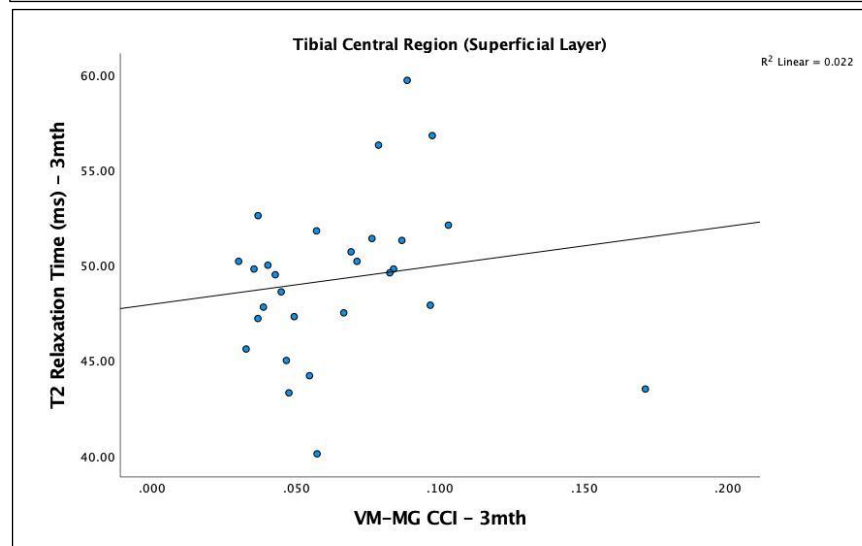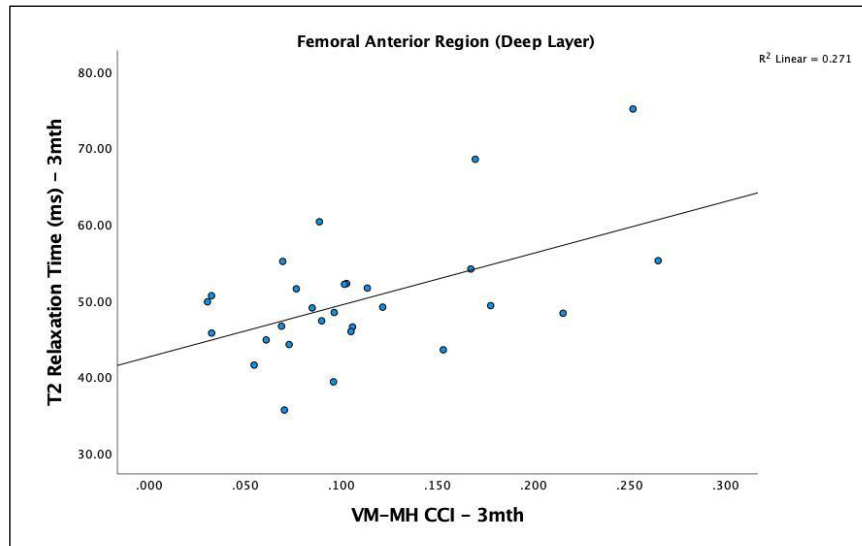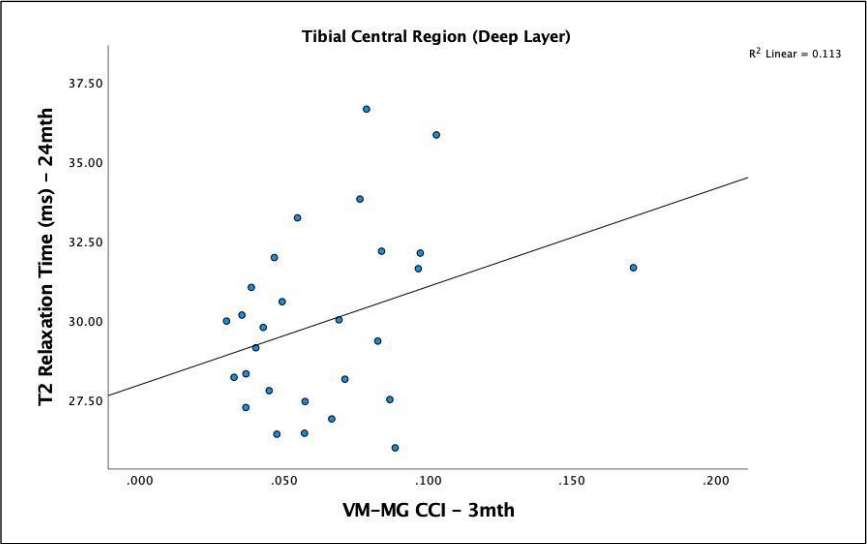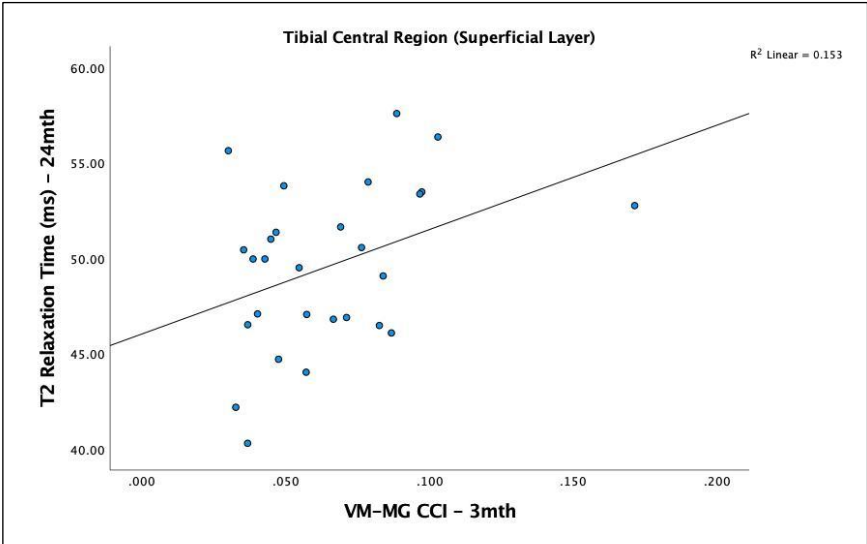
IRB / HUMAN SUBJECTS APPROVAL

Study title: Understanding the role of unloading in the knee in osteoarthritis (OA) following anterior cruciate ligament reconstruction (ACLR).

UNIVERSITY OF DELAWARE.

DATE: March 1, 2022

TO: Thomas Buchanan, PhD
FROM: University of Delaware IRB

STUDY TITLE: [868724-16] Understanding the role of unloading in the knee in osteoarthritis
                              (OA) following anterior cruciate ligament reconstruction (ACLR).
SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED FOR DATA ANALYSIS ONLY
APPROVAL DATE: March 1, 2022
EXPIRATION DATE: March 15, 2023
REVIEW TYPE: Expedited Review
REVIEW CATEGORY: Expedited review category # *(8)*

Thank you for your Continuing Review/Progress Report submission to the University of Delaware
Institutional Review Board (UD IRB). The UD IRB has reviewed and APPROVED the proposed research
and submitted documents via Expedited Review in compliance with the pertinent federal regulations.

As the Principal Investigator for this study, you are responsible for and agree that:

- All research must be conducted in accordance with the protocol and all other study forms as
  approved in this submission. Any revisions to the approved study procedures or documents must
  be reviewed and approved by the IRB prior to their implementation. Please use the UD amendment
  form to request the review of any changes to approved study procedures or documents.

- Informed consent is a process that must allow prospective participants sufficient opportunity to
  discuss and consider whether to participate. IRB-approved and stamped consent documents must
  be used when enrolling participants and a written copy shall be given to the person signing the
  informed consent form.

- Unanticipated problems, serious adverse events involving risk to participants, and all non-
  compliance issues must be reported to this office in a timely fashion according with the UD
  requirements for reportable events. All sponsor reporting requirements must also be followed.

Oversight of this study by the UD IRB REQUIRES the submission of a CONTINUING REVIEW seeking
the renewal of this IRB approval, which will expire on March 15, 2023. A continuing review/progress
report form and up-to-date copies of the protocol form and all other approved study materials must be
submitted to the UD IRB at least 45 days prior to the expiration date to allow for the required IRB review
of that report.

If you have any questions, please contact the UD IRB Office at (302) 831-2137 or via email at hsrb-
research@udel.edu. Please include the study title and reference number in all correspondence with this
office.

LOCATION, COMPENSATION, AND CONSENT FORMS

Study title: Understanding the role of unloading in the knee in osteoarthritis (OA) following anterior cruciate ligament reconstruction (ACLR).

QUANTITATIVE MAGNETIC RESONANCE IMAGING (qMRI) STUDY

MOTION ANALYSIS TESTING: STAR campus, Room # 107

540 S College Ave, Newark DE 19713

NOTE: Free parking

Contact: Jack Williams: jackrw@udel.edu, 1 (315) 794-5583
Majeed Alfayyadh: fayyadh@udel.edu, 1 (302) 750-3376

DATE: TIME:

MAGNETIC RESONANCE IMAGING: Center for Biomedical and Brain Imaging

77 East Delaware Ave, Newark DE 19713

NOTE: Free parking in reserved spot (Center for Brain and Biomedical Imaging) behind Spencer Building
(Additional parking also available at 134 E Main St: Located between E Main St and E Delaware Ave: 1 USD for 1 hour)

Contact: Jack Williams: jackrw@udel.edu, 1 (315) 794-5583
Kelsey Neal: kaeneal@udel.edu, 1 (505) 412-9275

DATE: TIME:

XRAY: Go-Care at Abby Medical

1 Centurian Drive, Suite 106, Newark DE 19713

NOTE: Free parking
Appointment NOT required
M-F: 9 AM - 9 PM,
SAT: 9 AM - 6 PM, SUN: 10 AM - 6 PM

Contact: Laurie Cox
lcox@gocaredelaware.com, 1.302.999.0331
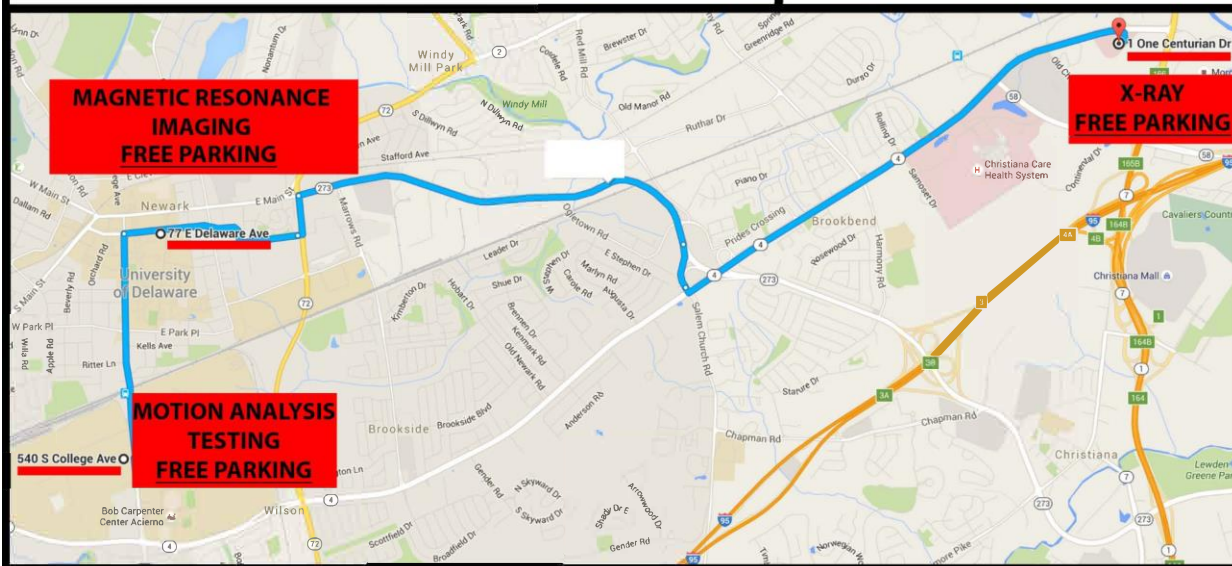
College of Health Sciences
RESEARCH PARTICIPANT COMPENSATION FORM

PLEASE PRINT CLEARLY

PARTICIPANT'S INFORMATION

First Name:_____ Last Name:_____ Phone:_____

Street:_____ Citizenship: ☐ U.S. citizen

City:_____ State:_____ Zip:_____ ☐ Permanent resident (UD W-8 required)

Email:_____ ☐ Non-resident (UD W-8 required)

UD AFFILIATION

If current employee, select one: ☐ Graduate student   ☐ Undergraduate student   ☐ Other

　　　　ID #:_____ Department:_____ HR Liaison:_____

☐ Past UD employee

☐ Never employed by UD

STUDY DATES AND COMPENSATION INFORMATION

Session Date	Compensation
MAL:	50.00
IMAGING 1:	50.00
IMAGING 2:	50.00
TOTAL	

Grant/Study Title: qMRI R01HD087459 BUCHANAN TS ACLR: qMRI & BIOMECH.

Purpose Code: DERI 322115

Account Code: 149250

Preferred Payment Method:

☐ Gift card (vendor):_____

　Card #:_____

☐ Payment by check
　For business office use:
　*Req ID:*_____

AUTHORIZATION

Submitted by: Jack Williams/Kelsey Neal/Majeed Alfayyadh _____ _____

　　　　　　　　　　　　PRINT NAME　　　　　　　　　　　　　　　　SIGNATURE　　　　　　　　　DATE

Supervisor/PI:_ TS BUCHANAN / MARLO GOSS _____ _____

　　　　　　　　　PRINT NAME　　　　　　　　　　　SIGNATURE　　　　　　　　　DATE

✂ **(Cut here)**

- -

IMPORTANT: If the SSN is collected, study personnel **must remove and shred** this section after submission to the departmental business office. **Do not retain the SSN in the study records**. Departmental business office **must remove and shred** this section before forwarding to grants administration.

Social Security #: _____-____-_____ (required for payment by check for non-UD employees or inactive UD employees)

Revised 5/29/16

INFORMED CONSENT/ASSENT/PARENTAL PERMISSION TO PARTICIPATE IN RESEARCH

Title of Project: Understanding the role of unloading in the knee in osteoarthritis (OA) following anterior cruciate ligament reconstruction (ACLR)

Principal Investigator(s): Dr. Thomas S. Buchanan

You or your child are being invited to participate in a research study. This consent form tells you about the study including its purpose, what you will be asked to do if you decide to take part, and the risks and benefits of being in the study. Please read the information below and ask us any questions you may have before you decide whether or not you want to participate as a volunteer or parent.

Participation is voluntary for your or your child and you can refuse to participate or withdraw at any time without penalty or loss of benefits to which you are otherwise entitled. If you decide to participate, you will be asked to sign this form and a copy will be given to you to keep for your reference.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this research study is understand the role of unloading in the knee in osteoarthritis (OA) following anterior cruciate ligament reconstruction (ACLR) surgery.

Though ACLR restores knee stability, it does not fully address abnormal knee movement and knee loading patterns, i.e. abnormal walking patterns where the ACLR knee is unloaded compared to the other non-surgical knee. Abnormal walking patterns are believed to be a mechanism leading to knee osteoarthritis (OA). Knee OA is a condition wherein the load bearing region of the knee, the cartilage, undergoes degradation. Reliable identification of knee OA requires the use of radiographs, commonly known as X-rays.

Motion analysis testing is the most common method used to analyze walking patterns. Motion analysis testing comprises of non-invasive walking experiments, often including surface electromyography (non-invasive muscle signal recording) to estimate muscle coordination patterns and force production.

In addition to walking patterns, biological and chemical changes (biochemical changes) in knee cartilage are also believed to affect the progression of OA. An imaging method, known as quantitative magnetic resonance imaging (MRI) allows for non-invasive estimation of these changes in knee cartilage. Quantitative MRI is being increasingly used over the past two decades. However, estimates of biochemical changes in knee cartilage, specific to an ACLR population, are not yet readily available in literature. It is also not known how these changes are related to walking patterns. Finally, changes in knee geometry, which can be studied using standard MRI, are also known to affect the progression of knee OA.

With that background, the purpose of this research project is to study changes in walking patterns and biochemical properties of knee cartilage after ACLR. Knee geometry measurements and OA related changes will also be evaluated. 75 subjects will be recruited 3 months after ACLR. Testing will be conducted for each subject at the following time points after ACLR:

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- 3, 6, and 24 months

Motion analysis testing will be used to assess walking patterns. Quantitative MRI will be used to estimate biochemical changes in knee cartilage, while standard MRI will be used to construct a geometric representation of the knee. Finally, the presence/absence of knee OA and progression of OA will be verified using radiographs.

In addition, 30 subjects, in the same age groups as ACLR subjects, but with no history of knee injury will also be recruited, to allow for comparison against subjects with ACLR. Control subjects will only be required to complete motion analysis testing and imaging (quantitative and standard MRI), similar to subjects with ACLR, and at one time point only, i.e. immediately after recruitment.

The ultimate goal of this research project is to use a mathematical model to reveal conditions that can affect the knee cartilage negatively, and result in knee OA. We also hope that an improved understanding of these conditions will eventually contribute to preventative therapeutic protocols.

WHY ARE YOU OR YOUR CHILD BEING ASKED TO PARTICIPATE?

We are asking you or your child to be in the study because you or your child has undergone ACLR approximately three months ago, and can be part of the ACLR group, or because you have no history of knee injury, and can be part of the control group. The age range for participation, for both groups, is between 16 and 45 years.

Exclusion criteria for ACLR group:

You could be excluded from volunteering for the study if you have sustained major leg injury, have undergone major leg surgery that requires serious medical management (i.e. fracture or re-injury), or an ACL injury/repair prior to the most recent procedure in either knee. You could also be excluded if you sustained major tears to other knee ligaments, or repairable meniscus injuries.

Exclusion criteria for control group:

You could be excluded from volunteering for the study if you have sustained major leg injury, have undergone major leg surgery that requires serious medical management (i.e. fracture), knee ligament injuries or knee meniscus injuries.

Exclusion criteria common to both ACLR and control groups:

You could be excluded if you have any condition that prevents you from walking, or laying still on your back.

Additionally, the conditions listed below, if met, will be grounds for exclusion because of standard precautions for imaging. Pregnancy is not a contraindication to MRI of the knee, but our scans may be taken by community providers who screen for and do not perform MRI on pregnant women when it is not medically necessary.

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- Joint replacement with metallic parts

- Surgical procedure that includes metallic components

- Extreme claustrophobia (fear of small, closed spaces)

- Pacemaker (a medical implant in the heart)

- Metal in the body (implants, screws, plates, shrapnel, etc.)

- Aneurysm clips (clips used to treat bulging blood vessels)

- Ear or Eye Implants

WHAT WILL YOU BE ASKED TO DO?

If you or your child want to participate, the information below lists the location and details about the study. All the procedures are non-invasive, i.e. nothing will be inserted in the body, and rather, components will be attached to the surface of the body, when required.

Motion analysis testing will be conducted at STAR campus at the University of Delaware (540 S College Avenue, Newark DE 19713).

MRI will be conducted at either of the following locations:

- University of Delaware's Center for Biomedical or Brain Imaging, located at 75 East Delaware Avenue, Newark DE 19716, OR

- Diagnostic Imaging Associates, located at L-6 Omega Drive, Newark DE 19713, OR

- Best Open MRI-Abby Medical Center, 1 Centurian Drive, Suite 107, Newark DE 19713

Finally, radiography (x-ray imaging) will take place at either of the following locations:

- Diagnostic Imaging Associates, located at L-6 Omega Drive, Newark DE 19713, OR

- Go-Care at Abby Medical Center, 1 Centurian Drive, Suite 106, Newark DE 19713, OR

- First State Orthopedics (4745 Ogletown Stanton Rd #225, Newark, DE 19713) OR

The information below provides a description of what the testing sessions will include.

<u>**Study questionnaire**</u>

<u>Relevant time points: 3, 6 and 24 months</u>

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You or your child will fill out a survey form that will be used to capture information related to injury and functional capabilities. This will enable us to get information about the current status of the knee. It generally takes an average of 5 minutes for the survey form to be filled out.

Motion analysis testing

Relevant time points: 3, 6 and 24 months

Surface electrodes taped to your (or your child's) skin will be used to record the electrical activity of your muscles (electromyography). After all electrodes have been placed, you will perform a maximum contraction of each muscle (i.e. applying maximum effort that is comfortable), with straps applied to your ankles to provide resistance. Nine electrodes will be secured to each leg and then plugged into a small (6" x 4" x 3") transmitter box that will be attached to the back of a vest with Velcro. The transmitter sends the signal to the computer so we can determine when the muscles are contracting during the activities. These measurements will also be taken during the walking trials of motion analysis testing.

Markers will be attached to your skin and sneakers on both legs using adhesive skin tape. Shells with markers on them will be placed on your pelvis, thighs and calves and will be held in place with elastic wraps. These markers will allow the cameras to track your leg positions. You will be asked to perform several walking trials in our laboratory. Walking trials will give us information about the way your hips, knees, and ankles move while you walk. You will be asked to perform 7 trials of walking at a comfortable, self-selected speed, although additional trials may be required to obtain enough data. During the trials, you will also walk over a force plate that is embedded in the floor. The force plate enables collection of loading data during walking.

The electrodes and markers will be removed at the end of the testing session. Motion analysis testing is a safe, non-invasive process. The entire testing session will last approximately two (2) hours.

Quantitative and Standard MRI

Relevant time points: 3, 6 and 24 months

This study involves measuring anatomy and estimating biochemical properties of the knee using magnetic resonance imaging (MRI).

You (or your child) will be required to lie completely still on the scanner bed that will slide into the center (bore) of the MRI scanner. A knee coil will be placed around each leg, alternately, to measure the signal emitted from the knee. Pillows and other cushions may be used to make you more comfortable. Several scans will be taken and you will be required to remain still on the table for about 5-10 minutes at a time. You will be given periodic breaks in which they will be able to relax but will be asked to remain on the scanner bed for the duration of the session, which should last about 45-50 minutes. Another similar MRI session will also be conducted, which can be on the same day, or a different day, depending on your preference.

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You (or your child) will be able to communicate with us via a built-in intercom. You will also be holding an emergency bulb that you can squeeze at any time to let us know you want to come out of the MRI scanner. If at any time you feel uncomfortable or unwilling to continue, no matter what the reason, you can request to immediately stop the study, and the operator will remove you from the scanner. All scans will be conducted by a certified MRI Technologist or other experienced personnel with relevant safety training.

These scans will provide information regarding biochemical and geometrical knee properties.

This is not a clinical evaluation

The images of the knee collected in this study are not intended to reveal illness, in part because this research protocol is not designed for clinical diagnosis. The images will not be routinely examined by a clinical radiologist. The personnel at the MRI Center are not qualified to medically evaluate these images. However, if, in the course of collecting images, we have any concerns, we may show scans to a clinical radiologist, who may suggest that you (or your child) obtain further diagnostic tests. Do not rely on this research MRI to detect or screen for abnormalities.

At our discretion, you may view their images and receive digital copies of them. These images will show the inside of the knee and you should be aware of the potential distress or discomfort that may occur by viewing these type of images.

<u>Radiographs</u>

<u>Relevant time points</u>: 3 and 24 months

Standing x-rays will be taken with the knee slightly bent. These x-rays will allow a radiologist to verify the presence or absence of OA, and to determine knee joint space width (JSW) measurements. This takes 5-10 minutes to be completed.

Radiographs at the 3 month time point will be useful for establishing a baseline, while radiographs at the 24 month time point will be useful to verify the presence/absence of knee OA, and OA progression. These x-rays will be locked in a cabinet for research purposes only.

If you (or your child) are part of the control group (i.e. no history of knee injury), you will only be required to complete motion analysis testing and imaging (quantitative and standard MRI), similar to subjects with ACLR, and at one time point only, i.e. immediately after recruitment.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS? (WHAT ARE THE POSSIBLE BAD THINGS ABOUT THIS RESEARCH?)

A few things about this study that could make a volunteer uncomfortable are listed below.

<u>Motion analysis testing</u>

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All motion analysis testing procedures involve a simple walking task that has been standardized at the University of Delaware. The risk of re-injury for the ACL-reconstructed population within the first 5 years after ACLR is approximately 5%. Of the hundreds of tests performed on ACLR individuals at the University of Delaware, no one has torn their surgical graft during testing. At the end of motion analysis testing, you may experience discomfort from the removal of tape holding markers and electrodes in place.

Quantitative and Standard MRI

MRI is an imaging technique that uses radio waves and magnetic fields to produce images of internal structures in the body. It is commonly used in hospitals. Unlike X-rays, the MRI does not use any ionizing radiation, and it does not use radioactivity, so there are no radiation related risks from having an MRI scan. Below there is a description of MRI related risks and what is being done to reduce any possible risks associated with them:

Metal: The MRI scanner produces a constant strong magnetic field, which may cause any metal implants, clips, or implanted medical devices within the body to shift position or malfunction. You (or your child) will not be allowed to participate in this study if you have any implanted metal, clips or devices. You will be screened to make sure that it is safe for you to enter a strong magnetic field. Please provide us with as much information as you can, for example if you had surgery in the past, so that we may decide whether it is safe for you to be a participant. Metallic objects brought into the MRI environment can become hazardous projectiles and can also interfere with the data quality. To minimize this risk, metal earrings, other piercings, necklaces and any other metal in contact with your body will be removed prior to the study. You will also be asked to remove all items from your pockets, including coins, electronics (including cell phones and hearing aids) and wallets. You will also be asked to remove belts with metal buckles, and may be asked to change into a gown that we will provide if your clothing contains significant metal, including metal underwire bras.

Pregnancy: Exposure to MRI scanning might be harmful to an unborn child. Although there are no established guidelines at this time regarding MRI and pregnancy, you (or your child) should be informed that there is a possibility of a yet undiscovered pregnancy related risk. If you know or suspect you may be pregnant or if you do not want to expose yourself to this risk, you will be excluded from participating in this study.

Inner ear damage: MRI scanning produces loud noises that can cause damage to the inner ear if appropriate hearing protection is not used. Earplugs and/or headphones will be provided to protect your (or your child's) ears.

Claustrophobia: When you (or your child) is inside the MRI scanner, the "bore" of the scanner will surround the knee that is being scanned. You will be positioned so that their knee is centered in the bore of the scanner. If you feel anxious in confined, spaces, you may not want to participate. If you are unsure, you can try a "mock" scanner when available, to evaluate the comfort level with the enclosed space of the

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magnet bore. If you decide to participate and begin to feel claustrophobic, you will be able to tell us via the intercom or the squeeze ball and we will discontinue the study immediately.

Burns: In rare cases, contact with the MRI transmitting and receiving coil, conductive materials such as wires or other metallic objects, or skin-to-skin contact that forms conductive loops may result in excessive heating and burns during the experiment. The operators of the MRI scanner will take steps, such as using foam pads when necessary, to minimize this risk. Tattoos with metallic inks can also potentially cause burns. In addition, you (or your child) are requested to let the MRI operator know immediately if you experience any heating or burning sensations during a scan. The scanning session will be stopped as soon as you tell the operator.

Nerve or muscle stimulation: While the scanner is operating, there is a small chance that the rapidly changing magnetic fields could cause a slight tingling sensation or a muscle twitch, usually felt in the upper arms or torso. While these sensations may be startling, they are not dangerous or a health risk, and they have no lasting consequences. The sensations should stop when the scan ends. Because these sensations may nevertheless be distracting or even possibly uncomfortable, you (or your child) will be able to squeeze the signal bulb to alert the scanner operator if you feel tingling or muscle twitching, and we will immediately stop the scan. You will then have the opportunity to choose to withdraw from the study or to continue.

Other Risks: Besides the risks listed above, there are no other known risks from the magnetic field or radio waves at this time. Although MRI scanning has been used for more than 20 years, long-term effects are unknown.

Radiographs

This research study involves exposure to radiation from a standard radiograph (x-ray). This radiation exposure is not necessary for your medical care and is for research purposes only. At each time point that the radiograph is obtained, the total amount of radiation that you will receive in this study is about 0.12 mSv (mili-Silvert) and is approximately equivalent to a uniform whole body exposure of 15 days of exposure to natural background radiation. This use involves minimal risk per National Institutes of Health guidelines, and is necessary to obtain the research information desired. To reduce exposure, you (or your child) will wear a lead apron to cover the rest of your body while the x-rays of your leg are captured.

WHAT IF YOU OR YOUR CHILD ARE INJURED DURING PARTICIPATION IN THE STUDY?

If you or your child are injured during research procedures, you will be offered first aid at no cost to you. If additional medical treatment is needed, the cost of this treatment will be your responsibility or that of your third-party payer (for example, your health insurance). By signing this document, you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

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WHAT ARE THE POTENTIAL BENEFITS? (WHAT ARE THE POTENTIAL GOOD THINGS ABOUT THIS RESEARCH?)

The proposed motion analysis testing procedure aims to study walking patterns after ACLR, and the change in these patterns over time. The data collected will be used to propose a mechanism for OA, and distinguish between abnormal versus normal walking patterns. A link between abnormal patterns and OA has not yet been established and validated. Hence, the proposed motion analysis testing procedure, by itself, cannot identify OA, and as such, no direct benefit to you is expected.

Similarly, the proposed MRI imaging procedure only aims to estimate biochemical properties of the knee cartilage, which has not yet been shown to predict OA. These properties will be used in a mathematical model to reveal conditions that can affect the knee cartilage negatively, and may result in knee OA. The proposed MRI imaging procedure, by itself, cannot predict OA, and as such, no direct benefit to you is expected.

However, we do hope that an improved understanding of the effect of the study measurements will provide information about knee OA in an ACLR population, and contribute to preventative therapeutic protocols in the future.

NEW INFORMATION THAT COULD AFFECT YOUR PARTICIPATION:

During the course of this study, we may learn new information that could be important to you. This may include information that could cause you to change your mind about participating in the study. We will notify you as soon as possible if any new information becomes available.

HOW WILL CONFIDENTIALITY BE MAINTAINED? (WHO MAY KNOW WHO PARTICIPATED IN THIS RESEARCH?)

No one other than the investigators will know that you or your child were in this study. If we tell other people about the research, we will not use names.

More details for adult participants and for parents/guardians of adolescent participants are provided below.

All information obtained during the study will be held in strict confidence to the fullest extent possible by law. In no case will personal identifiable information be shared with any other individuals or groups without your expressed written consent. Your (or your child's) images will be stored on secured computer servers and will be archived indefinitely. Non-identifiable images of your scans may be used for teaching purposes, be presented at meetings, published, and also shared in databases accessible to other researchers for further research and educational purposes. Your names or other identifying information will not be used in any publication or teaching materials without your specific permission.

Identities will be kept confidential by coding them with a subject identification number stored on a password protected computer. Only the investigators and research coordinator will have access to that file on the secure server.

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All data will be electronically encrypted and archived indefinitely for comparative analyses of scientific and clinical questions related to the ACL injury, surgery and knee OA. All research findings will be compared to knee cartilage properties, knee loading patterns and knee movement patterns reported via peer-reviewed academic journals and conferences that emphasize outcomes after ACLR.

While rare, an accidental breach of confidentiality is a risk. Should an accidental breach of confidentiality occur, the event will be reported to the institutional review board (IRB) immediately, and appropriate follow up steps will be taken based on IRB recommendations.

HIPAA AUTHORIZATION

State and federal privacy laws protect your PHI. These laws say that, in most cases, your health care provider can release your PHI for the purpose of conducting research only if you give permission by signing an Authorization.

The research team would like and appreciate access to your PHI, specifically regarding any knee injury and or surgery, to make the study as complete as possible; however, if you do not sign this Authorization, you may still participate in the research study.

Who May Disclose and Who may Use and/or Receive my PHI?

By signing this document, you are hereby permitting your physicians, medical care providers, and UD's physical therapy clinic to disclose the PHI described in this Authorization to the research team involved in this project; the study sponsor and its employees; the Institutional Review Board (IRB) and other regulatory agencies responsible for overseeing research.

Once your PHI is shared with these persons, you understand that the PHI may no longer be protected by federal or state privacy laws.

What PHI Will Be Disclosed and Used, and for What Purpose?

The following PHI may be disclosed to, collected by, used by, and shared with those listed above:

Operative report (about an operation) and Physical therapy records.

This only pertains to medical records related to your ACL injury and surgery.

This Authorization will expire at the conclusion of the research study. You may cancel this Authorization at any time before, during, or after your participation in this study by giving a written request with your signature on it to the Principal Investigator at buchanan@udel.edu. If you cancel this Authorization, your PHI obtained before that date may still be used for this research study.

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Participant's Initials _____

I hereby authorize the disclosure and use of **my Personal Health Information**

_____   _____

Signature of Patient or Authorized Representative   Date

Printed Name of Person Signing: _____

Relationship to Patient: _____

WILL THERE BE ANY COSTS TO YOU FOR PARTICIPATING IN THIS RESEARCH?

There are no costs associated with participating in the study.

WILL YOU RECEIVE ANY COMPENSATION FOR PARTICIPATION?

Participants will be compensated 50 USD for motion analysis testing, 50 USD for qMRI, and 50 USD for a radiograph (x-ray). Thus, there will be a total of 150 USD compensation associated with each time point, i.e. 3, 6 and 24 month time points.

DO YOU HAVE TO TAKE PART IN THIS STUDY? (CAN YOU CHANGE YOUR MIND ABOUT BEING IN THE STUDY?)

You do not have to say yes. Taking part in this research study is up to you or your child. If you choose to take part, you can change your mind and stop at any time. If, at any time, you decide to stop, please let us know by telling one of the researchers.

If you are a student volunteer and decide not to take part in this research, your choice will not affect your grades or your relationship with your classmates and your teachers.

We may ask you to stop participating if any leg injury that requires serious medical management (i.e. fracture or re-injury) has occurred before the testing session.

WHO SHOULD YOU CALL IF YOU HAVE QUESTIONS OR CONCERNS?

If you have any questions about this study, please contact the Principal Investigator, Dr. Thomas S Buchanan at buchanan@udel.edu or (302) 831-2410.

If you have any questions or concerns about your rights as a research participant, you may contact the University of Delaware Institutional Review Board at hsrb-research@udel.edu or (302) 831-2137.

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For adult participants (at least 18 years old):

Your signature below means that: 1) you are at least 18 years old; 2) you have read and understand the information given in this form; 3) you have asked any questions you have about the research and those questions have been answered to your satisfaction; 4) you accept the terms in the form and volunteer to participate in the study. You will be given a copy of this form to keep.

_____   _____   _____
Printed Name of Participant   Signature of Participant   Date

For adolescent participants (less than 18 years old):

If you want to participate, and we have answered all of your questions about it, please sign below.

_____   _____   _____
Printed Name of Participant   Signature of Participant   Date

For parents/guardians of adolescent participants (less than 18 years old):

You are making a decision whether or not to have your child participate in this study. Your signature indicates that you have read the information provided above and decided to allow your child to participate.

_____   _____ _   _____
Printed Name of Parent/Guardian   Signature of Parent/Guardian   Date

_____   _____   _____
Person Obtaining Consent   Person Obtaining Consent   Date
  (PRINTED NAME)   (SIGNATURE)

OPTIONAL CONSENT TO BE CONTACTED FOR FUTURE STUDIES:

Do we have your permission to contact you regarding participation in future studies? Please write your initials next to your preferred choice.

_____ YES   _____ NO

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Participant's Initials _____

Appendix E

PERMISSION

A permission granted from the Journal of Electromyography and Kinesiology to include the published manuscript of Aim 1 in my dissertation.

UD G Suite

Abdulmajeed Barakat M Alfayyadh <fayyadh@udel.edu>

Re: Permission to use the published manuscript in my thesis [230707-002136]

Journal of Electromyography and Kinesiology <JEK@elsevier.com>   Sun, Jul 9, 2023 at 10:32 PM
Reply-To: Journal of Electromyography and Kinesiology <JEK@elsevier.com>
To: fayyadh@udel.edu

Dear Dr. Alfayyadh,

Thank you for your email.

Please let the author know that it is fine for the published article to be used in the thesis, providing the thesis is not going to be produced commercially.

Please do not hesitate to contact me for further assistance.

Thanks & Regards,
Ragi Raj
Journal Manager
Tel : +91 44 4299 4748

From: Abdulmajeed Alfayyadh
Date: Thursday, July 06, 2023 07:29 PM GMT

I am a phd candidate, and since I am defending my thesis after a month, I would like to receive a permission of using my manuscript, that has been published in your journal, in my thesis document.

Attached is the article.

Thank you,
Abdulmajeed Alfayyadh