FEMORAL ARTICULAR CARTILAGE CHARACTERISTICS AND MECHANICAL KNEE JOINT LOADING IN INDIVIDUALS DURING EARLY PHASES OF RECOVERY FOLLOWING ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

By

Caroline Michele Lisee

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PUBLIC ABSTRACT

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Anterior cruciate ligament reconstruction (ACLR) is a risk factor for the development of accelerated post-traumatic knee osteoarthritis. Pre-radiographic assessments of early knee joint changes and understanding the impact of movement and activity contributing to worsening knee joint health may help health care providers identify and intervene before detrimental degenerative effects occur. The purposes of this dissertation comprised of 3 manuscripts with data collected from 2 studies were to: 1) establish the reliability 2 ultrasound assessment techniques of early knee joint structure and deformation, 2) assess knee joint structural differences and changes between surgical and non-surgical knees 4- and 6-months post-ACLR in individuals recovering from surgery, and 3) identify if walking movement patterns and amount of activity participation at 4-months post-ACLR contribute to knee joint structural changes assessed via ultrasound at 6-months post-ACLR. In the first study, ultrasound images of knee articular cartilage thickness were captured in the knees of 30 participants without a history of knee injury at rest and after 3,000 steps of walking. The second study was completed by 20 participants recovering from ACLR at 4- and 6-months after surgery. At 4 months post-ACLR, participants completed a walking movement pattern assessment and an ultrasound imaging assessment of knee articular cartilage thickness at rest in both the surgical and non-surgical knees. Participants were also instructed to wear a physical activity monitor for 7 days to assess average daily steps at this time. At 6-months post-ACLR, ultrasound images of knee articular cartilage thickness were captured at rest for all participants. For the first study, intra-rater and test-retest reliability was excellent for assessing resting knee articular cartilage thickness in all compartments ($ICC_{2,k}$=0.97-0.99). Knee articular cartilage changes after 3,000 steps of walking demonstrated good to excellent intra-rater reliability ($ICC_{2,k}$=0.84-0.94), but poor test-retest reliability ($ICC_{2,k}$=-0.36-0.46). For study 2, there were no significant differences or interactions between surgical and non-surgical resting knee articular cartilage thickness or between 4-and 6-months post-ACLR (*p* range =0.22-0.92). Additionally, individuals who with walked with lesser sagittal knee joint forces, but greater steps per day at 4 months post-ACLR had greater knee articular cartilage thickness (R^2= 0.39, *p*=0.03). Ultrasound assessment of knee articular cartilage thickness at rest is a reliable measure of knee joint structure that should be used in individuals at risk for knee osteoarthritis, but changes in knee articular cartilage thickness after walking are too inconsistent for application over multiple study sessions. Individuals with a history of ACLR do not demonstrate knee articular cartilage structural differences assessed via ultrasound at rest between knees within the first 6 months of recovery. After ACLR, individuals who participate in high amounts of activity before altered knee movement patterns are resolved demonstrated knee articular cartilage thickness associated with cartilage swelling. Future research should determine when ultrasound assessment of knee articular cartilage can be used to identify early knee articular cartilage structural changes after ACLR and if addressing altered walking movement patterns before increasing activity participation promotes long-term knee joint health in individuals after ACLR.

ABSTRACT

FEMORAL ARTICULAR CARTILAGE CHARACTERISTICS AND MECHANICAL KNEE JOINT LOADING IN INDIVIDUALS DURING EARLY PHASES OF RECOVERY FOLLOWING ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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Individuals with a history of anterior cruciate ligament reconstruction (ACLR) have a higher risk of developing accelerated knee osteoarthritis compared to individuals without a history of knee injury. It is necessary to establish reliable tools (i.e. ultrasound assessment) that assess knee joint health, determine how early these tools can identify poor knee joint health changes in high-risk populations, and examine which modifiable risk factors (i.e. mechanical knee joint loading) contribute to accelerated poor knee joint health development. The purposes of this dissertation were to: 1) establish the intra-rater and test-retest reliability of two ultrasound assessment techniques (resting cartilage and cartilage response to loading assessments) of femoral articular cartilage structure and deformation, 2) assess resting femoral articular cartilage structural differences between the involved limb and contralateral limb and changes over time from 4- to 6-months post-ACLR, and 3) determine the ability of cumulative knee joint loading (gait knee biomechanics and volume of loading) at 4-months post-ACLR to predict resting medial femoral articular cartilage structure at 6-months post-ACLR. In the first observational study, femoral articular cartilage structure and deformation were evaluated via the resting cartilage and cartilage response to loading ultrasound assessment techniques in 30 participants without a history of knee injury. In 2 identical testing sessions, the resting cartilage and post-loading cartilage images were capture after 30 minutes of rest and 3,000 steps of walking, respectively. A total of 20 participants post-ACLR completed the resting cartilage ultrasound assessment in their involved and contralateral limb at 4- and 6-months post-ACLR for the second longitudinal study. At 4-months post-ACLR, knee gait biomechanics (knee extension moment, knee abduction moment, and vertical ground reaction force) were assessed with motion capture and force plates, and volume of activity (steps/day) were assessed with a hip worn accelerometer over 7 days. All ultrasound images were processed using a semi-automated processing technique to divide the total cartilage cross-sectional area into medial, intercondylar, and lateral compartments normalized to compartment length for cartilage thickness (mm). Resting cartilage ultrasound assessment demonstrated excellent test-retest and intra-rater reliability ($ICC_{2,k}$= 0.97-0.99). Cartilage response to loading ultrasound assessment demonstrated poor test-retest reliability ($ICC_{2,k}$=-0.36-0.46), but good to excellent intra-rater reliability ($ICC_{2,k}$=0.84-0.94). Individuals 4- to 6-months post-ACLR did not demonstrate any significant limb main effects (p range=0.50-0.92), time main effects (p range=0.22-0.72), or interactions (p range=0.24-0.49) for resting medial, intercondylar, or lateral femoral articular cartilage compartmental thickness. Lesser knee extension moment (unstandardized β=2.82, p=0.02) and greater steps per day (unstandardized β=0.00, p=0.04) at 4-months post-ACLR predict greater medial femoral articular cartilage compartmental thickness at 6-months post-ACLR (R^2= 0.39, p=0.03). The resting cartilage ultrasound assessment is a reliable technique between multiple processing and testing sessions, but the cartilage response to loading ultrasound assessment is not reliable between testing sessions. Femoral articular cartilage structural differences between limbs or change over time may not be present before 6 months post-ACLR. Individuals with poor biomechanics who take more steps per day demonstrate articular cartilage structural changes indicative of articular cartilage swelling within 6-months post-ACLR. Cumulative mechanical knee joint loading is a multifactorial risk factor of knee joint health during the early phases of recovery after ACLR.

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CHAPTER 1: INTRODUCTION

STATEMENT OF THE PROBLEM

Knee osteoarthritis (OA) is a chronic health condition resulting in irreversible tissue damage to the synovial joint especially within the tibiofemoral articular cartilage.[20] Knee OA is a leading cause of disability in daily activities worldwide and individuals with the condition report poor quality of life.[1,2] Traumatic knee injury, including anterior cruciate ligament (ACL) injury, is a leading risk factor for the development of knee post-traumatic osteoarthritis (PTOA).[3] Greater than 50% of young, otherwise healthy individuals with ACL injury and/or subsequent reconstruction (ACLR) develop PTOA within 20 years.[3] Unfortunately, there is no cure for knee PTOA so individuals with ACLR often develop PTOA early in life, leading to many years lived with disability. Therefore, there is a critical need to identify individuals at elevated risk for knee PTOA and to develop interventions that help prevent or delay the development of poor synovial knee joint health, disability, and poor quality of life after ACLR. To address these problems, we must: 1) establish a reliable assessment of early knee joint health changes; 2) determine if this assessment technique can detect early knee joint health changes in ACLR populations; 3) identify which modifiable risk factors contribute to early, poor knee joint health changes after ACLR.

STATEMENT OF THE PURPOSE

Mechanical knee joint loading is one of three pathways along with joint metabolism and structure that contribute to the development of knee PTOA after ACLR. Mechanical knee joint loading refers to the internal and external forces acting on the knee joint during weightbearing activities. Healthy knee articular cartilage aids in absorbing these forces, but damaged knee articular cartilage responds poorly to the mechanical forces acting on the knee.[4] It is hypothesized that assessing knee articular cartilage response to mechanical loading may provide unique insights into early microstructural or compositional changes of the tissue that may precede radiographic evidence of knee PTOA.[5] Ultrasonography is an emerging tool used to assess femoral articular cartilage health. A resting cartilage ultrasound assessment technique that calculates the resting femoral articular cartilage compartmental thickness is a valid and reliable assessment in individuals with and without a history of knee injury.[6-8] Based on resting cartilage technique, the cartilage response to loading ultrasound assessment technique was developed to evaluate the change in femoral articular cartilage compartmental thickness from resting to post-loading characterized as the response of femoral articular cartilage after a period of mechanical loading (referred to as deformation).[7-9] Good to excellent intra-rater and test-retest reliability[7-9] have been established for the cartilage response to loading ultrasound assessment techniques applied to healthy populations when completed by an expert rater (> 5 years of experience), but a gap exists in the literature defining the intra-rater and test-retest reliability of a novice rater (<1 year of experience). Therefore, the purpose of the first manuscript was to determine the intra-rater and test-retest reliability of the cartilage response to loading ultrasound assessment technique in a novice rater. Novice raters must establish similar reliability quality to determine if this tool is can also be adopted by new healthcare providers.

Knee joint degeneration, especially in the tibiofemoral articular cartilage, is present as early as 6 months post-ACLR.[9-12] The earliest changes in knee joint health occur in the medial compartment compared to the intercondylar or lateral compartments due to greater mechanical loading forces acting in this region during weightbearing activities.[13] After ACLR, patients undergo 6-12 months of rehabilitation[14] and are progressively integrated into activities with increasing intensities of knee joint mechanical loading. A critical period in rehabilitation occurs at 4 and 6 months post-ACLR when patients are exposed to greater mechanical loading through exposure to more challenging therapeutic exercises. For example, patients are integrated into jogging and modified sports activities 4 months post-ACLR, and begin discharge from rehabilitation and unrestricted return to physical activity as early as 6 months post-ACLR.[14] Assessment of knee articular cartilage structure to determine knee joint health in individuals with ACLR is imperative during this rehabilitation period so healthcare providers can intervene if necessary. The purpose of the second manuscript was to assess between limb differences and changes over time in femoral articular cartilage structure captured via resting cartilage ultrasound assessment between the involved and contralateral limbs in patients with a primary, unilateral history of ACLR at 4- and 6-months post-surgery. Application of this technique in ACLR populations can help determine if articular cartilage structural changes or limb differences are present while patients remain under the care of healthcare providers.

Modifiable factors of knee joint mechanical loading include magnitude of loading (characterized by walking biomechanics) and volume of loading (characterized by volume of physical activity). Both of these factors contribute to the development of poor knee joint health[4,15] especially in the medial tibiofemoral compartment.[13] These factors of knee joint mechanical loading are often considered separately, but this is an unrealistic representation of daily mechanical loading placed on the knee. Instead, these factors of mechanical loading should be considered concurrently to reflect the daily cumulative mechanical loading occurring at the knee. After ACLR, individuals demonstrate aberrant walking biomechanics in knee extension moment, knee abduction moment, and vertical ground reaction forces during the initial phases of rehabilitation which may persist for years after ACLR.[11,16] Patients also demonstrate lesser volumes of physical activity quantified as steps per day compared to individuals without a history of knee injury within 5 years of surgery.[17] Altered magnitude and volume of mechanical knee joint loading are persistently present in individuals with a history of ACLR. However, it is unclear how these factors are associated with knee joint health while individuals with ACLR are integrated into activities with increasing mechanical loading and remain under healthcare supervision. The purpose of the third manuscript was to assess the ability of cumulative mechanical loading (walking biomechanics and volume of activity) at 4-months post-ACLR to predict medial knee articular cartilage compartmental thickness captured via resting cartilage ultrasound assessment 6-months post-ACLR. Identification of modifiable risk factors of mechanical loading during the rehabilitation process after ACLR may enable patient-specific secondary prevention of knee PTOA after ACLR.

RESEARCH QUESTIONS AND EXPERIMENTAL HYPOTHESES

MANUSCRIPT 1 RESEARCH QUESTIONS AND HYPOTHESES

Primary Purpose: The primary purpose of this study was to assess the intra-rater reliability of a novice assessor using the resting cartilage and cartilage response to loading ultrasound assessment techniques in healthy participants without a history of knee injury.

Secondary Purpose: The secondary purpose of this study was to assess the test-retest reliability of the novice assessor between 2 sessions of both ultrasound assessment techniques in healthy participants without a history of knee injury.

H 1.1. The primary hypothesis was that both ultrasound assessment techniques will demonstrate excellent intra-rater reliability for assessing medial, intercondylar, and lateral femoral articular cartilage compartmental thickness and deformation.

H 1.1. The secondary hypothesis was that both ultrasound assessment techniques will demonstrate excellent test-retest reliability for assessing medial, intercondylar, and lateral femoral articular cartilage compartmental thickness and deformation.

MANUSCRIPT 2 RESEARCH QUESTIONS AND HYPOTHESES

Primary Purpose: The purpose of this study was to assess between limb differences (involved limb and contralateral limb) and changes over time (4- and 6-months post-ACLR) in resting femoral articular cartilage characteristics (medial, intercondylar, and lateral femoral articular cartilage compartmental thickness) in individuals recovering after ACLR.

H 2.1. The primary hypothesis is that the involved limb will demonstrate greater resting medial femoral articular cartilage compartmental thickness, but no differences in intercondylar and lateral femoral articular cartilage compartmental thickness compared to the contralateral limb at 4-months and 6-months post-ACLR.

H 2.2. The secondary hypothesis is that the involved limb will demonstrate greater medial femoral articular cartilage compartmental thickness at 6-months compared to 4-months post-ACLR. Involved limb intercondylar and lateral femoral articular cartilage compartmental thickness will not be different between 4- and 6-months post-ACLR

H.2.3. The contralateral limb medial, intercondylar, and lateral femoral articular cartilage compartmental thickness will not be different between 4- and 6-months post-ACLR.

MANUSCRIPT 3 RESEARCH QUESTIONS AND HYPOTHESES

Primary Purpose: The purpose of this study was to assess the ability of cumulative mechanical knee joint loading (gait biomechanics and volume of activity) at 4-months post-ACLR to predict involved limb medial femoral articular cartilage compartmental thickness in individuals 6-months post-ACLR.

H 3.1. The primary hypothesis is that greater involved limb peak internal knee abduction moment, peak internal knee extension moment, and peak vertical ground reaction force during the stance phase of walking at 4-months post-ACLR will be associated with greater involved limb medial femoral articular cartilage compartmental thickness at 6-months post-ACLR. Peak internal knee abduction moment will demonstrate the strongest relationship.

H 3.2. The secondary hypothesis is that greater involved limb peak internal knee abduction moment and lesser daily steps at 4-months post-ACLR will predict greater involved limb medial femoral articular cartilage compartmental thickness 6-months post-ACLR in individuals recovering from ACLR.

SIGNIFICANCE OF THE STUDY

The proposed studies incorporate emerging ultrasound techniques to assess articular cartilage health which are accessible in orthopedic clinics and activity assessments which are accessible through consumer-grade technology. Early identification of tibiofemoral articular cartilage changes is key in secondary prevention efforts, but radiographic and MRI-based assessments are impractical or lack sensitivity which limits their feasibility in the rehabilitation environment. Ultrasound offers a repeatable assessment approach for longitudinal assessments that with more research has the potential to be integrated into healthcare clinics to identify individuals at risk for developing knee PTOA. If these assessments are adopted in healthcare clinics, it is important to understand how well individuals with limited experience effectively perform these assessments and the best way to perform this assessment technique. To our knowledge, this is the first study to longitudinally assess femoral articular cartilage structure via ultrasound after ACLR. Rehabilitation after ACLR is generally completed within a 6- to 9-month period. This study has a longitudinal design to better understand the relationship between factors of loading during rehabilitation and articular cartilage joint health when many individuals are actively engaged with a healthcare provider on a consistent basis and have not returned to unrestricted activity.

The proposed studies also take a multifaceted approach to addressing the early effects of cumulative mechanical loading on knee articular cartilage health after ACLR. Traditionally, walking biomechanics and volume of activity have been assessed individually after ACLR, but considering these factors conjointly provides a more comprehensive assessment of contributors to knee mechanical loading following surgery. In the current studies, we utilize research-grade activity monitoring technology for valid data collection to provide better context about the volume of activity via daily step counts. However, consumer-grade activity tracking technology is widespread in today's culture and outcomes like steps/day can easily be assessed and modified by clinicians and patients through consumer-grade devices such as smart watches or FitBit monitors. The results of the proposed studies will provide the first step in a line of research to characterize the effects of under- or over-loading behavior on articular cartilage health during critical points of the recovery process following ACLR to slow or mitigate the rapid development of PTOA commonly observed in this at-risk population. This also captures a period when rehabilitation clinicians can incorporate interventions to improve articular cartilage health. By identifying which load-related factors are associated with poor knee articular cartilage health, we may be able to develop and implement safe, progressive walking-based protocols during recovery with the goal of limiting sedentary behaviors and promoting healthy knee articular cartilage.

CHAPTER 2: REVIEW OF THE LITERATURE

INTRODUCTION

Many individuals with a history of anterior cruciate ligament (ACL) injury or reconstruction (ACLR) develop post-traumatic knee osteoarthritis (PTOA) at an accelerated and greater rate compared to individuals without a history of knee injury. The pathogenesis of symptomatic knee post-traumatic osteoarthritis (PTOA) after anterior cruciate ligament reconstruction (ACLR) occurs over an initiation phase and subsequent progression phase[18] due to a combination of biological, structural, and mechanical mechanisms.[15] The initiation phase is characterized by early, superficial articular cartilage damage and the progression phase is characterized by long-term, deeper articular cartilage degeneration.[18] Although these mechanisms are symbiotic and should be occurring concurrently in the overall development of knee PTOA, the Loading in OsteoArthritis Development (LOAD) model focuses on the mechanical mechanisms. Mechanical mechanisms[18] refer to the various forces of load applied to knee articular cartilage during movement or activity and can be assessed through biomechanical analysis and wearable technologies that are able to quantify activity.

Magnitude and volume of loading are 2 mechanical factors considered in the development of knee PTOA. Magnitude of loading considers the magnitude and location of biomechanical forces applied across the articular cartilage surfaces of the knee joint and volume of loading refers to how often the knee articular cartilage is loaded (or not loaded) during daily activities. After ACLR, individuals experience an abrupt change in movement patterns and extended periods of restricted knee joint loading activity. These alterations in both quality and quantity of mechanical loading expose knee articular cartilage to abnormal contact forces and cartilage composition which contribute to poor cyclical articular cartilage loading response and disruption.[4,19] Cartilage compositional changes may reflect lesser proteoglycan content, collagen disorganization or greater water content.[20-22] The conceptual model (Figure 1) that serves as the basis for this dissertation project focuses on the initiation phase of PTOA and considers a combination of previously proposed mechanical mechanisms to understand how different aspects of loading may impact knee articular cartilage after ACLR. This literature review explores the most recent evidence supporting this model.

First, the literature review will explore the epidemiology of ACL injury, ACLR, and knee OA. The review will also briefly describe the most relevant wet and dry biomarkers used to characterize the progression and severity of knee OA as it relates to knee articular cartilage health. Additionally, the review will summarize mechanical loading (walking biomechanics and volume of activity) in individuals with and without a history of knee pathology. Finally, the review will discuss the relationship between biomarkers of knee articular cartilage health and modifiable factors of mechanical loading.

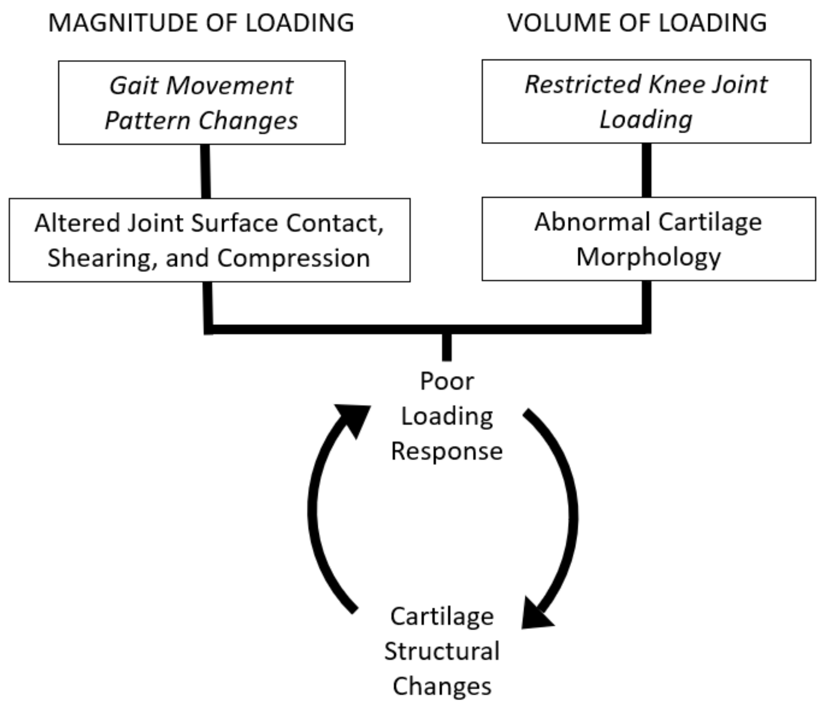

Figure 1. Loading in Osteoarthritis Development (LOAD) model supporting the mechanical pathways for developing of post-traumatic OA during the initiation phase after ACLR

EPIDEMIOLOGY OF ACL INJURY AND ACLR

ANTERIOR CRUCIATE LIGAMENT (ACL) ANATOMY

The tibia, femur and patella bones articulate to form the patellofemoral joint (PFJ) and tibiofemoral joint (knee). The knee is a synovial, modified hinge joint that moves through flexion, extension, and a minimal degree of internal and external rotation. Passively, the knee joint is supported by intra-articular ligaments (medial collateral ligament, lateral collateral ligament), extra-articular ligaments (anterior cruciate ligament and posterior cruciate ligament), and a synovial joint capsule. One of the commonly injured extra-articular ligaments in the knee is the anterior cruciate ligament (ACL). The ACL is divided into posterior-lateral and anterior-medial bundles that originate from the medial portion of the lateral femoral condyle and inserts on the intercondylar tibial eminence.[23] The primary role of the ACL is to resist anterior translation of the tibia, but the ligament also aids in rotary stability of the tibiofemoral joint.[24] The ligament primarily consists of type I collagen[23] and includes various mechanoreceptors to aid in knee joint proprioception including Ruffini corpuscles, Pacinian corpuscles, golgi tendon organs, and free nerve endings. The meniscus consists of fibrocartilage and is located superiorly to the tibia to aid in compressive force absorption for the tibiofemoral joint. The meniscus is divided into an oval shaped lateral meniscus and a crescent shaped medial meniscus which also attaches to the medial collateral ligament. The vascularity of the meniscus varies with the outer portion of the meniscus receiving the greatest blood flow and the inner portion of the meniscus receiving little to no blood flow.[25]

PRIMARY ACL INJURY

Primary ACL injuries are the result of contact (58.8%) and non-contact mechanisms (37.9%).[26] Contact mechanisms of injury appear to be more prevalent in high school-aged individuals,[26] and non-contact mechanisms are more prevalent in individuals older than 18 years.[27] Overall, men sustain more ACL tears compared to females[28] due to higher number of athletic event exposures and greater participation in contact sports, but females have a higher rate of non-contact ACL tears after controlling for exposures.[26] Girls have slightly higher ACL injury rates compared boys in sex-comparable sports at the high school level.[29] However, boys have a higher incidence of ACL tears after high school between the ages of 19 to 25 years, and girls have a higher prevalence during high school between the ages of 14 to 18 years.[28] In high school and collegiate level sports, the majority of ACL injuries occur during football and rugby in boys[26,27] in comparison to soccer and basketball in girls.[26] Non-modifiable risk factors of primary non-contact ACL injuries include young age (<20 years), female sex especially during the preovulatory phase, participation in sports with high levels of cutting and jumping, and narrow femoral intercondylar notch.[30] Modifiable risk factors of primary ACL injuries include dynamic valgus during sport specific movements, stiff landing mechanics at the hip and knee, poor lumbopelvic control, and weakness of hamstrings and hip abductors.[30] Primary injury prevention programs that focus on improving lower extremity strength, balance, flexibility and agility and incorporate plyometric exercise[31] may reduce the risk ACL injury by 50% in male and female youth athletes by targeting some of these modifiable risk factors.[32]

Other knee related pathologies may occur concurrently with isolated ACL tears.[28,33,34] The "unhappy triad" is a hallmark term describing concomitant injuries to the ACL, MCL and meniscus. MCL injuries are reported to occur in 22% of individuals with ACL injuries, but this number may be under-reported.[33] Meniscal pathologies in either the medial or lateral menisci may be as high as 60% in individuals with acute ACL tears.[28] Acute ACL injuries are also consistently associated bone contusions of the tibial plateau and femoral condyles and are reported to occur in 16 to 46% of the pathological population.[34] The highest prevalence of bone contusions occur in the lateral portion of the tibial plateau and the lateral femoral condyle.[33] This may result in early disruption of articular cartilage or subchondral bone depending on the severity of the injury. Both meniscal pathologies and bone bruises are potential risk factors for the development of poor articular cartilage health[35] and may be important confounding factors to consider when assessing articular cartilage health. Meniscal injury involvement will be discussed in greater detail during the “ACLR Surgical Technique” section.

MECHANISM OF NON-CONTACT ACL INJURY

Non-contact ACL injuries occur most commonly during sport specific movements including landing, cutting or deceleration tasks.[36] Due to the increasing incidence of non-contact ACL injuries, accumulating evidence suggests ACL injuries result from multiplanar injury mechanisms especially during jump landing and cutting tasks. Cadaveric studies indicate that ACL strain is greatest during 0-30° of knee flexion.[37] At 25° of knee flexion, anterior shear loads, abduction moments and internal rotation moments were applied to cadaveric knees at the same time as an axial load to simulate multiplanar landing conditions from a jump.[38] Combined forces of anterior shear loads of 268 N, internal rotation moments of 60 Nm or greater, and knee abduction moments of 75 N or greater resulted in increased ACL strains from baseline and from forces applied individually.[38] Knee abduction angle explained greater peak ACL strain variance (R^2=0.45) compared to knee rotation angle (R^2=0.32).[39] Additionally, cadaveric loading simulations with MCL tears demonstrate greater ACL strain regardless of tibial rotation position[39] indicating that the MCL plays a role in resisting multiplanar loads during ACL injuries. These results suggest that anterior translation, knee abduction moments and internal rotation moments should be considered when assessing mechanisms of non-contact ACL injuries.

A meta-analysis assessing video of ACL injury events during sport also supported these studies and reported that individuals demonstrated on average 16° of knee flexion with a maximum of 30° of knee flexion at the time of injury.[40] A prospective biomechanical analysis of ACL injury in young females athletes reported that young girls with greater peak knee abduction moments and vertical ground reaction force demonstrate greater risk of primary ACL injury.[41] Additionally, knee "valgus collapse" during landing and cutting tasks which is a combination of greater dynamic knee valgus, hip internal rotation and tibial internal or external rotation may also be a non-contact mechanism of ACL injury in women.[42,43] Expert opinion suggests that the best supported hypothesis of non-contact ACL injury mechanism is a multiplanar mechanism of ACL injury in the sagittal, frontal and transverse planes of motion.

ACLR EPIDEMIOLOGY

After ACL injuries, individuals may seek conservative treatment through rehabilitation or surgical intervention to regain knee joint stability. Approximately 130,000 ACL reconstructions (ACLRs) are performed annually in the United States[44] and are most commonly performed on individuals under the age of 20.[44] Since 2002, the incidence of ACLRs has increased especially in individuals 13 to 17 years old.[45] In this age group, isolated ACLRs, ACLRs with meniscal repairs, and ACLRs with meniscectomies have increase by 37%, 107%, and 63% resepectively.[45] The number of ACLRs performed in the United States has increased in both male and female populations.[44] Furthermore, the number of ACLRs has nearly doubled in female populations since 1994.[44]

ACLR SURGICAL TECHNIQUE

Autograft and allograft tendons are used to surgically reconstruct the ACL after ACL injuries. Approximately, 20-100% of surgeries are completed with an autograft tendon and 0-

80% of surgeries are completed using an allograft tendon.[46] Individuals under the age of 29 years are most commonly treated with autograft tendons, and individuals older than 40 years old are more commonly treated with allografts.[46] Hamstring tendon grafts (54%) are the most commonly used autograft tendons for primary ACLRs followed by bone-patellar tendon-bone grafts (45%) and quadriceps tendon grafts (less than 1%).[47] However, quadriceps tendon autografts are gaining popularity for use in young females since other autograft tendon choices may be too small or disrupt epiphyseal plate growth in pre-adolescent females.[48]

Individuals undergoing ACLR often have concomitant surgical procedures to address additional tissue damage. Overall, meniscectomies are the most common concomitant procedure,[45] but meniscal repairs and microfracture surgeries are also consistently performed. Determining the optimal surgical treatment approach for concurrent meniscal pathologies are important to short term[49] and long-term knee health and function after ACLR.[50] Individuals undergoing ACLR and meniscectomy are 3.54 (95% CI = 2.56-4.91) times more likely to development knee OA compared to individuals undergoing an isolated ACLR average time to follow-up ranging between 10.7 and 24.5 years post-surgery.[50] The meniscus plays a primary role in knee function especially for distributing forces places on the articular cartilage in the knee during weightbearing movement. Even minimal disruption in meniscal tissue due to a meniscectomy, may result in altered, high-risk walking mechanics and contact between the tibia and the femur associated with the development of knee OA.[49] Alterations in meniscal tissue resulting from injury or surgical procedure may shift articulating surface contact increasing the mechanical load during weightbearing to different regions of the articular cartilage and decreasing the mechanical load to other regions. Articular cartilage contact and load distribution changes may disrupt the homeostasis of the joint metabolic environment progressing towards tissue degeneration.[18]

Bone contusions or bruises may result in bone marrow lesions or chondral damage. Bone bruises are identified by greater signal intensity changes on MRIs representative of excessive connective tissue growth and increased vascularity.[51] Approximately 80% of patients are diagnosed with bone bruises in individuals who sustain an ACL injury which disrupts the articular cartilage.[52] Bone bruises occur in 86% of the medial and 87% of the lateral tibial condyles with the majority occurring in the posterior regions.[53] They also occur in 86% of the medial and 94% of the lateral femoral condyles with the majority occurring in the anterior and central regions.[53] Bone bruises are a risk factor for knee PTOA[54] and greater chondral damage is correlated with greater presence of radiographic tibiofemoral PTOA (r=0.411) 6 years post-ACLR.[54] Bone bruises may take up to approximately a year to fully heal[55] and due to their association with PTOA should be considered a confounding factor when assessing knee articular cartilage health.

SECONDARY ACL INJURY EPIDEMIOLOGY

ACLR is generally successful in regaining knee joint stability following ACL injury. However, over 20% of individuals under the age of 25 will sustain a secondary ACL injury including ipsilateral graft tears and contralateral ACL tears within 15 years after ACLR.[56] Conversely, a meta-analysis reported that only 15% of individuals sustain secondary ACL tears within the broader population of individuals 10 to 64 years old.[56] Within 2 years of surgery, individuals with a history of ACLR have a 6 times greater risk of sustain a secondary ACL injury in the involved or contralateral limb compared to those without a history of knee injury.[57] Approximately 75% of secondary ACL injuries occur as a result on non-contact mechanism.[58]

RISK FACTORS FOR SECOND ACL INJURY

Women with a history of ACLR are 2 times more likely to sustain a contralateral ACL tear compared to ipsilateral graft re-tears[57] and men with a history of ACLR have a 38% higher risk of sustaining an ipsilateral graft re-tear compared to women with a history of ACLR.[59] A multisite prospective cohort study reported[59] that young, male patients with a hamstring autograft were at increased risk of sustained an involved limb ACLR revision, but young, female patients were at increased risk of sustaining contralateral limb ACL tears.[59] Overall, patients with allografts had a significantly higher graft failure rate compared to patients with autograft tears especially from the ages of 10 to 19 years.[60] Time since surgery was also associated with risk of secondary ACL tears. Individuals returning before 9 months post-surgery were 50% more likely to sustain a secondary ACL tear compared to individuals returning after 9 months. These factors are important to consider when determining graft type and time of return to play after primary ACLR for individuals with different demographic backgrounds.

VOLUME OF ACTIVITY IN INDIVIDUALS WITH AND WITHOUT A HISTORY OF KNEE PATHOLOGY

Quantity of mechanical knee joint loading can be measured through volume of daily lower extremity weightbearing activity. Knee articular cartilage morphology and deformational behavior in response to mechanical load may change based on the volume of daily weightbearing activity performed,[5,8] especially in populations with a history of knee joint injury. Physical activity (PA) which encompasses many types of daily weightbearing activity, has been assessed in healthy and pathological individuals demonstrating protective health benefits to noncommunicable diseases including knee OA. Lesser volumes of daily activity that are apparent in ACLR populations years after surgery may negatively impact the knee articular cartilage morphology and deformational behavior, but these relationships have not been explored. This section of the literature review aims to understand the current state of physical activity behavior in general and pathological populations, how to measure activity (i.e. type, intensity, volume), and the relationship between activity and health outcomes.

PHYSICAL ACTIVITY IN THE GENERAL POPULATION

Recently, new national PA guidelines were published by the *2018 Physical Activity Guidelines Advisory Committee.*[61] The guidelines state that youth aged 6 to 17 should participate in 60 minutes of moderate to vigorous PA (MVPA) per day and muscle/bone strengthening exercises at least three days per week.[61] Adults should complete either 150 to 300 minutes of moderate intensity activity or 150 minutes of MVPA per week with 2 days of strength training.[61] While accumulating evidence suggests that PA is beneficial to improving an individual's psychological and physical health, less than 30% of population in the United States meet recommended guidelines for physical activity.[61]

Improving PA participation at all ages is imperative for promoting healthy lifestyles. Longitudinal evidence suggests low to moderate evidence of PA behavior stability from childhood and adolescence to adulthood indicating that individuals that were more active in their childhood or adolescence remained more active into adulthood.[62] In terms of sports participation, individuals who participated in organized sport during childhood were 1.75 times (95% CI = 1.11-2.76) more likely to report healthy living habits in adulthood including PA compared to individuals that didn't participate in organized sport activity during childhood.[63] Individuals who participated in organized sport during childhood and adolescence also more frequently reported achieving recommended PA guidelines during adulthood compared to individuals who did not participate in organized sport during childhood and adolescence.[63] This demonstrates the importance of promoting PA and sports participation in children and adolescence to increase PA engagement in adulthood.

BENEFITS OF PHYSICAL ACTIVITY PARTICIPATION

PA participation has various impacts on an individual's health including improvements in mental health[64] and reducing an individual's risk of developing a myriad of chronic, non-communicable diseases. A prospective study reported that youth and adult populations that participate in greater volume of PA are 10% (95% CI = 83%-98%) and 22% (95% CI = 70%-87%) less likely to develop depression compared to youth and adult populations that participate in lesser amounts of PA.[64] Additionally, individuals who are physically inactive have a higher risk of developing coronary artery disease, type 2 diabetes, and certain types of cancer compared to individuals who are active worldwide.[65] Improving PA participation throughout an individual's lifespan can have long lasting benefits.

MEASURING PHYSICAL ACTIVITY

Detailed evaluations of activity are best characterized using the FITT principle which considers the frequency, intensity, time, and type of activity when quantifying PA.[61] Frequency indicates how often activity is performed, intensity describes the rate of energy expended during activity (i.e. metabolic equivalent of a task) and time quantifies the duration of activity that is performed. Volume of activity is an outcome describing the frequency and duration of activity and can be used to quantify different activity intensities such as MVPA.[61] FITT characterizations of PA are commonly measured via self-reported surveys responses or accelerometry. Commonly utilized measurements of self-reported PA utilized in individuals with a history of ACLR include the Marx Activity Scale (Marx Scale, Appendix Marx Scale), the Tegner Activity Scale (TAS) and the International Physical Activity Questionnaire Short Form (IPAQ-SF), and more recently accelerometry to measure activity in free-living conditions has also been used in this population.

SELF-REPORTED TYPE AND VOLUME OF ACTIVITY – THE MARX ACTIVITY SCALE

The Marx Scale consists of 4 items scored on a 5-point Likert scale (0-5). The questionnaire measures how often (< 1 time per month to >3 times a week) participants participate in running, cutting, deceleration, and pivoting tasks. The Marx scale was created based on expert opinions from sports medicine health care providers and feedback from patients with knee injuries.[66] The test-retest reliability for the Marx scale was excellent (ICC = 0.97). The Marx scale also achieved face and content validity as determined by physicians and allied health care professionals in the rehabilitation profession.[66] Construct validity of the scale was determined through assessing the relationships to other self-reported activity.[66] The Marx scale demonstrated a moderate relationship with the TAS (r=0.66),[66] but a poor relationship (ρ=0.15) with MVPA assessed via accelerometry.[67] Divergent validity was established based on an inverse relationship between the Marx scale (r=-0.48, p=0.002) and age according to the hypothesis that individuals will become less active as they age.[66] The Marx scale is a valid and reliable scale for measuring type and volume of activity especially in individuals with knee injuries.

SELF-REPORTED TYPE OF ACTIVITY – THE TEGNER ACTIVITY SCALE

The TAS (Appendix Tegner Activity Scale) consists of 1 item ranging on an 11-point Likert scale (0-11). This self-reported activity scale was traditionally created to be used by physicians to measure activity changes early on after ACL surgery to when an individual may be returning to work or later on when returning to some level of recreational activities or sport.[68] A score of zero indicates that individual is unable to participate in activity due to knee disability, scores 1-5 indicate progressing levels of work intensity participation, and scores 5-10 indicate progressing levels of recreational and sport activity participation. The TAS was validated in individuals with a history of ACLR 2, 6, 9, 12 and 24 months post-surgery.[69] In ACLR populations, the TAS demonstrated acceptable test-retest reliability (Intraclass Coefficient = 0.82 95% CI=0.66-0.89) and a minimal detectable change of 1 on the Likert scale.[69] Based on pre-operative data, the TAS demonstrated acceptable construct validity indicate less than 30% of patients demonstrating floor (8% of participants reported score of 0) or ceiling effects (3% of participants reported score of 10), weak criterion validity compared to the Short Form-12 (ρ=0.2, p<0.05), and acceptable construct validity on all 6 proposed constructs.[69] Between most time points, the TAS demonstrated large effect sizes except at 6 months when the TAS score demonstrated moderate effect sizes for survey responsiveness.[69] The TAS demonstrates acceptable validity and reliability for measuring type of activity in ACL injured and ACLR populations. However, the TAS is poorly correlated to MVPA assessed via accelerometry in individuals with (ρ=0.31) and without a history of ACLR (ρ=0.07).[67]

SELF-REPORTED INTENSITY AND VOLUME OF ACTIVITY – THE IPAQ-SF

The IPAQ-SF (Appendix IPAQ-SF) is a self-reported 9-item questionnaire assessing the amount of time spent in vigorous, moderate, walking and sitting activities over the course of one week. This questionnaire is a widely used scale especially in epidemiological and longitudinal studies of activity in various populations. The IPAQ demonstrates good test-retest reliability with an overall ICC of 0.86 (vigorous activity ICC = 0.86, moderate activity ICC = 0.71, walking activity ICC = 0.89) in adults.[70] However, test-retest reliability was poor to moderate in adolescents ranging in age from 13 to 18 (ICC = 0.10-0.62).[71] The IPAQ has been validated against various PA measures including accelerometers[72] and doubly labeled water which is considered a more accurate and objective measurement of activity.[73] The IPAQ-SF demonstrated negligible to small relationships with accelerometers when assessing total physical activity and demonstrated moderate relationships when assessing walking.[72] Overall, the IPAQ-SF tends to overestimate volume when compared to accelerometers.[72] When validated against the doubly labeled water technique, the IPAQ could only adequately distinguish those who participate in large amounts of activity from physically inactive individuals.[72] The IPAQ-SF demonstrates acceptable reliability in adults, but should be used cautiously in adolescents and children due to its variable test-retest reliability in this population.[72] Researchers using the questionnaire should also be aware that it tends to over-estimate PA behavior.

FREE LIVING VOLUME OF ACTIVITY – ACCELEROMETRY

Utilizing accelerometers to measure activity is more time consuming and requires technical expertise by the researcher in selecting parameters chosen to enable appropriate data processing. Accelerometry can be effectively used to measure activity in free-living conditions to overcome the barriers of personal bias in self-report measures.[74] A variety of consumer- and research-grade activity monitors exist, but this portion of the literature review will focus on the research utilizing research-grade Actigraph GT3X and Link GT9X monitors based on their inclusion in the proposed study methodology. The GT3X and Link monitors are triaxial capacitive accelerometers that determine number of counts per minute based on a proprietary algorithm that accounts for changes in acceleration along three different axes.[75] Activity counts were traditionally based on accelerometer data obtained from the vertical axis, but more recent Actigraph monitors can assess data based on vector magnitude (VM) which is the square root of the sum of squares of data from each of the three axes. Activity counts take into consideration the intensity of an individual’s movement. For example, more intense movements (jogging) are represented by more activity counts per minute when compared to less intense activities (walking).

Selection of appropriate activity monitoring methodology and processing data parameters are essential to establish before data collection. For adults, it is recommended that accelerometer methodology include waist worn monitors sampling at 30, 60 or 90 Hz with a normal filter using an epoch length of 60 seconds for at least 10 hours per day for at least 4 days (1 weekend day).[74,76] Various activity cut-point metrics based on counts per minute (cpm) can be used to assess different intensities of activity and steps completed while wearing the monitors. Two commonly utilized cut-point measurements in adults are the Freedson 1998[77] and Freedson 2011 VM bouts.[78] The Freedson 1998 cut-points utilize counts from the vertical axis to determine sedentary (0-100 cpm), light (101-1951 cpm), moderate (1952-5724 cpm) and vigorous (<5724 cpm) activity intensities.[77] Freeson 2011 VM cut-points utilize counts from the VM data to determine sedentary (0-200 cpm), light (201-2690 cpm), moderate (2691-6166 cpm), and vigorous (>6166 cpm) activity intensities.[78] Total activity counts (TACs) can be calculated based on total VM counts or counts from axis 1 to assess total amount of activity. TACs can be helpful in measuring total activity during the wear period while Freedson 1998 or Freedson 2011 VM cut-points may be used to identify total activity at a specific intensity (i.e. sendentary, light, moderate, and vigorous). Although not perfect, it can help provide context about the intensity of the exercise because more intense activity results in greater activity counts. However, it is also important to normalize this measure appropriately to wear time or amount of days worn to account for different varying times in activity monitor wear time.

Various studies have used different methods to determine the reliability and validity of the Actigraph monitors. When worn for 4 days for at least 10 hours per day, the WGT3X demonstrates acceptable test-retest reliability over 1 to 3 week periods (ICC =0.80-0.90)[79] It is recommended that men and women wear the activity monitors for at least 4 days to achieve 80% reliability when assessing MVPA or TACs.[79] However, seasonal variability in activity may affect longitudinal reliability of activity wear. For example, activity is greatest during the summer months compared to the winter months.[80] As previously stated, doubly labeled water is a recommended validation test for activity energy expenditure, and demonstrated weak to moderate correlations with TACs and steps (TACs ρ = 0.33-0.44, Steps ρ =0.42).[73] The doubly labeled water technique can be used in free-living situations, in this case over the period of a week, to determine total energy expenditure based on the rate that carbon dioxide leaves an individual's body.[73] Actigraph monitors tend to underestimate step counts at lower speeds (2.4-3.2 km/h) when compared to a manual step count, but demonstrate 83% sensitivity and 89.6% specificity at identifying MVPA compared to indirect calorimetry.[81] Actigraph monitors demonstrated acceptable reliability when testing time points are one month apart, but season variability must be taken into consideration when assessing longitudinal time points farther apart. Validity of the devices varies based on the validation activity to which the monitor is being compared.

ACTIVITY MEASUREMENT STRENGTHS AND WEAKNESSES

Self-reported and accelerometry measures of activity each have their own strengths and weaknesses. The TAS, Marx scale, and IPAQ can be administered quickly and do not require any technical skills or training to administer. However, the nature of the Marx scale and the IPAQ require individuals to retrospectively determine their level of PA within the past week or year subjecting the results to recall bias. The TAS and Marx scale demonstrate adequate test-retest reliability[66,69] in adults over time which can be beneficial to understand if individuals are returning their pre-injury level of activity, but accelerometry measures of activity lack reliability longitudinally when utilized in free-living conditions across different seasons.[80] The Marx scale was validated in adults (>18 year old), but demonstrates good test-retest reliability in children and adolescents, but has a large ceiling effect.[82] The TAS was also only validated in adults (>18 year old) and demonstrated lower comprehensibility in children and adolescents.[83] Because the IPAQ is self-reported, it is subject to recall bias and often overestimates an individual's amount of activity[72] and is not as reliable in individuals under 18 years old.[71] The Physical Activity Questionnaire for Adolescents (PAQ-A) is a more valid and reliable method for determining physical activity level in high school aged adolescents.[84] The Actigraph collects data in free-living situations at all times during the day, but is only worn for short periods of time (1 week) which may not reflect an individual's true physical activity behavior. Due to this fact, accelerometry-based measures physical activity tends to be underestimate actual physical activity participation volume.

Both the TAS and the Marx scale are catered towards identifying individuals that participate in sport-related activities, while the IPAQ and the Actigraph activity monitor assess activity regardless of whether or not its sports related. Individuals that participate in recreational activity or achieve the National PA guidelines without participating in sport will report low to mid-range scores on the TAS and Marx scale. For example, the Marx scale is effective for identifying sport specific activities but may not be good at differentiating individuals that meet PA guidelines with strength and conditioning and moderate to vigorous physical activity. Individuals who are recreationally active, jog, and strength train three times a week to meet the national PA guidelines would only be recorded as a 3 out of 16 total points on the scale. On the contrary someone who plays soccer or basketball would report a 16 out of 16.[85] The IPAQ and the Actigraph capture an individual's participation in all types of activity, even walking, which may be considered light or moderate activity. The limitations of these activity measurements are important to take into consideration when interpreting results of studies that use them.

MEASURING VOLUME AND TYPE OF ACTIVITY AFTER ACLR

The definition of successful recovery after ACLR varies among individuals based on their individualized post-operative goals. Many individuals undergoing ACLR are physically active before injury and describe successful recovery as returning to sport-based PA (recreational, non-elite or competitive, elite) after surgery. Approximately 81% of individuals return to any level of sport-based PA, 65% of individuals return to pre-injury level of sport-based PA, and only 55% of individuals return to competitive levels of sport-based PA.[86] Return to sport-based activity is often used as a surrogate measure for PA in ACLR populations. Some of the most commonly used tools to assess self-reported return to activity in individuals with a history of ACLR include the TAS and Marx scale. However, these classifications of activity cannot determine the extent to which an individual is physically active or if they meet national PA guidelines. The TAS only determines the type of activity in which individuals participate and the Marx scale determines the frequency of sport specific activities, but not the intensity or volume. More recent studies[67,87] have included the self-reported IPAQ to help better define the quantity and quality of activity in individuals with ACLR since it allows individuals to report amount of time spend in vigorous, moderate, and walking activity. Due to improvements in research grade technology, accelerometry as a means of assessing objective activity in this population has been recently explored. The IPAQ and objectively measured PA provide more comprehensive approaches to evaluating activity assessing frequency, intensity and duration of activity. It can also be used to determine if individuals are meeting the recommended national

PA guidelines; however application of these measurement techniques has been limited in the ACLR population to this point.[61]

VOLUME OF ACTIVITY DIFFERENCES IN INDIVIDUALS WITH AND WITHOUT A HISTORY OF ACLR

Methods of activity assessment in ACLR populations include the TAS, the Marx, the IPAQ and accelerometry. No differences in TAS or Marx scores were reported between the ACLR and healthy control groups within five years of ACLR.[87] Additionally, step count was weakly correlated to the TAS (r=0.36, p=0.04) score, but not Marx score (r=0.16, p=0.27) in individuals after ACLR. However, individuals after ACLR reported decreased Tegner scores compared to healthy age and gender matched controls (ACLR=4, Control=6, p=0.001) but no differences in IPAQ scores (ACLR=1563, Control=1893 p=not significant) on average 20 years (range 17-28 years) after surgery.[88] It is important to note that correlations between the TAS, Marx, and objectively measure activity are weak and non-significant (ρ range = -0.03-0.31, p range = 0.10-0.89).[67] These results highlight a discrepancy between an individual's perception and reality of his or her activity engagement. Individuals with and without a history of ACLR tend to overestimate their activity when asked to self-report activity. These limitations are important to consider when interpreting the results of studies using self-reported activity.

In comparison, individuals with a history of ACLR tend to demonstrate poorer accelerometry measured activity outcomes compared to individuals without a history of knee injury, but no differences in self-reported activity outcomes. Individuals with ACLR demonstrated lesser accelerometry measured minutes of MVPA per day (ACLR=79.37±23.95 min/day, Control=93.12±23.94 min/day, p<0.02, d=-0.72, 95% CI=[-1.21, -0.22]), and steps per day (ACLR=8,158±2780 steps/day, Control=9769±980.38 steps/day, p<0.02, d=0.68, 95% CI=[-

1.18, -0.18]) compared to age and sex matched healthy controls.[87] Individuals with a history of ACLR were also 2.36 times (95% CI=[1.09-5.08]) less likely to meet national physical activity guidelines compared in individuals without a history of knee injury.[89] Other recommendations include meeting 10,000 steps per day.[90] However, only 24% of ACLR participants met 10,000 steps per day guidelines compared to 42% healthy controls.[89] Overall, participants with ACLR spent 15 minutes of MVPA less and 1,611 steps less per day compared to healthy controls.[87] As stated previously, approximately 70% of the Americans demonstrate poor levels of activity. It is problematic that individuals with a history of ACLR participate in lesser activity on a weekly basis compared to individuals that are representative of the general population. Limited activity participation resulting in low quantities of knee articular cartilage loading may contribute to the mechanical pathway of poor knee joint health and the premature development of knee PTOA after ACLR in addition to the already well described role it plays in the development of chronic disease and the occurrence of premature mortality.

RELATIONSHIPS BETWEEN VOLUME OF ACTIVITY AND CLINICAL OUTCOMES AFTER ACLR

Lesser quadriceps strength[91,92] and poor self-reported knee function based on the Knee Injury and Osteoarthritis Outcome Score (KOOS)[93] are reported in individuals with a history of ACLR and knee OA. Due to the importance of these clinical outcomes in the development of knee OA, relationships between lower extremity strength, self-reported function, and activity levels have been assessed in individuals after ACLR who are at increased risk of developing knee PTOA. Discrepancies between objective and self-reported measurements of activity may influence these relationships. In this population, the Marx scale was weakly correlated with KOOS stiffness and pain, KOOS symptoms, KOOS daily living and KOOS sports and recreation activities (range *rho*=0.37-0.39, range *p*=0.03-0.04). The TAS was moderately correlated with KOOS sports and recreational activities (*rho*=0.42, *p*=0.02).[67] Objective measures of MVPA were not correlated with self-reported knee function or peak isometric and isokinetic knee extension torque[67], but individuals with greater isotonic and isokinetic quadriceps limb symmetry strength at 3 months were 1.96 times (95% CI = [1.18-3.25]) and 1.68 times (95% CI = [1.10-2.56]) more likely to report greater than a 6 on the TAS approximately months after ACLR.[94] Activity in high-risk populations may play a minor role in poor clinical outcomes commonly measures in individuals with knee OA. Unfortunately, these relationships are inconsistent and longitudinal studies are necessary to better understand the relationship between knee function and activity after ACLR.

VOLUME OF ACTIVITY AND KNEE OSTEOARTHRITS

Individuals with knee OA are less physically active compared to individuals without knee OA[95] completing on average 3,000 less steps per day.[96] However, evidence is conflicting regarding how volume of activity contributes to the progression and development of knee OA.[97] In general, participating at recommended levels of MVPA,[17] as assessed by either self-reported or measured by accelerometer-based physical activity monitors,[98] does not increase an individual's risk of developing knee OA and may have a protective effect. The effects of physical inactivity and very high levels of activity on knee OA development are inconclusive.[17,99,100] The cohorts in these studies[17,98,100] are generally older (>45 years old) than the average age individuals who are included in studies evaluating outcomes in individuals after ACL injury. One systematic review reported that the prevalence of knee OA is highest in elite and professional runners (13.3%) and lowest in recreational runners (3.5%) with sedentary, nonrunners (10.2%) between those groups.[101] The majority of this data was collected from cohort, cross-sectional and case-control studies and a causative relationship cannot be determined. Despite the lower quality evidence, this meta-analysis[101] highlights a potential relationship between volume of activity and knee OA development. Specifically, under- or over-loading of knee based on limited or excessive volumes of activity may impact the deformational behavior of knee articular cartilage in high-risk populations.

BMI IN INDIVIDUALS WITH AND WITHOUT A HISTORY OF KNEE PATHOLOGY

BMI is a general health outcome that is often used as a measurement of body size which considers an adult's height and weight using the equation (Equation 1):[102]

$$Equation\ 1. BMI = \frac{body\ mass\ (kg)}{height^2\ (meters^2)}$$

The same equation is used for children and adolescents, but BMI values are compared to national percentiles based on age and sex.[102] Greater BMI is positively associated with various noncommunicable disease comorbidities including high blood pressure, high non-HDL cholesterol, diabetes, ischemic heart disease, and stroke.[103] Higher BMI, especially those classified as overweight or obese, is also a risk factor for the development of clinical, symptomatic and/or radiographic knee OA.[97] BMI is hypothesized to contribute to the development of symptomatic knee OA through greater mechanical loading, catabolic hormonal and growth factors, and genetics.[104] Greater body mass places greater mechanical on the knee joint during weight-bearing activities which may exceed knee articular cartilage structural properties over time.[104] Young obese and overweight adults also demonstrate lesser knee flexion excursion, greater instantaneous vGRF, and are more likely to walk with heel strike transient during walking as compared to individuals with normal weight.[105,106] The differences in gait biomechanics may alter the distribution of forces on the knee articular cartilage to areas that are not accustomed to high loads.[105,106] While BMI is a clinically applicable quantification of body size, it is a limited measure because it fails to differentiate between fat mass and fat-free mass. For example, individuals with high lean muscle mass may be classified as overweight or obese, but have low fat mass. This is an important limitation to acknowledge because greater presence of adipose tissue in the body may increase the production of growth factor hormones that may negatively impact articular cartilage health.[104]

BMI IN THE GENERAL POPULATION

For adults, BMI less than 18.5 kg/m^2 is considered underweight, 18.5 to 24.9 kg/m^2 is considered healthy or normal weight, 25.0 to 29.9 kg/m^2 is considered overweight and greater than 30.0 kg/m^2 is considered obese.[102] For children and adolescents, BMI less than the 5th percentile is considered underweight, 5th to 85th percentile is considered normal or healthy weight, 85th to 95th percentile is considered overweight and greater than the 95th percentile is considered obese.[102] In general, BMI is moderately associated with better validated measurements of body fat including dual exergy x-ray absorptiometry.[107] However, individuals with larger muscles mass (i.e. professional athletes) are often categorized as overweight or obese based on the BMI classifications despite normal body fat percentages. Average BMIs for young adult men and women in the United States are 27.9 kg/m^2 (20-29 years = 26.6 kg/m^2) and 28.2 kg/m^2 (20-29 years = 26.8 kg/m^2) respectively.[108] Approximately, 36% of American young adults are obese.[109] Median BMI for adolescents is 23.6 kg/m^2.[110] Approximately 34% of adolescents are greater than the 85th percentile (either overweight or obese) and of those individuals 18% are greater than the 95th percentile (only obese).[110] This study suggests that on average young adults between the ages of 20-29 years may be overweight. Due to the relationship between higher BMI and knee OA risk, BMI is a potential covariate to consider in individuals at this age.

BMI AFTER ACLR

BMI has not been adequately tracked longitudinally after ACLR so the casual relationships between ACLR and greater BMI have not been established. In general, adults with a history of ACLR have higher BMI compared to pediatric patients (Adult = 27.2±0.7 kg/m^2, Pediatric = 24.3±1.1 kg/m^2, $p<0.01$).[111] This trend was similar in both male and female patients.[111] One study, tracked BMI at one, three and 6 months post ACLR, but separated low and high BMI groups *a priori*.[112] No significant differences in BMI were reported between time points in the low or high BMI group after ACLR indicating that average BMI in the low and high groups were relatively unchanged across time points.[112] A limitation of this study is that it fails to consider individual fluctuations in BMI and whether specific groups of individuals are more likely to demonstrate BMI changes after ACLR.[112] On average 20 years after ACLR, individuals had higher BMI compared to healthy, age- and gender-matched controls.[88]

Individuals with a history of ACLR are at elevated risk of developing knee OA and greater BMI may act as a confounding factor that increases the mechanical load on knee articular cartilage during movement over time. It is hypothesized that BMI may increase the forces of mechanical loading occurring at the knee joint. During a walking task, obese participants demonstrate greater vGRF compared to normal weight young adults suggesting greater mechanical loading.[105,106] Others hypothesize that an increase in BMI may alter metabolic factors involved in the development of knee OA. Obese individuals demonstrate greater odds of developing hip, knee and hand OA compared to normal weight individuals.[113] Hip and knee joints may experience greater mechanical loading during weightbearing activities regularly, but hand joints do not experience the same frequency of mechanical loading.[113] These findings suggests other factors known to increase the risk of developing OA such as metabolic factors may play a role in obese individuals.

BMI AND SELF-REPORTED KNEE FUNCTION AFTER ACLR

Lower BMI was weakly and negatively associated with higher IKDC scores (-0.08, p=0.04), higher TAS scores (-0.08, p<0.05) and younger age (r=0.23, p<0.05) in individuals ranging from 3.9 to 301.2 months post-surgery.[114] Women with ACLR demonstrated weak associations between BMI and IKDC scores (r=-0.13, p=0.009), but no relationships were reported in men.[114] Individuals with patellar tendon autografts demonstrated weak associations between BMI and IKDC scores (r=-0.16, p<0.01), but no relationships were reported in individuals with allografts or hamstring tendon autografts. After ACLR, individuals who were underweight or normal had a 1.45 higher odds (95% CI = [1.05,1.99]) of achieving healthy normative IKDC scores compared to individuals who were overweight and obese.[114] While these results are statistically significant, these relationships are negative and weak indicating that individuals with higher BMI report poorer knee function. The cross-sectional approach to this study should be considered as a limitation in the interpretation of these results and highlights the need for longitudinal studies assessing the relationship between BMI and self-reported knee function after ACLR.

One study assessed the longitudinal nature of BMI on knee pain and symptoms 2- and 6-years post-surgery. Higher BMI scores at time of surgery were more likely to report poorer knee function scores 6 years (OR = 0.79, 95% CI = [0.69, 0.91]) post-ACLR.[115] Between 5 to 20 years after surgery, higher BMI at the time the survey was administered is associated with poorer self-reported quality of life and greater reporting of depressive symptom.[116] These results differ in comparison to the results of Pietrosimone et al. that reported weak associations ($r = 0.8$, p =

0.04) between BMI and self-reported knee function,[114] but the longitudinal study considers the effects of BMI over time. Greater BMI over longer periods of time may progressively impact articular cartilage health and this relationship may not be apparent in cross-sectional study designs. Physical deficits may also be associated with BMI after ACLR. Individuals who were overweight or obese one month after surgery demonstrated poorer quadriceps strength, single leg hop, and balance testing 6 months post ACLR compared to individuals who were underweight or normal range BMI.[112] Self-reported knee disability and presence of symptoms are considered in the clinical diagnosis of symptomatic knee OA along with radiographic imaging. Self-reported knee function is persistently poorer compared to individuals without a history of knee injury[117] and it is necessary to understand which modifiable risk factors such as BMI may relate to long-term perceived knee disability.

GAIT BIOMECHANICS IN INDIVIDUALS WITH AND WITHOUT A HISTORY OF KNEE PATHOLOGY

Multiplanar knee kinematics and kinetics, and vertical ground reaction forces (vGRFs), differ between individuals with and without a history of ACLR during gait.[118] Knee joint kinematics are defined as angular displacements of the tibiofemoral joint without regards to forces acting on the joint.[119] Knee joint kinetics are defined as internal (i.e. muscular, ligamentous, joint capsule) and external forces (i.e. ground reaction force) acting on the joint during movement.[119] vGRF is defined as the vertical forces of the ground acting on the body.[119] This section will explore gait biomechanical differences in individuals with and without a history of ACLR, and how they may be important in the development of knee PTOA. Table 1 summarizes multiplanar gait biomechanical differences between the involved limb of ACLR patients compared to the contralateral limb and limb of healthy controls which will be discussed in greater detail throughout this section.

Table 1. Summary of gait biomechanics in the involved limb of individuals with a history of ACLR compared to the contralateral limb and the limbs of healthy controls from post-op to greater than 4 years post-surgery

	< 6 months	6-12 months	1-2 years	>4 years
Knee flexion angle	• Greater than healthy control limb • Less than contralateral limb	• Greater than healthy control limb • Inconclusive compared to contralateral limb	-	-
Knee Extension Moment	• Lesser than healthy control limb • Less than contralateral limb	• Lesser than healthy control limb • No differences between involved and contralateral limbs	-	-
Knee adduction angle	-	-	• Greater than healthy control limb • Greater than contralateral limb	• Lesser than healthy control limb • Lesser than contralateral limb
Knee adduction moment	Conflictive Evidence of differences between involved contralateral and healthy control limbs			
Knee internal rotation angle	-	-	• Lesser than contralateral limb • Lesser than control limb	-
Knee internal rotation moment	-	-	• Lesser than contralateral limb • Lesser than control limb	-
vGRF	• Lesser than contralateral limb	• Greater than contralateral limb	-	-

Abbreviations: vGRF = vertical ground reaction force

MULTIPLANAR KNEE BIOMECHANICS DURING GAIT AFTER ACLR

Both greater and lesser knee flexion angle and knee extension moments have been associated with poor articular cartilage joint health and radiographic presence of PTOA.[16,120-122] Some authors speculate that greater knee extension moment and knee flexion angle during gait are indicative of greater quadriceps force which increases tibiofemoral contact.[16] Other authors hypothesize that lesser knee flexion angle results in more anterior tibiofemoral joint contact in the medial compartments where articular cartilage is thinner resulting in greater shear and compressive forces.[122] Changes in sagittal plane knee kinematics and kinetics are inconsistent across time in individuals with a history of ACLR[118,123] and may explain why both greater or lesser sagittal plane knee kinematics and kinetics are associated with knee PTOA.

Two recent meta-analyses[118,123] reported differences in sagittal plane knee joint walking gait biomechanics between the involved limb and contralateral limb of individuals after ACLR and the limbs of healthy controls less than 6 months after surgery. Mean peak knee flexion angles are 23.65º in the contralateral limb, 23.85º in the healthy control limb, and range between 13.41º to 24.4º in the ACLR limb.[123] Individuals with a history of ACLR have greater knee flexion angles compared to healthy controls, but lesser knee flexion angle compare to their contralateral limb before 6-months post-ACLR.[118] After 12 months, both studies reported lesser knee flexion angle in the involved limb of individuals with ACLR compared to the limbs of healthy controls after 12 months[118] for as long as 60 months[123] post-ACLR, but evidence of differences between the involved limb and contralateral limb at this time point is conflicting.[123] Mean peak knee extension moments are 2.74 Nm/kg in the contralateral limb, 2.73 Nm/kg in the healthy control limb, and range between 1.70 to 2.86 Nm/kg in the ACLR limb.[123] In comparison to healthy controls and the contralateral limb, individuals with ACLR demonstrate lesser knee extension moments before 6 months and after a year post-ACLR.[118] After three years post-ACLR, individuals demonstrate no differences compared to their contralateral limb, but lesser knee extension moment compared to healthy controls.[118,123] Therefore, both surgery and time since surgery play a role in determining gait patterns among this population.

Greater knee adduction moments during gait associated with the progression of knee OA.[4] Increases in knee adduction moment shift the weightbearing compression forces from the lateral tibial plateau to the medial tibial plateau increasing the mechanical load placed on the medial compartment.[15] Slater et al. [123] reported greater knee adduction angles between the involved limb of individuals with ACLR compared to their contralateral limb and the limb of healthy controls 11 and 20 months post-ACLR, but smaller knee adduction angles at 48 and 64 months post-ACLR between groups. Hart et al. reported no differences in external knee adduction moments between the involved limb and the contralateral limb of individuals after ACLR and the limbs of healthy controls at any time point.[118] However, Slater et al.[123] suggests that individuals with ACLR report smaller external knee adduction moments compared to the involved limb of ACLRs and limbs of healthy controls at 9, 26 and 34 months post-surgery.

Smaller knee internal rotation angle changes after ACLR alter contact surfaces and shearing forces between the tibia and femur during walking.[4] During this process, articular cartilage surface areas that have adapted to enduring large repetitive shearing forces are not exposed to those forces as often and other articular cartilage surface areas are exposed to increased shearing forces.[4] Transverse plane knee kinematics during walking after ACLR were scarcely and inconsistently reported in the literature.[118] Results for transverse plane knee kinematic and kinetic differences were inconsistent before one year after ACLR.[118] Knee internal-rotation angle and transverse plane knee moments were smaller in the involved limb of individuals with ACLR compare to the contralateral limb and healthy control limb more than 2 years after ACLR.[123] Overloading tissue with shearing forces that is unprepared to resist repetitive shearing due to smaller knee internal rotation angles may result in early degenerative articular cartilage changes.

Under- or overloading the knee joint through compressive forces (i.e. vGRF or loading rate) while walking is also associated with poor knee articular cartilage health.[124] In a cross-sectional study of individuals after ACLR, vGRF was greater in the contralateral limb compared to the involved limb after controlling for time since surgery.[125] When considering time since surgery, individuals with worse self-reported knee function demonstrated lesser peak vGRF less than a year out from surgery, but greater peak vGRF more than 2 years out from surgery.[126] This study demonstrates a shift from underloading early on post-surgery to overloading the knee joint in individuals self-reporting greater knee disability once returning to normal activities after ACLR. Obese individuals without a history of knee injury also demonstrate altered loading characteristics during gait.[106] Obese individuals walked with greater instantaneous loading rates compared to those with normal BMI.[106] Greater BMI may place greater mechanical load on the knee while walk, but may also contribute to altered knee joint kinetics that is detrimental to knee articular cartilage health.

Healthy knees respond positively to consistent loading patterns during activities of daily living. This results in increased thickness in the articular cartilage especially in the posterior lateral compartment and the anterior medial compartment.[4] During the initiation phase of PTOA after ACLR, areas that were once used to a certain degree of shear and compressive forces of loading are now faced with greater or lesser mechanical load in different articular cartilage compartments due to a biomechanical shift during various movements.[15] Greater internal joint moments and knee joint angles result in greater shear forces and may shift tibiofemoral joint surface contact to areas that are ill-equipped to handle the same magnitude or repetition of shear forces, while greater vGRFs result in greater compressive forces. The opposite is true for lesser internal moments, knee joint angles, and vGRFs. For example, greater knee abduction angle after ACLR and lesser vGRF may results in greater shear, but lesser compressive forces in the medial tibial and femoral compartment. It is hypothesized that areas of articular cartilage that are not capable of adapting to new or shifting loading parameters experience superficial fibrillation and collagen breakdown.[15] The cycle continues to negatively spiral as areas with greater fibrillation result in increased articular surface friction and shearing causing deeper tissue degeneration.[15] Hence, movement changes after ACLR may contribute to the early development of PTOA.

EPIDEMIOLOGY OF KNEE OA

OA of various joints affects approximately 1 in 7 people[127] resulting in an average annual cost of approximately $485 billion in the United States.[128] In addition to economic burden, OA results in significant disability contributing to one-third of days of lost work for any reported medical condition[128] and report poorer mental health and limitations in activities of daily living on a weekly basis.[2] Knee OA, specifically, is one of the most debilitating and potential, long-term consequences of ACL injury and reconstruction. The primary purpose of this section is to review the anatomy and development of knee joint articular cartilage. The secondary purpose of this section is to review epidemiology of knee OA in the general population and individuals with a history of ACLR.

ARTICULAR CARTILAGE ANATOMY

Articular cartilage of the knee, also known as hyaline cartilage, is made of fibrous connective tissue located at the distal end of the femur, proximal end of the tibia, and posterior side of the patella. The primary role of articular cartilage in the knee is transmit and distribute compressive forces and reduce friction at the articular surfaces of the knee.[129] In general, articular cartilage lacks vascular and neural innervation.[129] Therefore, articular cartilage receives its nutrients from joint synovial fluid that is diffused into the cartilage as a result of cyclical loading such as walking.[129] Articular cartilage is primarily made of water and consists of an extracellular matrix filled with cells called chondrocytes, collagen fibers (mainly type II collagen) and a collection of proteins called proteoglycan (PG) aggrecans.[130] Chondrocytes produce collagen, proteoglycans, and GAGs, but also aid in cartilage resorption.[130] PG aggregans include core proteins, link proteins, 2 types of glycosaminoglycans (GAGs) called chondroitin sulfate and keratin sulfate which bind to hyaluronan chain.[130] Collagen fibers and proteoglycans are hypothesized to resist tensile and compressive forces respectively.[129]

Articular cartilage can be classified into 4 zones that are structured appropriately to optimize function.[131] The superficial zone consists of type II collagen fibers aligned parallel to the surface, some presence of proteoglycans and flat chondrocytes, and the most amount of water compared to the other zones.[129] This zone resists tensile and shearing forces which occur during knee movement as the articulating surfaces move across each other.[129] In the middle zone, collagen is randomly structured, there are more proteoglycans, and sphere-shaped chondrocytes.[129] The deep zone has collagen fibers aligned perpendicular to the surface, column shaped chondrocytes, the greatest amount of proteoglycans, and the smallest amount of water.[129] The calcified zone has chondrocytes and is separated from the deeper vascular bone by the tide mark.[129] The deeper the zone, the better the articular cartilage is at resisting compressive forces.[129] Articular cartilage is the primary tissue that is compromised in individuals with a history of knee OA.

The bony endplate separates articular cartilage from subchondral bone in the tibia and femur and has neural and vascular innervations unlike the articular cartilage endplate.[132] The bony endplate and subchondral bone play a pivotal role in helping transmit compressive forces throughout the articular cartilage.[132] When the endplate and subchondral bone become compromised as a result of bone contusion, bone marrow lesion or natural tissue aging, the tissue may become sclerotic and demonstrates reduced capabilities in distributing mechanical forces throughout the articular cartilage.[132] Damage to the bony endplate or subchondral after ACLR results in primary damage to the tissue which often heals within a year of injury, but also contributes to altered mechanical loading at the knee both contributing factors to the progression of knee PTOA.[133]

ARTICULAR CARTILAGE AND GROWTH

Normal changes in articular cartilage growth occur as individuals age. MRI-based imaging of longitudinal articular growth over one year in adolescents reported a 0.8% increase in boys and a 1.4% increase in girls of the total tibiofemoral compartment.[134] The largest longitudinal change (<2.5% for girls and <1.5% for boys) occurred in the medial femoral condyle.[134] In comparison, adults (mean age = 30 years old) demonstrated minimal decreases (<3%) in knee articular cartilage thickness changes over one year.[134] The effects of age on knee articular cartilage changes varies depending on maturity. The results of this study[134] are limited because determination of maturity was not defined and there was significant heterogeneity within the adolescent population. The only maturity-based assessment completed in this study was evaluation of epiphyseal plate openness or closure.

Another longitudinal study assessed changes in tibial and patellar articular cartilage thickness changes over 1-2 years.[135] This study utilized Tanner staging to determine sexual maturity of the participants aged 9 to 18 years old.[135] Tanner staging was weakly and negatively correlated with articular cartilage changes indicating smaller changes in more sexually mature individuals, with the greatest change occurring in Tanner stage 2 (average age = 10-13 years old).[135] Medial and lateral tibial articular cartilage decreased less than 10% in individuals defined in Tanner stage 4 and 5. This study[135] is also limited because sexual maturity does not account for bone growth changes and skeletal maturity would be the most appropriate maturity-based indicator for this study methodology. Longitudinal studies assessing articular cartilage in adolescent patients collectively report minimal increases in articular cartilage thickness changes over 1-2 years but lack appropriate identification of skeletal maturity.[135] Adolescents demonstrate slight increases in knee articular cartilage, while presumed skeletally and sexual mature adults demonstrate slight decreases in knee articular cartilage. Studies assessing knee articular cartilages in young participants should account for normal growth changes over time.

KNEE OA

The prevalence of knee OA has increased by nearly one-third since 2005 and is one of the leading causes of disability worldwide.[136] The prevalence of knee OA in North America is 5% in females (lower uncertainty interval (UI) = 3.9%, upper UI = 4.6%) and 3.1% in males (lower UI = 2.4%, upper UI = 4.0%)[136] and the peak age of reporting knee OA is 50 years.[1] Individuals with knee OA spend on average over $140,000 in health care costs in their lifetime.[137] Worldwide years lived with disability as a result of knee OA has increased by over 60% in the past decade.[1] Those with knee OA report poorer health-related quality of life, greater disability and greater pain compared to individuals without a history of knee OA.[138] Knee OA is a disease pathology characterized by measurable structural articular cartilage damage (radiographic OA) and illness pathology referring to patient-reported symptoms (symptomatic OA).[139]

Structural or compositional damage to the joint can include the bone, cartilage, meniscus or the joint capsule, and is often diagnosed with radiographic or MRI imaging.[139] Patient-reported symptoms most often include knee pain, stiffness, and disability with activities and is diagnosed through patient reported outcomes or clinical presentation.[140] However, not all individuals with radiographic presence of knee OA demonstrate symptomatic presentations of knee OA and vice versa.[141] The prevalence of radiographic knee OA is greater than symptomatic knee (37.4% vs. 12.1% in adults older than 60 years old).[142] However, there is some relationship between radiographic and symptomatic knee OA especially in individuals with more advanced forms of knee OA.[143] Individuals with symptomatic knee OA report greater disability with activities of daily living like walking and climbing stairs.[142] The Osteoarthritis Research Society International-Food and Drug Administration (OARSI-FDA) Initiation recommends that radiographic and symptomatic knee OA should be utilized in research studies to help guide effective prevention strategies, diagnostic tools, and treatment.[139]

PTOA

Previous knee trauma, including ACL injury, is a significant risk factor of knee OA and this condition is commonly referred to as PTOA. Individuals with a history of knee injury are 4.2 times more likely to develop knee radiographic OA compared to individuals without a history of knee injury.[144] PTOA may be classified as radiographic or symptomatic similar to knee OA in the general population Prevalence of radiographic knee PTOA in either the tibiofemoral or patellofemoral joint ranges between 0-100% of the involved limb and 2-38% of the contralateral limb 10 to 24 years post-ACLR.[145] Prevalence of radiographic and symptomatic tibiofemoral and patellofemoral OA was 35% and 15% respectively 10 to 15 years post-ACLR. Comparisons to the prevalence of knee radiographic PTOA is much higher than the prevalence of symptomatic knee PTOA which is a similar trend of knee OA prevalence in the general population.[145] Some authors argue the relevance of identifying radiographic knee OA as opposed to symptomatic knee OA because a combination of both will be used in the diagnosis of clinical knee OA.[145] However, radiographic knee OA will precede symptomatic knee OA and may be beneficial in identifying the earliest stages of poor articular cartilage degeneration.

ACLR was once hypothesized to reduce the risk of PTOA after ACL injury, but this hypothesis has been disproven. Current best evidence indicates that surgical reconstruction does not protect against increased development of radiographic knee PTOA and that ACLR may actual result in increased harm and risk of PTOA compared to individuals remaining ACL-deficient (Numbers Needed to Harm = 3, 95% CI = [2, 6]; Relative Risk Increase = 44%, 95% CI = [29, 59].[3] Previous literature reports that 12% of individuals develop clinically diagnosed knee PTOA within 5 years of ACLR[10] and as many as 50% of individuals develop radiographic PTOA within 20 years of ACLR.[3] PTOA after ACLR is concerning due to the young age that individuals may develop PTOA.

Potential risk factors of PTOA in individuals with a history of knee injury include older age, high BMI, chondral injury, and meniscal pathology.[146] Individuals receiving surgery at 35 years old demonstrate 2.44 greater odds (95% CI = [2.1-2.8]) of developing clinically-diagnosed PTOA 5 years post-ACLR compared to younger individuals.[10] Meniscectomy, a common concomitant surgical procedure accompanying ACLR, increases an individual's risk of developing radiographic PTOA by 3.5 times (95% CI = [2.56-4.91]) 10 to 25 years post-ACLR compared to individuals without a meniscectomy.[50] Due to the early and accelerated development of PTOA in individuals with history of ACLR, health care professionals must determine was modifiable risk factors can be addressed to slow down the progression of the diseases. Furthermore, collecting reliable information about concomitant procedures such as meniscal procedures is necessary when exploring PTOA as potential confounders or sensitivity analyses.

MEASURES OF KNEE OA PROGRESSION AND SEVERITY

Radiographic imaging is used for traditional clinical diagnoses of knee-related OA by identifying osteophytes, cysts, stiffening of subchondral bone, and knee joint space narrowing.[147] While useful for ruling in conditions once they are present, radiographs capture articular cartilage with irreversible damage during the later stages of the pathology. Other assessment techniques have been explored to assess the metabolic and histological state of the articular cartilage before irreversible damage has occurred. "Wet" biomarkers may help assess knee joint metabolism and inflammation during the early phases of OA development and progression[148] through synovial, blood and urine biomarkers. Image-based, "dry" biomarkers, including MRI and ultrasound, may also have the capabilities of identifying pre-radiographic changes in articular cartilage. Currently, wet biomarkers are not used to diagnose knee OA in clinical settings because they are expensive, time consuming to process, and require extensive training to analyze. Early identification of individuals with pre-radiographic changes in knee OA, may help health care professionals intervene through injections of targeted biologics or rehabilitation exercise to slow the progression of articular cartilage degeneration in high-risk individuals such as those with a history of knee injury.

RADIOGRAPHIC KNEE ARTICULAR CARTILAGE ASSESSMENT

The Kellgren and Lawrence scale is used by physicians to diagnosis the radiographic presence and categorize the severity of knee OA. Classification of knee OA is graded on a scale of 0 to 4 based on presence of joint space narrowing, osteophyte formation, and increasing stiffness of articular cartilage.[147] The Kellgren and Lawrence scale demonstrates variable inter- and intra-rater reliability ranging from moderate to good.[147,149] The scale is criticized because it relies heavily on osteophyte formation and joint space size to diagnose OA despite the variable presentations of the condition.[147] The scale also fails to evaluate patellofemoral joint articular cartilage health and does not consider the severity of knee OA in relation to patient-reported symptomology disability.[147] This assessment technique is effective once tibiofemoral knee OA is present, but fails to consider the earliest signs of articular cartilage structural breakdown. In order to prevent or delay the development of knee OA, wet and dry biomarkers may be more effective in identifying early signs of articular cartilage changes.

WET BIOMARKERS AND KNEE ARTICULAR CARTILAGE ASSESSMENT

Biological mechanisms[15] refer to the biochemical environment in the synovial joint that can be measured through various inflammatory and cartilage protein biomarkers. Biological mechanism of knee PTOA may include prolonged inflammation and altered responses to mechanical stimuli which disrupt cellular metabolism homeostasis resulting articular cartilage degeneration.[15] Biomarkers can be collected from various sources including synovial fluid within the knee joint, blood, and urine. Synovial, blood and urinary biomarkers including cartilage oligomeric matrix product (COMP), collagen type II cleavage product (C2C), C-terminal cross-linked telopeptide of type II collagen (CTX-II) and matrix metalloproteinase 3 (MMP-3) are elevated when greater collagen degradation occurs in the body, while elevated procollagen II C-propeptide (CPII) is elevated when greater collagen synthesis is occurring. In order to understand homeostasis between collagen breakdown and formation, ratios of C2C to CPII are often assessed. Larger ratios indicate greater collagen degradation to synthesis. Blood biomarker interleukin 6 (IL-6) cytokines are associated with pro-inflammatory states within in the body. It is recommended that researchers use synovial biomarkers assessing cartilage degradation (i.e. COMP, C2C, CTX-II, MMP-3) because they are the most consistently altered after ACLR.[150]

In individuals with a history of ACLR, serum biomarker COMP and IL-6 decreased from pre-operative to approximately one week post-operatively, but no changes were present from 1 to 2 years post-operatively.[150] Similarly, urinary biomarker CTX-II decreased from 4 weeks to 4 months post-operatively, but was greater compared to sex- and age-matched healthy controls up to 4 years after ACLR.[151] Urinary biomarker ratio C2C:CPII was not different compared to controls up to 4 years after ACLR, but synovial biomarker ratio was C2C:CPII greater more than a year out.[150] Synovial MMP-3 has not been assess in ACLR patients, but is greater in ACLD patients compared to healthy controls for years after surgery.[150] Overall, many biomarkers indicative of cartilage breakdown and pro-inflammatory processes are elevated after immediately after surgery and up to 3.5 years post-ACLR providing evidence of a catabolic knee joint environment.[150]

Synovial fluid biomarkers are drawn directly from the joint of interest for localized assessment and provide a more direct measurement of joint metabolism and inflammation. However, these assessments are not commonly performed because they can be very painful and expose the patient to an elevated risk of intracapsular infection. Blood and urine biomarkers may be less invasive, but may identify other metabolic states in the body that could be interacting to elicit changes in protein content. For example, an inflammatory-based blood biomarker may be increased in individuals with concurrent injuries and infections that are not related to knee articular cartilage health. Unfortunately, all of these approaches are expensive, require technical expertise to complete and are inconsistent among studies based on a lack of consensus of the best biomarkers that identify poor articular cartilage health.[150] Assessment of dry biomarkers may overcome clinical barriers to identifying early knee articular cartilage degeneration.

MRI-BASED KNEE ARTICULAR CARTILAGE ASSESSMENT

MRI-based imaging provides a more direct assessment of knee articular cartilage structure as compared to blood and urinary biomarkers, and certain techniques identify early articular cartilage changes compared to radiographic imaging. Some MRI imaging techniques assess the structure outcomes of articular cartilage including joint space width, cartilage volume, cartilage thickness, cartilage area, cartilage roughness, cartilage homogeneity, and cartilage curvature.[152] Joint space width (AUC=0.73) and cartilage roughness (AUC=0.80) demonstrate the best diagnostic capability of all measures to appropriately identify individuals with radiographic knee OA defined in the Kellgren and Lawrence scale compared to healthy individuals. A composite score combining all MRI structural outcomes and wet biomarker CTX-II outcome had the best diagnostic accuracy (AUC=0.84, $p<0.05$).[152] MRIs are also used to identify bone marrow lesions which are identified by increased water signal on an MRI in the tibia or femur.[51]

Other MRI imaging techniques utilize T1ρ and T2 relaxation time of images to assess the energy exchange between water and macromolecules (i.e. proteins) within the articular cartilage[153] and mobility water within the articular cartilage.[154] These measures have been associated with histological changes in articular cartilage as opposed to structural changes. Greater MRI-defined T1ρ relaxation times are sensitive to detecting lesser proteoglycan and greater water content in articular cartilage.[155,156] Greater T2 relaxation times are sensitive to detecting collagen content, collagen disorganization and water content in articular cartilage.[156] T1ρ is more sensitive to early changes in articular cartilage structure compared to T2 relaxation.[157]These changes are indicative of early articular cartilage degeneration[158] before radiographic changes such as joint space narrowing and osteophytes can be identified.

Multiple studies have evaluated changes over time and differences between individuals with and without a history of ACLR of tibial and femoral T1ρ and T2 relaxation from 6 months to 2 years after ACLR. According to MRI-based imaging, changes to the medial compartment may begin within the first year after ACLR and as early as three weeks.[157] Significant increases in involved limb femoral T1ρ and T2 relaxation times have been reported from pre-op to 6 months, 1 year and 2 years post-ACLR indicating lesser proteoglycan content and greater water content within the articular cartilage,[13,16,159] but outcomes remained the same from 6 to 12 months post-ACLR.[159] Significant differences were also reported in medial femoral T1ρ relaxation times between involved limb of ACLR and healthy control limb at 1 year post ACLR.[160] Similar changes were reported in the contralateral limb of individuals after ACLR.[13] Individuals with ACLR undergoing concomitant meniscectomy and meniscal repair surgery demonstrate greater involved limb T2 relaxation times compared to individuals who did not undergo meniscal surgery.[161] Fibrocartilage pathologies are important covarying factors that may affect articular cartilage health and should be considered as confounding factors. Based on these overall findings, early proteoglycan and water content changes of involved limb femoral articular cartilage are evident after ACLR within the first 2 years of surgery and changes may also occur in the contralateral limb. However, these MRI-based imaging techniques and process are technically demanding and time consuming. While these types of images appear to be the most sensitive to changes, they are not clinically feasible for clinicians because image processing is time consuming and requires extensive technical training.

ULTRASONOGRAPHY AND KNEE ARTICULAR CARTILAGE ASSESMENT

Diagnostic US is an accessible tool that may overcome the budget and time barriers associated with MRI assessment of knee joint cartilage health. There are 2 popular US assessment techniques utilized to assess femoral articular cartilage outcomes. One technique involves assessing the cross-sectional area and thickness of resting medial, intercondylar and lateral femoral articular cartilage compartments.[9] US-based assessment resting anterior femoral articular cartilage demonstrates good agreement in medial condyle thickness (ICC=0.719), but poor agreement in lateral condyle (ICC=0.284) and intercondylar thickness (ICC=0.267) compared to macroscopic cadaver femoral articular cartilage full thickness measurements.[6] After eliminating one highly osteoarthritic knee, agreement improved to 0.883, 0.795, 0.732 in the femoral medial, lateral and intercondylar regions, respectively.[6] In general, US assessment tended to underestimate articular cartilage thickness.[6] In individuals without a history of ACLR, this technique demonstrates strong intra- and intersession reliability between sessions at least seven days apart.[8] In individuals with a history of ACLR, US assessment of resting knee articular cartilage demonstrates strong intra- (ICC[2,1]=0.98) and inter-session reliability (ICC[2,k]=0.95) and acceptable precision.[9] Overall, these studies indicate that resting femoral articular cartilage thickness US assessment is valid and reliable.

The second diagnostic US technique assesses the change in cross-sectional area and thickness of the femoral articular cartilage before and after a bout of exercise such as walking.[7,8] This technique is hypothesized to identify early changes in cartilage composition by understanding the deformational behavior of the cartilage through how it responds to loading during activity.[7,8,157,162] Reliability and validity for this technique have not been established.[5] Strong correlations ($\rho=0.82$, $p<0.001$) between US and MRI assessment of resting knee articular cartilage thickness have previously been reported indicating that similar results may be demonstrated in US-based assessments.[163] US-based femoral articular cartilage thickness and cross-sectional area change from pre to post activity in the medial, lateral and intercondylar regions of healthy participants after walking, running, and drop landing indicating femoral articular cartilage deformation.[7,8] Medial, lateral and intercondylar femoral articular cartilage deformation is significantly greater after walking, running, and drop-landing tasks compared to a control resting condition in individuals without a history of knee injury.[7,8] Knee articular cartilage thickness deformation may better capture the ability of articular cartilage to withstand mechanical load compared to resting knee articular cartilage thickness because it represents the response of the tissue.

Knee articular cartilage thickness before and after walking assessed via MRI decreased after walking.[164] Van Ginkle et al. assessed MRI-defined morphological deformation changes before and after 30 minutes of walking in individuals with history of ACLR at time of return sport.[162] Femoral and tibial deformational changes did not differ between participants with ACLR and controls, but participants with ACLR had a slower recovery of knee articular cartilage thickness after running.[162] US-based femoral articular cartilage deformation has not been assessed in individuals with a history of ACLR. Deformation outcomes should be used in studies exploring factors of mechanical load after ACLR such as volume of activity or gait biomechanics related to knee PTOA.

ULTRASONOGRAPHY OF RESTING KNEE ARTICULAR CARTILAGE IN INDIVIDUALS WITH A HISTORY OF ACLR

Harkey et al.[9] explored differences in resting femoral articular cartilage thickness and cross-sectional area between individuals with a history of ACLR and healthy matched controls.

This study identified initial differences in ultrasound based femoral articular cartilage size between ACLR patients and individuals without a history of knee injury. Involved limb knee articular cartilage had greater anterior femoral articular cartilage CSA compared to the contralateral limb (p=0.01, Cohen's d=0.46) and a healthy control limb (p<0.001, Cohen's d=0.50).[9] Specifically, the involved limb had greater medial condyle thickness compared to the contralateral limb and greater medial and lateral condyle thickness compared to healthy controls.[9] While knee OA is defined by joint space narrowing and articular cartilage thinning, thickening of the articular cartilage may occur during the early phases of OA progression.[165] The authors hypothesize that greater articular cartilage thickness may be the result of swelling, increased water content or hypertrophy of articular cartilage.[9] While this may seem counterintuitive, the beginning stages of knee OA have been defined by increases in knee articular cartilage thickness following by articular cartilage thinning and degeneration in later stages.[166] One limitation to this study is that the population is heterogenous and individuals with ACLR varied in time since surgery (time since surgery range = 7 to 103 months).[9] Moderate correlations (r=0.47, p=0.04) were reported between time since surgery and articular cartilage cross-sectional area limb symmetry in individuals with a history of ACLR indicate that individuals greater in time since surgery had greater femoral articular cartilage cross-sectional area in the involved limb compared to the contralateral limb. These findings highlight a need for longitudinal studies utilizing US to assess femoral articular cartilage size after ACLR to understand when these changes begin to occur and how femoral articular cartilage thickness and cross-sectional area vary over time.

KNEE ARTICULAR CARTILAGE HEALTH AND FACTORS OF MECHANICAL LOADING AFTER ACLR

Researchers have benefitted from advancing technological assessment of knee articular cartilage to explore the progression of knee OA in individuals at high-risk of knee OA such as those with a history of ACLR. Factors which influence mechanical loading hypothesized to contribute to the development of PTOA include volume of loading during activity, BMI, and gait biomechanics. Volume of loading assessment explains both the frequency and intensity of loading that occurs at the knee over time. BMI considers the amount of body mass (including fat and fat free mass) that exerts compressive force placed on the knee during all weight bearing activities. Mechanical loading during gait accounts for the shifts in knee joint contact forces as a result of injury and surgical reconstruction. Together these factors represent a more complete picture of the loads placed on the knee that may help to understand how mechanical load contributes to the early development of knee PTOA after ACLR.

VOLUME OF LOADING AND KNEE ARTICULAR CARTILAGE HEALTH AFTER ACLR

Both under- and overloading volumes of activity have been associated with poor knee articular cartilage health. After ACLR, individuals go through extended periods of immobilization and partial weight bearing in addition to restricted activity during rehabilitation. They also continue to demonstrate lesser daily PA and a lower daily step count after they've finished rehabilitation and have been cleared for full participation.[87] Animal and human models indicate that periods of immobilization results in acute periods of articular cartilage thinning.[165,167] Exercise combats the deleterious effects of immobilization and may increase articular cartilage thickness[167,168] which some author's hypothesize may be due to increase in proteoglycan content.[5] In animal models, articular cartilage thickness is not different compared to controls once loading is reinitiated after periods of immobilization.[165] While these periods may be necessary for tissue healing and cannot be avoided, they may acutely weaken the articular cartilage during normal compression. Once patients return to progressively intensive weightbearing activities, the weakened articular cartilage may not be adept at handling significant volumes of load quickly potentially requiring a period of acclimatization as weightbearing loads with progressively challenging activities (i.e. walking, running, sprinting) are introduced throughout rehabilitation. Therefore, authors suggest that graded activity is best for promoting healthy biochemical and mechanical loading environments when integrating individuals into weight-bearing activities.[169] Graded exposure protocols based on healthy cartilage recovery used to return patients to weight-bearing activities have not been established in individuals after ACLR.

New evidence suggests that there may be an optimal volume of load associated with healthy knee articular cartilage. Middle aged individuals without history of OA longitudinally demonstrates a quadratic relationship between accelerometry-measured and self-reported physical activity and T2 relaxation time of the tibiofemoral joint.[17,19] These studies suggest that individual's participating in both excessive amounts of activity and those exhibiting disproportionate amounts of sedentary behavior are associated with greater degeneration in knee articular cartilage as opposed to those participating in moderate levels of activity.[17,19] A healthy and consistent volume of load over time through daily activities may help protect knee articular cartilage from consequential morphological changes. After ACLR, individuals demonstrate lesser volumes of load compared to healthy sex- and aged- matched controls,[87] but it is unclear if this lower volume of activity falls outside of the optimal volume of loading that may promote healthy articular cartilage.

BMI AND KNEE ARTICULAR CARTILAGE HEALTH

While greater BMI is a risk factor for knee OA in individuals without a history of knee injury, the predictive relationship between BMI and PTOA after ACLR is unclear. This is especially the case when understanding the relationship between BMI and early articular cartilage degeneration. At 6 months post-ACLR, BMI was not associated with blood biomarkers associated with proteoglycan content.[170] Conversely, greater BMI on average 3 years after ACLR was moderately correlated with blood biomarkers indicative of greater collagen breakdown to collagen synthesis ratio, but the results were not significant.[124] Other studies report overweight and obese individuals at time of surgery are at 2 to 5 times greater odds of displaying MRI-based OA features including cartilage defects, bone marrow lesions and osteophytes in the tibiofemoral and patellofemoral joints 1 to 5 years post ACLR.[12,171] BMI may be related to articular cartilage changes in later phases after ACLR compared to earlier phases because weight changes may occur as a result of an increased accumulation of loading over time. Within five years of ACLR, individuals who gained weight as opposed to those who maintained weight demonstrated a weight increase of 8.8 kg.[172] This indicates that a subgroup of individuals experience significant weight changes after ACLR which may impact articular cartilage health over time.

BMI may also indirectly affect to the development of knee PTOA through mechanical loading. Individuals with lower BMI were more likely to participate in higher levels of PA (interquartile range OR: 1.37; 95% CI: [1.04-1.82]) based on the Marx scale within 2 years of ACLR[85] indicating that individuals with greater BMI and ACLR may participate in less daily activity. Obesity has also been associated with changes in gait movement patterns in young adults. Obese young adults demonstrate greater instantaneous loading rates, lesser knee flexion excursion, and greater incidence of impulsive loading compared to normal weight young adults.[105,106] Impulsive loading and lesser knee flexion angle have been associated with poor articular cartilage health.[120,173] Therefore, the complex interaction of greater BMI and different factors of mechanical loading after ACL injury may contribute to the development of knee PTOA.

GAIT BIOMECHANICS AND KNEE ARTICULAR CARTILAGE HEALTH AFTER ACLR

As with volume of activity, it is unclear if under- or overloading as expressed through gait kinetics such as knee joint moments and ground reaction forces are associated with the development of knee PTOA. Some of the most commonly explored gait biomechanics include knee extension moment, knee adduction moment, and vertical ground reaction force. Tibial rotation angle, knee flexion angle, and knee abduction angle have also been assessed, but to a lesser extent. A culmination of the relationships between knee gait kinetics and kinematics are reported in Table 2.

Greater internal knee extension moment resulting in greater sagittal plane knee joint shear forces was associated with lesser femoral proteoglycan content within the first 2 years post-ACLR,[16] but lesser internal knee extension moment resulting in lesser shear forces was associated with lesser femoral proteoglycan content and radiograph evidence of OA 3 years post-ACLR.[121,122] Declining knee extension moment could be evidence of quadriceps weakness over time which is a risk factor for knee OA.[174] Lesser external knee adduction moment resulted in lesser shear forces 6 months post-ACLR was associated with metabolic changes indicative of articular cartilage degeneration,[175] but greater external knee adduction moment and angles result in greater shear forces were associated with MRI and ultrasound-defined poor articular cartilage health 6 months to 3 years post-ACLR.[11,176] Minimal external knee adduction moment result increases after ACLR result in shifts of loading on the knee articular cartilage resulting in greater shear forces on the medial tibial plateau compared to the lateral tibial plateau.[15] Both greater and lesser peak vGRF during gait is associated with poor metabolic and imaging-based articular cartilage health due to greater and lesser compressive forces, respectively. Authors speculate that greater vGRF at initial contact or heel strike result in impulsive loading on the knee after ACLR.[173] Any changes in gait have the potential to alter articulating joint contact to areas that are thinner and have not adapted to load changes.[177] A more reasonable explanation is that a combination of tri-planar under- and overloading during different phases of the gait cycle collectively contribute to poor quality of knee loading.

Table 2. Relationship between gait biomechanics and measures of knee articular cartilage health after ACLR

Gait Outcomes	Radiographs	Wet Biomarkers	MRI Imaging	Ultrasound Imaging
Lesser Peak vGRF (involved or LSI)		↑ COMP; 49.3±27.3 mo. post-ACLR[178] ↑ C2C:CPII ratio; 37.9±29.27 mo. post-ACLR[124]	↑ medial and lateral femoral T1ρ; 6 mo. post-ACLR[11] ↑ medial tibial T1ρ; 3 years post-ACLR[121]	
Greater Peak vGRF (involved)		↑ IL-6; 6 mo. post-ACLR[175]	↑ medial femoral T1ρ and T2; 1-2 years post-ACLR[16]	↑ thicker femoral medial condyle; 60±24.8 mo. post-ACLR [176]
Lesser Knee Adduction Moment LSI		↑ MMP-3; 6 mo. post-ACLR[175] ↑ C2C:CPII ratio; 6 mo. post-ACLR[175]		
Greater Knee Adduction Moment (involved)			↑ lateral femoral and medial tibial T1ρ; 6 mo. post-ACLR[11]	↓ femoral medial condyle thickness; 60±24.8 mo. post-ACLR[176]
Greater Knee Adduction Angle (involved or LSI)			↑ medial femoral T1ρ; 3 years post-ACLR[121]	↓ femoral medial condyle thickness; 60±24.8 mo. post-ACLR[176]
Greater knee flexion moment (involved)			↑ medial femoral T1ρ; 1-2 years post-ACLR[16]	

Table 2. (cont’d)

Lesser knee flexion moment (involved or LSI)	KL grade >2; 5 years post-ACLR[122]		↑ medial tibial and femoral T1ρ; 3 years post-ACLR[121]	
Greater knee flexion angle (involved)	Presence of symptomatic lateral compartment knee OA; 12±7 years post-ACLR[120]		↑ medial femoral T1ρ and T2; 1-2 years post-ACLR[16]	↑ thicker femoral medial condyle; 60±24.8 mo. post-ACLR[176]
Lesser knee flexion angle (involved)	KL grade >2; 5 years post-ACLR[122]			
Greater Knee Flexion Excursion (involved)				↑ thicker femoral medial condyle; 60±24.8 mo. post-ACLR[176]
Lesser Knee Internal Rotation Angle (involved)	Presence of symptomatic lateral compartment knee OA; 12±7 years post-ACLR[120]			

↑ = greater; ↓ = lesser
Abbreviations: vGRF = vertical ground reaction force, LSI = limb symmetry index, MRI = magnetic resonance imaging, ACLR = anterior cruciate ligament reconstruction, KL = Kellgren-Lawerence Classification of Osteoarthritis, OA = osteoarthritis

CONCLUSION

PTOA is a common consequence of ACLR and mechanical loading may play a role in accelerating the disease process. Two mechanisms of mechanical loading include cumulative load on the joint during daily activities which is decreased after ACLR and altered surface contact driven through changes in gait movement patterns after ACLR. Both under- and overloading of the knee joint measured through volume of activity and gait knee joint kinetics may contribute to PTOA, but a gap exists in the literature understanding how these mechanical factors of loading interact to impact poor articular cartilage health. Ultrasonography is a pre-radiographic tool to assess resting femoral articular cartilage and femoral articular cartilage response to loading that may overcome the barriers of wet and dry biomarker-based assessments. Early identification of articular cartilage changes and effective interventions are necessary to slow the disease progression considering articular cartilage damage is irreversible.

CHAPTER 3: KNEE ULTRASOUND ASSESSMENTS OF RESTING CARTILAGE AND CARTILAGE RESPONSE TO LOADING IN HEALTHY PARTICIPANTS: A RELIABILITY STUDY

ABSTRACT

Reliable and pre-radiographic assessments of knee articular cartilage health are necessary to identify early changes in cartilage health that precede development of knee osteoarthritis. Ultrasonography of resting femoral cartilage structure or femoral articular cartilage response to loading provide alternatives to traditional imaging. The purpose of this study was to assess the intra-rater and test-retest reliability of resting cartilage and cartilage response to loading ultrasound assessments. Thirty participants (13 Male/17 Female, age=21.8 years, gait speed=1.3 m/s) sat with knees unloaded for 30 minutes before standard ultrasound assessment was captured bilaterally by a single assessor. Next, participants walked 3,000 steps on a treadmill at their habitual gait speed. Post-loading assessments were captured bilaterally by the same assessor. The same procedures were repeated by the same assessor at least 72 hours later. A single blinded rater segmented cartilage images using a semi-automated processing technique to calculate femoral articular cartilage compartmental cross-sectional area (CSA) and thickness. Deformation was calculated as the percentage difference between resting and post-loading CSA or thickness. The same blinded rater processed all images 1 month later. Intraclass correlation coefficients were used to determine intra-rater and test-retest reliability for all cartilage outcomes. All cartilage outcomes during resting and post-loading demonstrated excellent test-retest ($ICC_{2,k}$=0.97-0.99, $p \leq 0.001$) and intra-rater reliability ($ICC_{2,k}$= 0.99, $p \leq 0.001$). All cartilage deformation outcomes demonstrated poor test-retest reliability ($ICC_{2,k}$=-0.36-0.46, *p*-range=0.01-0.87), but good to excellent intra-rater reliability ($ICC_{2,k}$=0.84-0.94, $p \leq 0.001$). The resting cartilage assessment is reliable when capturing images across multiple visits and between image

processing sessions. Caution should be exercised when assessing cartilage deformation over more than one study visit using the cartilage response to loading assessment. Femoral cartilage response to loading may not consistently deform over time and future research should explore why these differences occur.

INTRODUCTION

Knee osteoarthritis (OA) is a chronic disease resulting in irreversible damage to the synovial joint including tibiofemoral articular cartilage. This disease is the 11th most dominant cause of disability worldwide.[1] Radiographic imaging and clinical presentation are the gold standard for diagnosing individuals with knee OA,[147,179] but this approach may lack the sensitivity to detect early synovial joint structural changes that may progress to symptomatic knee OA. Diagnostic ultrasonography, which is available in many orthopedic clinics, is an emerging tool that may help assess early stages of knee articular cartilage change that may precede radiographic findings indicative of knee OA.[4] Ultrasound assessment may also overcome some of the accessibility and invasive barriers associated with other assessment types such as blood and synovial wet biomarkers[148] or T1 rho and T2 relaxation time MRI imaging[158] that are effective in identifying early decline in articular cartilage metabolism or composition.

Currently, resting cartilage and cartilage response to loading ultrasound assessment techniques have emerged to characterize the structure and loading response of femoral articular cartilage.[8,176] Changes in tibiofemoral articular cartilage structure based on conventional MRI imaging across time have been associated with radiographic indications of early development of knee OA[180] and provides the basis for exploring femoral articular cartilage structure using the resting cartilage ultrasound technique.[181] This technique involves assessing resting femoral articular cartilage compartmental thickness and cross-sectional area (CSA) in an unloaded condition. Previous research indicates the resting cartilage assessment technique has excellent intra-session (ICC = 0.98-0.99) and inter-session (ICC = 0.95-0.98) reliability for measurement of femoral articular cartilage compartmental thickness and CSA outcomes within a single unblinded, expert assessor.[8,9] Additionally, excellent inter-rater reliability (ICC =0.94-0.99) has been established between unblinded novice and expert assessors for imaging segmentation of medial, intercondylar, and lateral femoral articular cartilage compartmental thickness.[182] However, a gap exists in the literature exploring the reliability of the resting cartilage assessment technique in a blinded, novice rater. As this ultrasound technique develops, it is important to understand if the measure is reliable across individuals with various levels of training before it can be widely adopted clinically in a variety of healthcare and research settings.

Recently a novel ultrasound technique, which assesses the response of femoral articular cartilage compartmental thickness and CSA to weightbearing loading of walking activity, has been described and applied to individuals without a history of knee injury. This response in articular cartilage size is referred to as deformation.[8] Knee articular cartilage composition may undergo compositional changes such as decreases in proteoglycan content and increases in water content during the beginning stages of knee OA development.[158] Changes in the articular cartilage extracellular matrix may alter its ability to respond to loading (i.e. compression and shear forces) during weightbearing activities.[5] With the cartilage response to loading ultrasound technique, greater deformation following a period of loading indicates poor articular cartilage response to loading[183] and may indicate that cartilage composition has been compromised.[184] Therefore, this technique may provide unique information during the initial stages in knee OA development that may not be apparent on more traditional forms of imaging such as radiographs. Intra-rater and test-retest reliability of the cartilage response to loading ultrasound technique assessing femoral articular cartilage deformation after activity has not been reported. The purpose of was study is to assess intra-rater reliability, test-retest reliability, and agreement of femoral articular cartilage outcomes of a blinded, novice rater using the resting cartilage and cartilage response to loading ultrasound assessment techniques in healthy participants. We hypothesize that the both the resting cartilage and cartilage response to loading ultrasound assessment technique will demonstrate excellent intra-rater and test-retest reliability for assessing femoral articular cartilage compartmental thickness and CSA outcomes.

METHODS

This observational laboratory study was approved by Michigan State University's Institutional Review Board and conducted over 2 identical study sessions at least 72 hours apart. All participants ≥ 18 years old provided written informed consent at the beginning of the first study session. Participants who were < 18 years old provided written informed assent and their parents or guardians provided written informed consent.

PARTICIPANTS AND SCREENING PROCESS

A convenience sample of participants was recruited through flyers, emails, and word of mouth across the faculty and students on the university campus. After providing consent, participants were screened for inclusion criteria and completed a general health history form during the first session. Participants were included in this study if they were between 16 and 30 years old and reported no previous history of intra-articular knee injury or surgery. They were excluded from the study if they reported any other history of lower extremity orthopedic injury in the past 6 weeks (i.e. ankle sprains, muscle strains, etc.), rheumatoid arthritis, or any other chronic illnesses that may impede their ability to complete the tasks required of the study. As part of the screening process, a participant's hydration status was assessed by providing a urine sample at both study sessions because hydration status is reported to impair articular cartilage imaging.[154] An Atago 3730 digital refractometer (ATAGO U.S.A., Inc., Bellevue, WA) was used to measure a participant's urine specific gravity (USG) to determine if the participant was adequately hydrated. Participants were rescheduled if their USG exceeded 1.025 which indicates potential dehydration.[185]

GAIT SPEED ASSESSMENT

Participants performed a gait speed assessment along a 6-meter track between 4 TF100 infrared timing gates (TracTronix, Belton, MO) during the first session only. Participants were asked to walk between the timing gates and along the track at a normal walking speed for 10 trials to determine average habitual gait speed (meters/second)[8] which was converted to miles per hour and used as the treadmill gait speed in the walking protocol described later in the methods.

RESTING CARTILAGE ULTRASOUND IMAGING ASSESSMENT

During both sessions, participants sat in a long-sitting (i.e. legs straight on table) position to unload the knee joints for 30 minutes to minimize the effects of loading experienced prior to the assessment.[7] Sitting normalization time was determined based on a study reporting no differences in femoral articular cartilage compartmental thickness 30 minutes post-activity between individuals who walked and controls who did not walk.[8] Ultrasound images of anterior femoral articular cartilage were captured in both knees with a Vivid iQ ultrasound machine and 12L-RS linear probe (GE Healthcare, Boston, MA). The assessor (C.L.) received in-depth ultrasound assessment training from an expert assessor with 7 years of experience utilizing the same imaging technique.[7-9,183] The proposed imaging technique has been validated using cadaver models[6] and in comparison to MRI imaging of femoral articular cartilage structure.[186] After the 30 minute normalization time, participants were instructed to sit with their back flat against the wall and bend their dominant knee to 140° of knee flexion which was identified manually by a goniometer. The position of the posterior aspect of the calcaneus of the flexed knee was recorded using a tape measure affixed to the table to ensure similar knee positioning post-loading and between sessions (Figure 2A). To image the resting femoral articular cartilage structure, the ultrasound probe was placed perpendicular to the anterior surface of the femoral condyles and aligned with the most anterior aspects of the medial and lateral femoral condyles, superior to the patella (Figure 2B).[8] A transparency grid placed over the monitor display of the image was used to record the position of the medial femoral condyle, lateral femoral condyle, and intercondylar notch to improve reliability of knee images post exercise and between sessions.[8] A total of three images were collected on the dominant limb and the non-dominant legs.

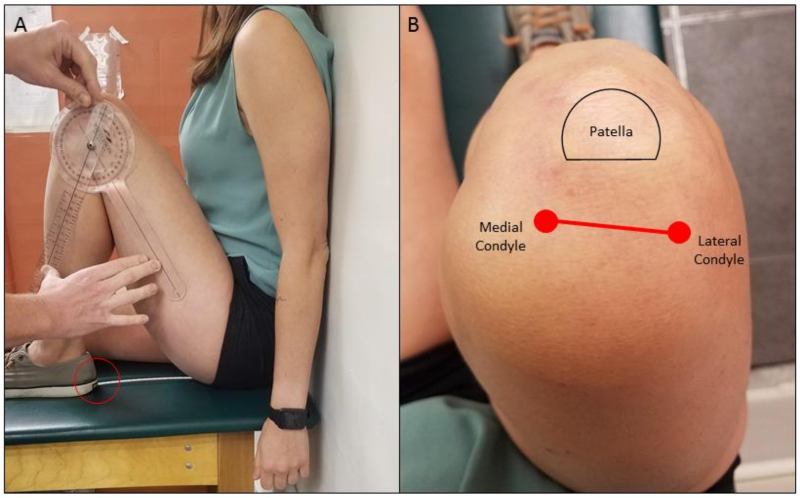

Figure 2. A.) Patient position for all phases of ultrasound assessment with back against wall and knee at 140° of flexion identified by manual goniometer. Red open circle indicates area where position of posterior aspect of calcaneus is recorded and used for all assessments; B.) Positioning of ultrasound head for image capture. Red circles represent medial and lateral condylar landmarks, the red line indicates where ultrasound head is placed in between condyles

STANDARDIZED LOADING PROTOCOL

After the resting ultrasound assessment, participants completed 3,000 steps of walking on a treadmill at their average habitual walking speed. This volume of loading was adopted based on reporting that 3,000 steps is an optimal volume of loading to assess femoral articular cartilage tibiofemoral CSA deformation.[187] The table was located approximately 3.75 meters from the treadmill requiring approximately five additional steps to the step count when leaving and returning to the table. Participants wore Fitbit Charge 2 monitors (Fitbit, Inc., San Francisco, CA) on their dominant wrist to track their step counts in real time with the Fitbit app. The Fitbit Charge 2 was chosen over other activity tracking technology because of its ability to track steps in real time via the Fitbit mobile application and Bluetooth syncing capabilities. When participants achieved 2,995 steps, they were instructed to walk five more steps and place their feet on the side rails of the treadmill so the total steps and duration of the activity could be recorded. Participants returned to lab table for post-loading ultrasound imaging assessment.

CARTILAGE RESPONSE TO LOADING ULTRASOUND IMAGING ASSESSMENT

After engaging in the walking protocol, a total of three images were immediately collected on both the dominant and non-dominant legs using the same protocol reported for the ultrasound assessment of the resting femoral articular cartilage structure.

FOLLOW-UP STUDY VISIT

The second study visit was completed at least 72 hours after the first study visit. Identical methods were used to complete the resting ultrasound imaging assessment, the walking protocol and the post-loading ultrasound imaging assessment. Average habitual gait speed and knee flexion placement based on posterior calcaneus position, and transparency grid with anatomical landmarks from the first study session were used to complete the second study visit.

IMAGE PROCESSING

Ultrasound images were processed with a previous defined semi-automated technique[182] using open source Image J software (National Institute of Health, Bethesda MD). All images were deidentified and randomized by an independent study team member to blind the rater for image processing. Total CSA, defined as the space between the outer borders of the medial and lateral condyles in addition to the superior synovial-cartilage border and the inferior cartilage-bone border (Figure 3A),[7-9,183] was measured by blinded novice (C.L.) rater. The central point of the femoral articular cartilage was manually identified by the blinded rater as the middle of the synovial-cartilage border of the articular cartilage separating the medial and lateral upslopes (Figure 3A). After identifying the total CSA and the central point of the femoral articular cartilage, the images were processed using a custom MATLAB code (Version 9.2, Mathworks, Natik, MA) which segmented the total femoral articular cartilage CSA into medial, intercondylar, and lateral compartments. The intercondylar segment length was defined as the middle 25% of the femoral articular cartilage extending from the manually identified central point (Figure 3B). The medial compartment length was defined from the medial border of the intercondylar compartment to the outer border of the medial condyle and the lateral compartment length was defined from the lateral border of the intercondylar segment to the outer border. Average medial, intercondylar and lateral thicknesses (mm) normalized to segment length and CSAs (mm^2) were calculated for each image of the femoral articular cartilage (Figure 3B). Percentage change[8] between resting femoral articular cartilage and post-loading femoral articular cartilage was used to determine femoral articular cartilage deformation of all outcomes (Equation 1).

$Equation\ 1.$

$$Deformation = \frac{Average\ Post\ Loading\ Outcome - Average\ Resting\ Outcome}{Average\ Resting\ Outcome}$$

Greater deformation is indicated by a negative percentage change value. The singular rater in this study received training in processing the images from the expert rater who has established excellent intra-session and test-retest reliability using traditional manual processing technique.[8,9] The rater for this study and expert rater established excellent inter-rater reliability for processing resting femoral articular cartilage CSA and average thickness outcomes ($ICC_{2,k}$ = 0.994-0.997) using the semi-automated processing technique described in this paper.[182] The same rater (C.L.) completed a second round of blinded image processing approximately 1 month from the first round of image processing in order to establish intra-rater reliability.

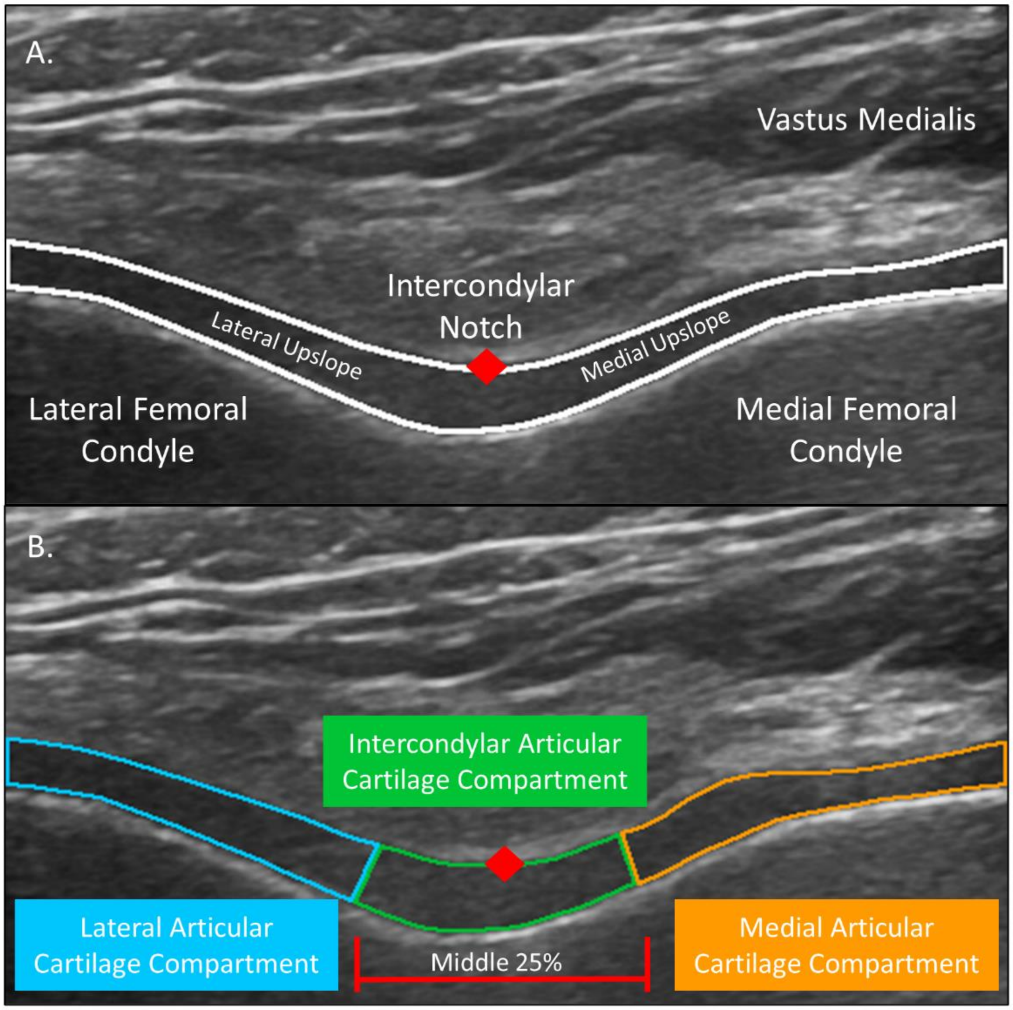

Figure 3. A.) Total cross-sectional area of anterior femoral articular cartilage is outlined by the white line and the center point of the articular cartilage is represented by the red diamond; Figure B.) Medial (orange), intercondylar (green) and lateral (blue) articular cartilage compartments representing the segmented CSA of anterior femoral articular cartilage; central point = red diamond; intercondylar length of the middle segment = red line

STATISTICAL ANALYSIS

Differences in walking characteristics including steps calculated by the Fitbit and total treadmill walking time were analyzed using paired t-tests if data were normally distributed and Wilcoxon signed rank tests if data were not normally distributed. Resting, post-loading, and deformation of femoral articular cartilage CSA and thickness for all three compartments were used for the reliability analyses. Separate intra-class correlation coefficient ($ICC_{2,1}$) were calculated to determine intra-session reliability of the outcomes between the 3 images captured in each knee during the first round of image processing for both visits. Separate intra-class correlation coefficients ($ICC_{2,k}$) were also calculated to determine test-retest reliability and intra-rater reliability of average femoral articular cartilage outcomes from the 3 images capture in each knee between the first and second visit, and the first and second round of rating by a single rater, respectively. ICC values are classified as poor (ICC<0.49), moderate (ICC=0.5-0.74), good (ICC=0.75-0.89) and excellent (ICC>0.9).[188] Standard error of measurement (SEM)[8,189] and minimal detectable change based on 90% confidence[8,189] (MDC_{90}) were also calculated to determine the precision and clinically relevant change of femoral articular cartilage outcomes for intra-rater reliability, inter-rater reliability, and test-retest reliability. MDC_{90} was used to compare to previous literature assessing standard and novel ultrasound assessments of femoral articular cartilage.[8]

$$Equation\ 2. SEM = Standard\ Deviation\sqrt{1 - ICC}$$

$$Equation\ 3. MDC_{90} = 1.654 \times SEM \times \sqrt{2}$$

Bland Altman plots with 95% limits of agreement were generated to analyze trends in agreement for all outcome measures between testing sessions and processing rounds.[190] The mean difference and the average of each articular cartilage outcome between the testing sessions or processing times were plotted on the Y axis and X axis, respectively. Systematic trends of overestimation or underestimation for the articular cartilage outcomes were determined using the Bland Altman plots if the majority of data points are greater than or less than the mean difference, respectively.[190] Trends of overestimation or underestimation based on cartilage size variation were also assessed. For example, smaller outcomes on the plot's x-axis may be evenly distributed between positive and negative mean differences on the plot's x-axis, but larger outcomes on the plot's x-axis may trend in the positive direction. These results would indicate trends of overestimation in participants with large CSA or thickness.

SAMPLE SIZE ESTIMATION

An *a priori* sample size estimation was determined using open-source software, RStudio (Version 1.1.453, RStudio Inc., Boston, MA) and CRAN Package 'ICC.Sample.Size'. We determined a total of 21 participants would be necessary to achieve adequate power (β=0.80) and alpha level (α=0.05) with 2 raters (k=2). This *a priori* power analysis is based on recent publications reporting good (0.83) to excellent intra-rater (0.99) reliability between 2 sessions of the standard ultrasound assessment technique.[8,191] For a conservative estimate, the hypothesized ICC value was set to 0.83 based on the lowest ICC value extracted in the previous study using the standard ultrasound assessment technique,[8] and the null hypothesis ICC was set to 0.49 which indicates poor reliability.

RESULTS

PARTICIPANT DEMOGRAPHIC, WALKING, AND CARTILAGE CHARACTERISTICS

A total of 31 participants enrolled in the study, but only 30 participants were retained in our analysis. The participant removed from the analysis did not return for the second session and did not provide a reason for dropping out. Participant and study session characteristics are reported in Table 3. Step and walking time data were not normally distributed so Wilcoxon 2-sample rank-sum tests were used to assess differences between testing sessions. The number of steps (p=0.68) and time in which participants completed the treadmill walking task (p=0.27) were not different between visit 1 and 2 (Table 4). Femoral articular cartilage outcomes for both visits during round 1 and round 2 of processing are reported in Tables 5 and 6, respectively.

Table 3. Participant and study session characteristics (N=30)

Sex	Males = 13, Female = 17
Age (years)	21.8 ± 3.8 [16, 28][a]
BMI for Adults(kg/m^2)	24.8 ± 4.4 [19.0, 33.5][a,c]
BMI for Adolescents (Percentile)	82% [71%, 90%][b,d]
Days Between Testing Sessions	6.4 ± 2.3 [3, 13][a]
Tegner Activity Level	7 [4, 10][b]
Gait Speed (m/s)	1.3 ± 0.2 [1.0, 1.1][a]

[a]= reported as Mean ± Standard Deviation [Minimum, Maximum]; [b] = reported as Median [Minimum, Maximum], [c] = based on N=25, [d] = based on N=5

Table 4. Participant walking characteristics (Mean ± SD [Range])

Walking Characteristics	Visit 1	Visit 2	*p*-value
Step Count (steps)	3008.6 ± 8.5 [2998, 3032]	3007.8 ± 7.5 [2997, 3034]	0.68
Treadmill Walking Time (min.)	28.4 ± 3.0 [25.1, 42.1]	28.4 ±2.1 [25.5, 36.3]	0.27

[a]= $p<0.05$

Table 5. Femoral articular cartilage characteristics for round 1 image processing of both visits (Mean ± SD)

Outcome	Compartment	Visit 1			Visit 2		
		Resting (mm)	Post Loading (mm)	Deformation (%)	Resting (mm)	Post Loading (mm)	Deformation (%)
Cross-sectional Area	Medial	34.63 ± 6.77	34.61 ± 6.83	0.12 ± 5.77	34.52 ± 6.54	34.26 ± 6.23	0.48 ± 5.66
	Intercondylar	20.61 ± 4.89	20.77 ± 4.93	0.90 ± 6.32	20.49 ± 4.77	20.38 ± 4.78	-0.34 ± 6.55
	Lateral	35.45 ± 7.44	35.44 ± 7.67	-0.04 ± 5.22	35.09 ± 7.29	35.23 ± 7.52	0.35 ± 4.91
Average Thickness	Medial	2.13 ± 0.40	2.12 ± 0.40	-0.21 ± 5.35	2.11 ± 0.39	2.09 ± 0.38	-0.68 ± 4.96
	Intercondylar	2.55 ± 0.60	2.57 ± 0.61	0.86 ± 6.30	2.54 ± 0.58	2.52 ± 0.59	-0.35 ± 6.48
	Lateral	2.08 ± 0.36	2.09 ± 0.39	0.20 ± 4.70	2.08 ± 0.36	2.08 ± 0.36	0.44 ± 4.51

Table 6. Femoral articular cartilage characteristics for round 2 image processing of both visits (Mean ± SD)

Outcome	Compartment	Visit 1			Visit 2		
		Resting (mm)	Post-Loading (mm)	Deformation (%)	Resting (mm)	Post-Loading (mm)	Deformation (%)
Cross-sectional Area	Medial	34.75 ± 7.16	34.51 ± 6.87	-0.40 ± 5.36	34.67 ± 6.69	34.30 ± 6.46	-0.84 ± 4.90
	Intercondylar	20.34 ± 4.97	20.56 ± 4.93	1.32 ± 6.19	20.17 ± 4.78	20.15 ± 4.77	0.07 ± 5.89
	Lateral	35.28 ± 7.26	35.40 ± 7.74	0.18 ± 4.75	35.04 ± 7.35	35.13 ± 7.51	0.31 ± 5.28
Average Thickness	Medial	2.12 ± 0.41	2.11 ± 0.40	-0.50 ± 5.12	2.11 ± 0.39	2.09 ± 0.36	-0.73 ± 4.77
	Intercondylar	2.52 ± 0.61	2.54 ± 0.61	1.24 ± 6.19	2.50 ± 0.59	2.49 ± 0.59	-0.09 ± 5.97
	Lateral	2.08 ± 0.37	2.09 ± 0.39	0.19 ± 4.83	2.07 ± 0.37	2.08 ± 0.37	0.33 ± 4.53

INTRA-SESSION RELIABILITY RESULTS

Intra-session reliability for the resting and post-loading outcomes between the 3 images in each knee was excellent during visit 1 and visit 2 ($ICC_{2,1}$ range = 0.91-0.97) for all outcomes (Table 6). Intra-session reliability for femoral articular cartilage deformation between the 3 images in each knee was poor for visit 1 and visit 2 ($ICC_{2,1}$ range = 0.12-0.38) for all outcomes (Table 6).

TEST-RETEST RELIABILITY RESULTS

Test-retest reliability between visit 1 and 2 for average femoral articular cartilage compartmental thickness and CSA during resting and post-loading was excellent ($ICC_{2,k}$ = 0.97-0.99) (Table 7). Test-retest reliability was poor for average femoral articular cartilage compartmental deformation ($ICC_{2,k}$ = -0.36-0.46) (Table 7). Standard error of measurement and minimal detectable change for each cartilage compartment are reported in Table 7. Based on the Bland Altman plots, there was good agreement between visits and systematic trends in error or based on cartilage size variations between visits were not noted for resting, post-loading, and deformation femoral articular cartilage CSA and thickness (Figure 4 and 5).

INTRA-RATER RELIABILITY RESULTS

Intra-rater reliability between the 2 rounds of processing for visit 1 was excellent during resting and post-loading ($ICC_{2,k}$= 0.99) (Table 9). For deformation, intra-rater reliability for all average femoral articular cartilage outcomes ranges from good to excellent ($ICC_{2,k}$= 0.84-0.94) (Table 9). Standard error of measurement and minimal detectable change between processing rounds for each cartilage compartment are reported in Table 9. Between image processing rounds 1 and 2, good agreement was noted in the Bland-Altman plots, and systematic trends based on cartilage size variation were not noted for resting, post-loading, and deformation femoral articular cartilage CSA and thickness (Figure 6 and 7).

Table 7. Intra-session reliability ($ICC_{2.1}$ and 95% Confidence Intervals) of individual femoral articular cartilage images for all compartments

Outcome	Compartment	Visit 1			Visit 2		
		Resting	Post-Loading	Deformation	Resting	Post-Loading	Deformation
Cross-Sectional Area	Medial	0.93[a] [0.90, 0.96]	0.93[a] [0.90, 0.96]	0.22 (p=0.002) [0.07, 0.39]	0.95[a] [0.93, 0.97]	0.93[a] [0.89, 0.95]	0.34[a] [0.18, 0.50]
	Intercondylar	0.97[a] [0.96, 0.98]	0.96[a] [0.94, 0.98]	0.26[a] [0.10, 0.43]	0.96[a] [0.94, 0.98]	0.96[a] [0.93, 0.97]	0.38[a] [0.22, 0.54]
	Lateral	0.93[a] [0.90, 0.96]	0.94[a] [0.92, 0.96]	0.13 (p=0.049) [-0.02, 0.30]	0.94[a] [0.91, 0.96]	0.95[a] [0.92, 0.97]	0.15 (p=0.03) [-0.00, 0.32]
Average Thickness	Medial	0.95[a] [0.92, 0.97]	0.95[a] [0.92, 0.97]	0.30[a] [0.14, 0.46]	0.95[a] [0.93, 0.97]	0.95[a] [0.92, 0.97]	0.22 (p=0.003) [0.06, 0.39]
	Intercondylar	0.97[a] [0.96, 0.98]	0.96[a] [0.94, 0.98]	0.23 (p=0.001) [0.08, 0.40]	0.96[a] [0.94, 0.98]	0.95[a] [0.93, 0.97]	0.38[a] [0.22, 0.54]
	Lateral	0.91[a] [0.87, 0.94]	0.94[a] [0.90, 0.96]	0.12 (p=0.064) [-0.03, 0.29]	0.93[a] [0.89, 0.95]	0.94[a] [0.92, 0.96]	0.18 (p=0.01) [0.02, 0.35]

[a] = intra-session reliability $p \leq 0.001$; Intra-session reliability for all resting and post-loading outcomes were significant ($p<0.05$)

Table 8. Test-rest reliability ($ICC_{2,k}$ and 95% Confidence Intervals), standard error of measurement (SEM) and minimal detectable change (MDC) for resting, post-loading, and deformation femoral articular cartilage outcomes

Outcome	Compartment	Resting (mm)			Post-Loading (mm)			Deformation (%)		
		ICC	SEM	MDC	ICC	SEM	MDC	ICC	SEM	MDC
Cross-Sectional Area	Medial	0.97[a] [0.95, 0.98]	1.13	2.64	0.96[a] [0.93, 0.97]	1.11	2.58	0.27 (*p*=0.12) [-0.23, 0.57]	3.71	8.63
	Intercondylar	0.99[a] [0.98, 0.99]	0.48	1.12	0.97[a] [0.95, 0.98]	0.83	1.93	0.07 (*p*=0.39) [-0.56, 0.44]	4.47	10.40
	Lateral	0.98[a] [0.97, 0.99]	1.03	2.40	0.98[a] [0.96, 0.99]	1.06	2.47	-0.35 (p =0.87) [-1.30, 0.20]	3.84	8.94
Average Thickness	Medial	0.97[a] [0.97, 0.95]	0.07	0.16	0.97[a] [0.95, 0.98]	0.07	0.15	0.46 (*p*=0.01) [0.09, 0.68]	3.05	7.10
	Intercondylar	0.99[a] [0.98, 0.99]	0.06	0.14	0.97[a] [0.96, 0.98]	0.10	0.24	0.03 (*p*=0.45) [-0.62, 0.42]	4.49	10.44
	Lateral	0.98[a] [0.97, 0.99]	0.05	0.12	0.98[a] [096, 0.99]	0.05	0.12	-0.36 (p=0.87) [-1.30, 0.20]	3.51	8.16

[a] = test-retest reliability $p<0.001$; test-retest reliability for all resting and post-loading outcomes were significant ($p<0.05$)

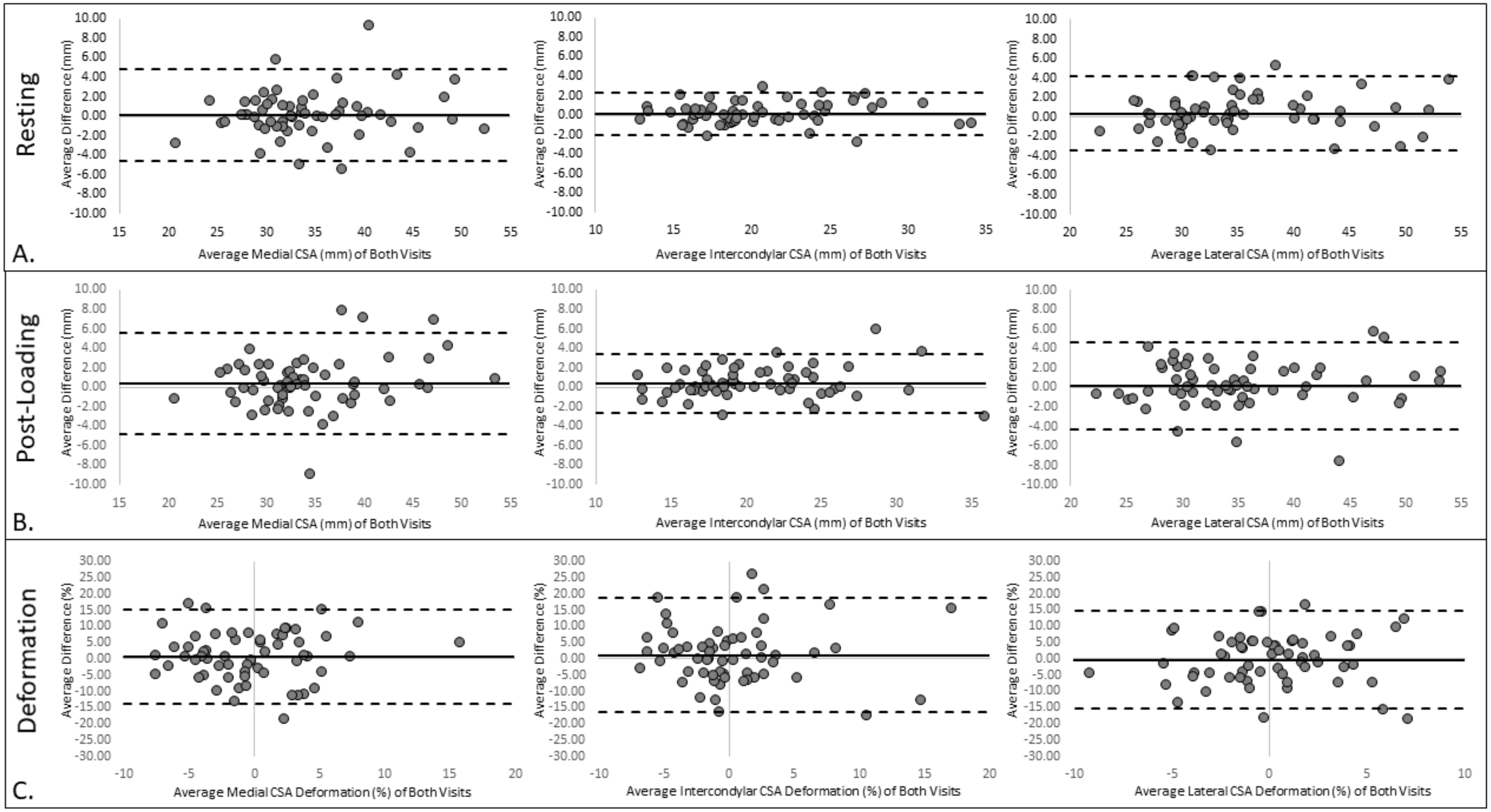

Figure 4. Average femoral articular cartilage CSA differences between visit 1 and visit 2 in A.) Resting, B.) Post-Loading, and C.) Deformation. The solid line represents the average mean difference between visit 1 and 2 for each outcome. The dotted lines represent the 95% upper and lower limits of agreement. Positive average differences indicate that the second visit had greater cartilage CSA

Figure 4 (cont'd)

compared to the first visit. Negative average differences indicate that the second visit had lesser cartilage CSA compared to the first visit

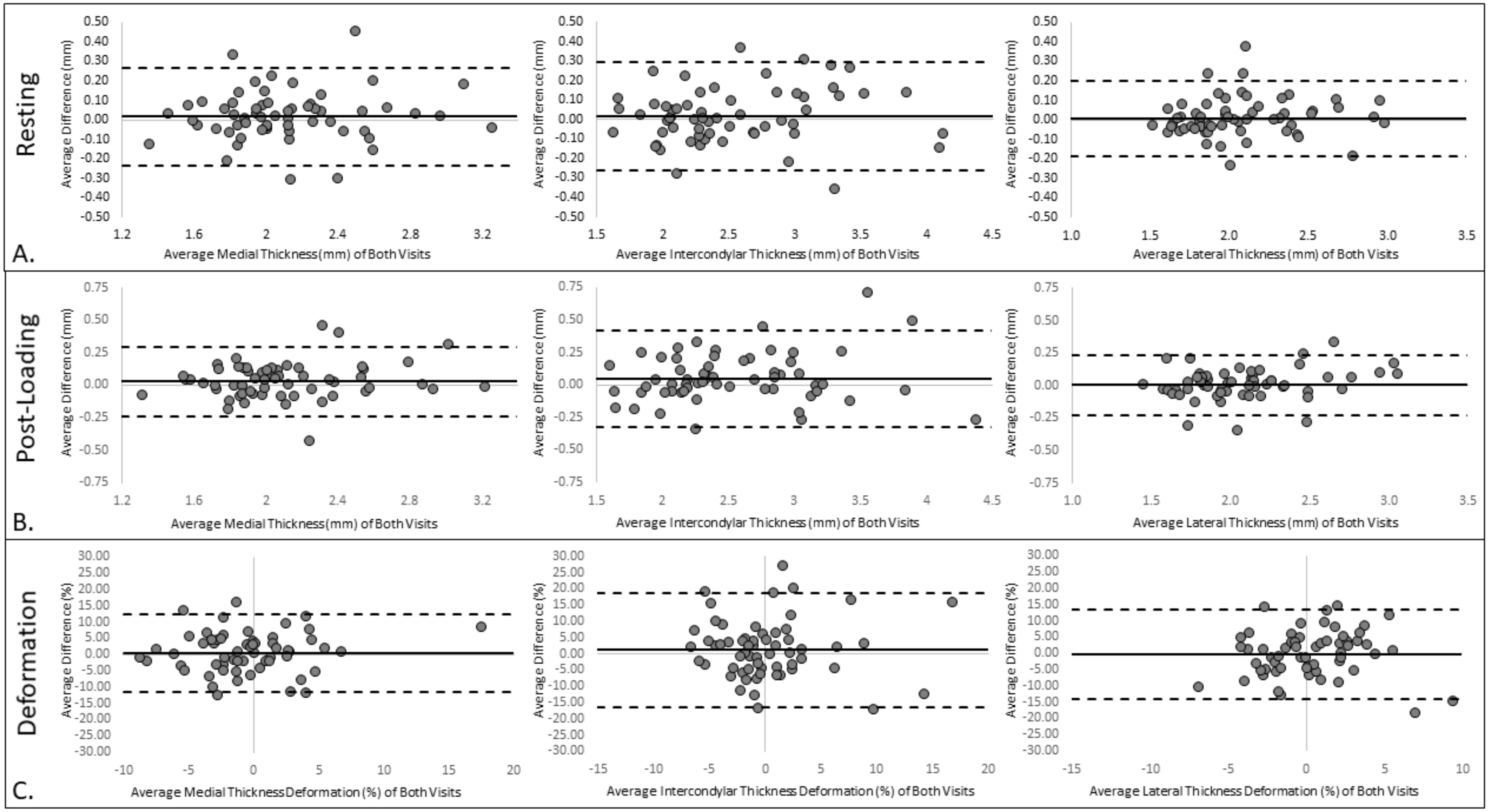

Figure 5. Average femoral articular cartilage thickness differences between visit 1 and visit 2 in A.) Resting, B.) Post-Loading, and C.) Deformation. The solid line represents the average mean difference between visit 1 and 2 for each outcome. The dotted lines represent the 95% upper and lower limits of agreement. Positive average differences indicate that the second visit had greater cartilage thickness

Figure 5 (cont'd)

compared to the first visit. Negative average differences indicate that the second visit had lesser cartilage thickness compared to the first visit

Table 9. Intra-rater reliability ($ICC_{2,k}$ and 95% Confidence Intervals), standard error of measurement (SEM) and minimal detectable change (MDC) for resting, post-loading, and deformation femoral articular cartilage outcomes

Outcome	Compartment	Resting (mm)			Post-Loading (mm)			Deformation (%)		
		ICC	SEM	MDC	ICC	SEM	MDC	ICC	SEM	MDC
Cross-Sectional Area	Medial	0.99[a] [0.98, 0.99]	0.69	1.61	0.99[a] [0.99, 1.00]	0.68	1.57	0.87[a] [0.79, 0.93]	1.89	4.41
	Intercondylar	0.99[a] [0.99, 1.00]	0.49	1.15	0.99[a] [0.99-1.00]	0.49	1.15	0.94[a] [0.90-0.96]	1.49	3.46
	Lateral	0.99[a] [0.98, 0.99]	0.73	1.71	0.99[a] [0.98-0.99]	0.77	1.78	0.84[a] [0.73, 0.90]	1.85	4.31
Average Thickness	Medial	0.99[a] [0.99, 1.00]	0.04	0.09	0.99[a] [0.99, 1.00]	0.04	0.09	0.92[a] [0.86, 0.95]	1.42	3.31
	Intercondylar	0.99[a] [0.99, 1.00]	0.06	0.14	0.99[a] [0.99-1.00]	0.06	0.14	0.94[a] [0.90, 0.96]	1.48	3.45
	Lateral	0.99[a] [0.99, 1.00]	0.04	0.09	0.99[a] [0.99-1.00]	0.04	0.09	0.92[a] [0.86, 0.95]	1.29	3.01

[a] = intra-rater reliability $p<0.001$; intra-rater reliability for all resting, post-loading, and deformation outcomes were significant ($p<0.05$)

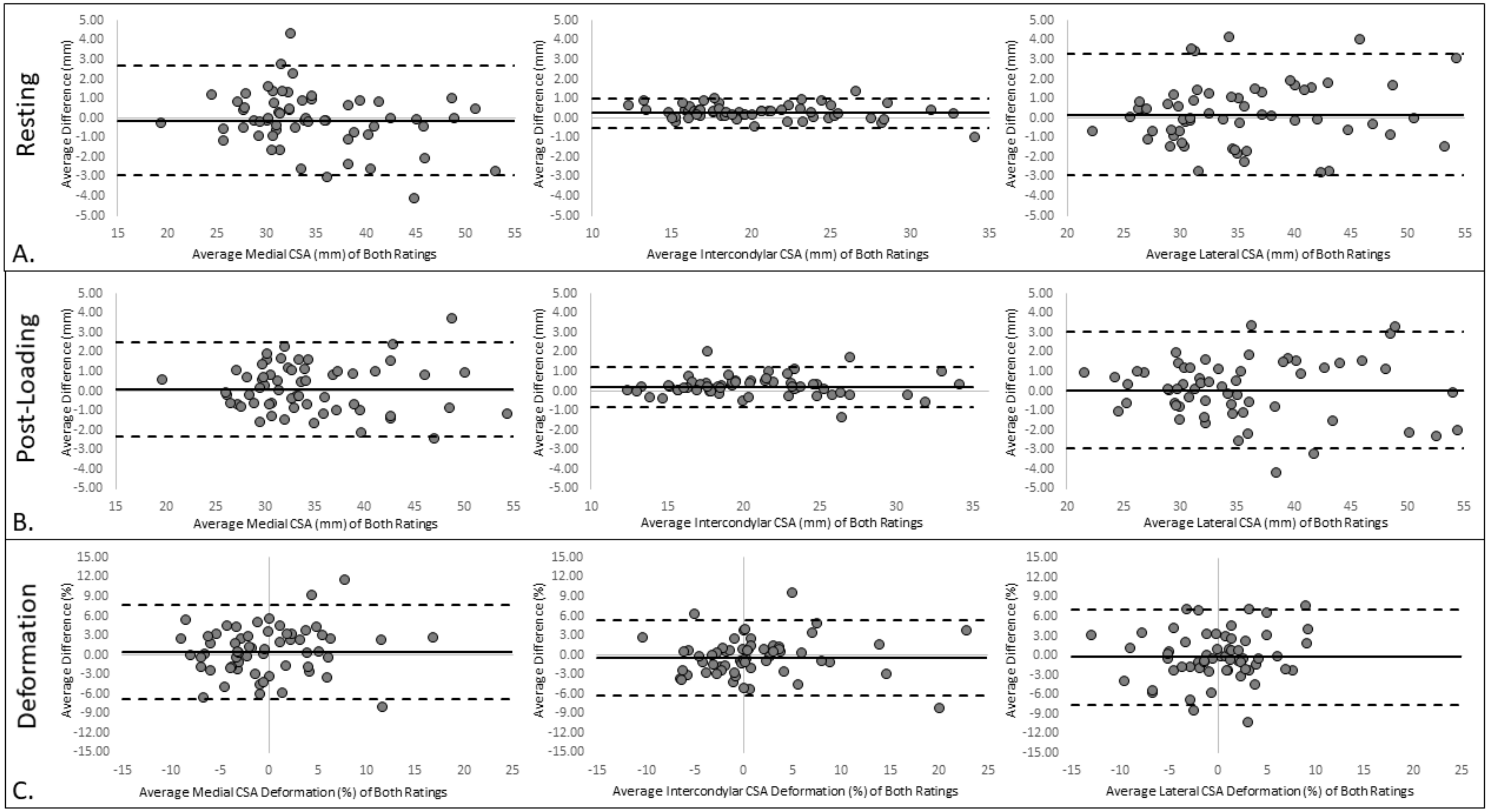

Figure 6. Average femoral articular cartilage CSA differences between image processing round 1 and 2 in A.) Resting, B.) Post-Loading, and C.) Deformation. The solid line represents the average mean difference between image processing round 1 and 2 for each outcome. The dotted lines represent the 95% upper and lower limits of agreement. Positive average differences indicate that the

Figure 6 (cont'd)

second round had greater cartilage CSA compared to the first round. Negative average differences indicate that the second round had lesser cartilage CSA compared to the first round

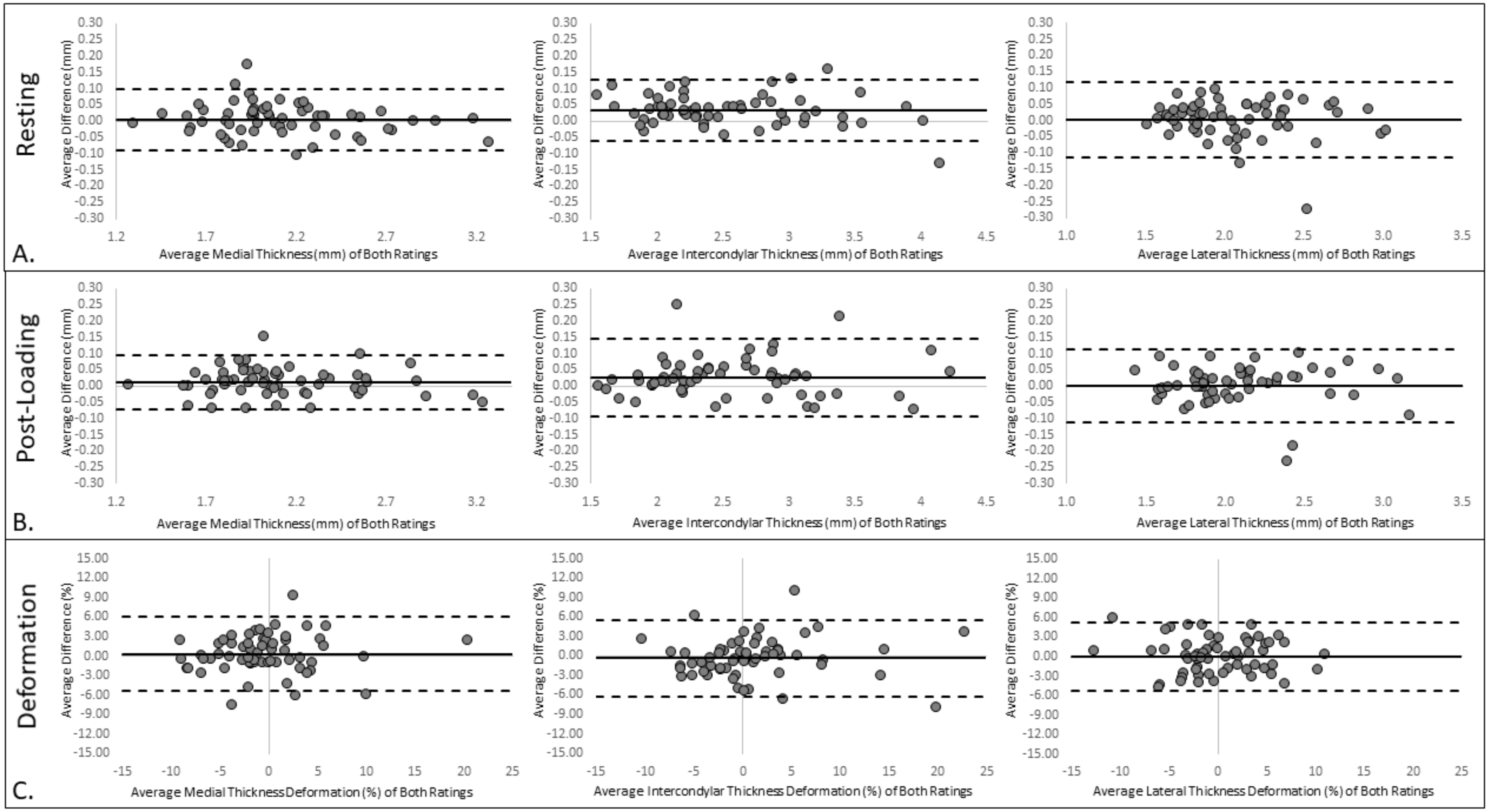

Figure 7. Average femoral articular cartilage thickness differences between image processing round 1 and 2 in A.) Resting, B.) Post-Loading, and C.) Deformation. The solid line represents the average mean difference between image processing round 1 and 2 for each outcome. The dotted lines represent the 95% upper and lower limits of agreement. Positive average differences indicate that the

Figure 7 (cont'd)

second round had greater cartilage thickness compared to the first round. Negative average differences indicate that the second round had lesser cartilage thickness compared to the first round

DISCUSSION

Resting cartilage and cartilage response to loading ultrasound assessments of resting, post-loading, and deformation femoral articular cartilage CSA and thickness have been conducted in individuals with[9,192] and without knee pathologies.[8,186,187,193,194] As research progresses, assessment of articular cartilage structure and response to loading may provide unique and pre-radiographic assessments to characterize knee joint health, but measurement properties must be established before future application of the technique can be applied more broadly with confidence. In the current study, excellent intra-rater and test-retest reliability were demonstrated for resting and post-loading cartilage outcomes. Similar SEM and MDC thickness outcomes were also observed when compared to previous research (Tables 5 and 6).[8] Good to excellent intra-rater reliability was also established for cartilage deformation outcomes, but poor test-retest reliability was reported. The results of this study suggest that resting and post-loading ultrasound femoral articular cartilage outcomes are reliable measures with small, acceptable SEM and MDC but differences in deformation outcomes over multiple study sessions should be interpreted with caution as they are less reliable.

This is the first study to assess post-loading and deformation femoral articular cartilage reliability using a semi-automated processing technique. The results of the current study confirm the previously reported excellent intra-rater reliability for resting femoral articular cartilage thickness in all compartments[182] and report the excellent test-retest reliability of resting and post-loading cartilage outcomes. CSA and thickness MDCs reported in this study can be applied in pathological populations to determine clinically meaningful longitudinal resting femoral articular cartilage compartmental changes. Resting femoral articular cartilage compartmental thickness MDCs between visits in this study (MDC = 0.12-0.16 mm) were similar to Harkey et al. (MDC =

0.14-0.18 mm),[8] but were larger in the intercondylar compartment when compared to previous reports (current study = 2.52-2.54 mm; Harkey et al.[8]=2.24-2.28 mm). These outcomes may vary because the semi-automated technique calculates femoral articular cartilage compartmental thickness across the compartment by normalizing compartment CSA to compartment length instead of measuring thickness at a single location. The technique utilized to measure femoral articular cartilage compartmental thickness in this study was developed based on standardized MRI assessments of tibiofemoral articular cartilage thickness.[195] This technique may provide a more thorough assessment of total femoral articular cartilage compartment thickness when compared to thickness estimated at a single point in the articular cartilage. The current study also included 5 adolescent participants younger than 18 years old. Adolescents have greater intercondylar cartilage thickness (average thickness range in healthy teenagers = 2.87 to 3.47 mm),[196] which may account for small differences in average cartilage thickness size when compared to adult populations (>18 years old). Normative resting femoral articular cartilage compartment thickness may vary by age or skeletal maturation stage[134] and should be considered as an important co-variate in future research assessing resting femoral articular cartilage compartmental CSA or thickness.

Articular cartilage deformation was inconsistent between visits, which limits the ability of researchers or clinicians to determine meaningful differences in deformation over time using this technique. In the case of ultrasound assessment, intra-rater reliability assesses the rater's ability to consistently segment total femoral articular cartilage CSA during image processing within a single study visit or session. Test-retest reliability assesses the assessor's ability to capture similar areas of the anterior cartilage by aligning the ultrasound probe consistently between 2 separate study visits or sessions. In this study, intra-rater reliability for cartilage deformation was categorized as good to excellent. However, test-retest reliability for deformation was categorized as poor. These results suggest that femoral articular cartilage may not uniformly deform as previously reported.[8,183] Contrary to our expectations, some compartments decreased in size, while others increased in size following the standardized loading protocol. Approximately 47% of limbs consistently deformed across all compartments and only 23% of participants consistently deformed in all compartments in both limbs. In the medial compartment alone, only 52% of limbs decreased or increased thickness consistently between visits. Based on these findings, caution should be exercised when comparing cartilage deformation outcomes between multiple visits or multiple assessments within the same study visits. Future research should determine if these inconsistencies are dependent on the cartilage deformation specific to individual study participants or ultrasound assessment methodology (i.e. length of time for knee unloading before assessment or time of day of assessment). When designing training for assessors, more time should be spent on post-loading image capturing training, and deformation reliability should be established before integration into data collection.

Poor femoral articular cartilage deformation test-retest reliability was reported in this study. This finding may have resulted from the use of a suboptimal loading protocol and limitations in our step tracking approach. Pfeiffer et al.[187] reported that participants' femoral articular cartilage tended to increase in size after walking 1,000, 2,000, 4,000 and 5,000 steps on a treadmill. However after 3,000 steps, 93% of the participants demonstrated decreases on total femoral articular cartilage CSA.[187] As a result, participants were required to achieve 3,000 steps in the current study to achieve optimal loading for deformation. It is unclear why this articular cartilage response occurs in healthy individuals, but the authors suggested that greater resting femoral articular cartilage structure is reported as a positive tissue adaptation to adequate and consistent loading.[187] In regards to standardizing the loading protocol, we used wrist-worn Fitbit accelerometers to track step counts because the device provides real-time data syncing via Bluetooth which was optimal for monitoring out participants' progress during the loading protocol. Unfortunately, wrist-worn Fitbits have moderate test-retest reliability (ICC = 0.70-0.73)[197] meaning the step counts reported in this study may have under- or overestimated the number of actual steps taken by each participant. Therefore, participants may not have achieved optimal loading conditions resulting in deformation as defined by Pfeiffer et al.[187] If some participants in our study accumulated steps greater than or less than 3,000 steps while others accumulated steps closer to 3,000, this may result in the inconsistent post-loading outcome findings. Future studies should utilize more reliable equipment to ensure that participants are loading the recommended number of steps for optimal deformation such as pedometer with capabilities to display real time feedback (i.e. OneTweak) used in a previous study.[187]

As previously stated, this was the first study to assess post-loading and deformation reliability of femoral articular cartilage outcomes among a sample that included adolescent participants. Knee OA typically impacts older individuals, but young individuals with a history of knee injury have an elevated risk of developing early post-traumatic knee osteoarthritis.[3] Early detection of knee OA development though pre-radiographic assessments are essential in younger populations. While only a few adolescent participants were included in our study, adolescent or young adult participants in our study may be skeletal immature, which may affect longitudinal assessments.[134] Implementation of gold standard skeletal maturity assessments requires increased exposure to radiation through radiographs or longitudinal assessments to determine peak height velocity.[198,199] We were unable to determine a participant's stage of skeletal maturity, but future research should assess longitudinal differences in ultrasound femoral articular cartilage assessments in skeletally immature participants.

CONCLUSION

Resting femoral articular cartilage compartmental CSA or thickness outcomes demonstrate excellent intra-rater and test-retest reliability. The results of this study reinforce the utility of semi-automated ultrasound-based measurement of resting femoral articular cartilage compartmental CSA or thickness for assessment between multiple study visits. In comparison, femoral articular cartilage deformation outcomes are inconsistent over multiple assessments and should be used with caution. Femoral articular cartilage may not consistently deform as previously hypothesized. Future research should assess physiological explanations for differences in cartilage response. Valid and reliable step-tracking equipment should be used when assessing cartilage response to loading to ensure optimal loading (3,000 steps) for more consistent cartilage deformation results.

CHAPTER 4: AVERAGE RESTING FEMORAL ARTICULAR CARTILAGE THICKNESS IN INDIVIDUALS AFTER ACLR: A LONGITUDINAL STUDY

ABSTRACT

Individuals with a history of anterior cruciate ligament reconstruction (ACLR) are at elevated risk for accelerated development of post-traumatic knee osteoarthritis. Many biomarkers used for assessing knee joint health in individuals with a history of ACLR are not sensitive to early structural changes in the joint or they are impractical for use by health care providers. Diagnostic ultrasonography may provide a pre-radiographic assessment of knee joint health, but it is unclear if changes in articular cartilage structure can be detected over time among individuals who have recently undergone ACLR. The purpose of this longitudinal study was to compare femoral articular cartilage thickness via ultrasound in the involved and contralateral limb at 4 and 6 months post-ACLR. A total of 20 participants recovering from ACLR (10 Male/10 Female, age=21.1±5.7) completed 2 identical testing sessions at 4- and 6-months post-ACLR. After 30 minutes of participants unloading their knees, three ultrasound-based femoral articular cartilage images were captured in the involved and contralateral knees at both time points. Average medial, intercondylar, and lateral femoral articular cartilage compartmental thicknesses were determined using a semi-automated processing technique. Two-way repeated measure ANOVAs were used to compare femoral articular cartilage thickness outcomes between limbs over time. Paired t-tests were used to assess difference between groups if interactions were identified. Individuals with a history of ACLR did not demonstrate statistically significant main effects for limbs (p-range=0.50-0.92) or time (p-range=0.22-0.72), or interactions (p range = 0.24-0.49) for any femoral articular cartilage compartment. Ultrasound assessment of femoral articular cartilage

thickness may not detect between limb differences or changes over time between 4 to 6 months post-ACLR.

INTRODUCTION

Approximately 12% of patients are clinically diagnosed with post-traumatic knee osteoarthritis (PTOA) only 5 years after anterior cruciate ligament reconstruction (ACLR)[10], and 35% of patients experience symptomatic PTOA within 15 years of ACLR.[145] Imaging assessment techniques, such as radiographs and magnetic resonance imaging (MRIs) of static tibiofemoral articular cartilage are used to assess synovial joint health in individuals with a history of ACLR.[120,122] Traditionally, these imaging techniques capture resting images of the knee providing essential information about the structure of the synovial joint especially the articular cartilage (i.e. presence of osteophytes, joint space narrowing), and are helpful for diagnosing PTOA after degeneration has occurred. However, traditional imaging assessments may not be able to identify early onset synovial joint changes before permanent tissue damage has occurred.

Despite the inability of traditional imaging assessments to detect early articular cartilage changes, other biomarkers may assess early metabolic, compositional, and structural cartilage changes that may precede the irreversible degenerative tissue damage. Serum, synovial, or urinary biomarkers indicative of cartilage breakdown and pro-inflammatory processes are elevated within the first few weeks or months post-ACLR providing evidence of a catabolic knee joint environment.[150] Furthermore, research grade MRI outcomes (T1ρ and T2 relaxations times) associated with diminished proteoglycans and increased water content of tibiofemoral articular cartilage are present in the medial compartment of the involved limb of individuals with a history of ACLR compared to their contralateral limb within the first 6 months to a year after surgery.[13,160] Unfortunately, these tools are impractical and difficult to use to perform longitudinal assessments. Diagnostic ultrasonography is an emerging, pre-radiographic assessment of knee articular cartilage health found in most orthopedic clinics that may overcome some of the barriers associated with assessing the other biomarkers.[148,200] A recent cross-sectional study reported differences in involved limb femoral articular cartilage thickness compared to the contralateral limb in individuals approximately 3 years post-ACLR providing preliminary evidence for the use of this technique.[9] However, it remains unclear how early after ACLR ultrasound assessments can detect between limb differences.

Declines in tibiofemoral joint health are present during the initial phases of recovery after surgery[13,150,160] and may be impacted by changes in mechanical loading through weight-bearing activities.[11,124,201] Time points 4 and 6 months post-ACLR mark milestones in the recovery process when health care professionals often recommend distinct changes in patient activity that may increase mechanical loading occurring at the knee.[202] At 4 months post-ACLR, approximately 75% of physical therapists report returning patients to jogging and 50% report returning patients to modified sports activity.[14] At 6 months post-ACLR, healthcare professionals often begin to make decisions regarding participation in unrestricted activity and many patients cease rehabilitative care.[14] Changes in activity around these time points may increase mechanical loading occurring at the knee and impact knee articular cartilage health. However, a gap exists in the literature understanding changes in knee articular cartilage structure during these periods of increased mechanical loading. There is a critical need to determine if this promising ultrasound assessment of femoral articular cartilage thickness can detect changes during this period of increased mechanical loading after ACLR.

The purpose of this longitudinal study is to compare changes in medial intercondylar, and lateral femoral articular cartilage compartmental thickness between the involved limb and contralateral limb of individuals recovering from ACLR at 4- and 6-months post-surgery. We hypothesize that individuals will demonstrate greater medial femoral articular cartilage compartmental thickness in the involved limb compared to the contralateral limb at both time points post-ACLR. Secondly, we hypothesize that individuals will demonstrate greater medial femoral articular cartilage compartmental thickness in the involved limb at 6 months post-ACLR compared to 4 months post-ACLR. Medial, intercondylar, and lateral femoral articular cartilage compartmental thickness will not differ in the contralateral limb between 4 and 6 months post-ACLR.

METHODS

This longitudinal study was completed over 2 testing sessions assessing femoral articular cartilage structure via ultrasound in individuals with a history of ACLR at 4-months (± 2 weeks) and 6-months (± 2 weeks) post-surgery.

PARTICIPANTS

Participants were recruited by 4 fellowship trained orthopedic surgeons at the Michigan State University sports medicine clinic and on Michigan State University campus via flyers, emails and word of mouth. Participants between the ages of 16 and 35 years old with a history of primary, unilateral ACLR and full knee range of motion were included in the study. Participants were excluded from the study if they had a previous history of intra-articular knee injury not related to the current ACL injury (i.e. meniscal pathology, articular cartilage pathology, previous ACL injury) or rheumatoid arthritis. Participants were not excluded if they had other surgical procedures (meniscal, articular cartilage or MCL related surgical procedures) completed at the time of ACLR.

SAMPLE SIZE ESTIMATION

A priori sample size estimations were completed using G*Power (Version 3.1.9.2, Henrich Heine Universität Düsseldorf, Brunsbuttel, Germany) assuming an alpha level of 0.05 and a statistical power of 0.80. The sample size estimation was based on a large effect (Cohen's *d*=0.79) indicating differences in ultrasound-defined resting medial femoral articular cartilage compartmental thickness between the injured limb of individuals on average 3 years post-ACLR and healthy controls which indicated that a minimum of 12 participants would be required to detect differences in femoral articular cartilage size over time within this population.[9]

PARTICIPANT SCREENING PROCESS

This study was approved by Michigan State University's Institutional Review Board and all participants ≥ 18 years old provided written informed consent before engaging in study activities. Participants under the age of 18 provided informed assent and their parents or guardians provided informed consent prior to engaging in any study related procedures. Previous research suggests that dehydration may negatively impact articular cartilage imaging.[154] Following the consent process, participants were required to provide a urine sample to assess their hydration status. A participant's urine specific gravity (USG) was assessed via an Atago 3730 digital refractometer (ATAGO U.S.A., Inc., Bellevue, WA). Participants were considered dehydrated if their USG was greater than 1.025.[185] Participants who were dehydrated were rescheduled to another day to eliminate hydration status as a confounding factor.

RESTING CARTILAGE ULTRASOUND IMAGING ASSESSMENT

After providing written informed consent, participants sat in a long-seated position (i.e. knees supported by table in extended position) for 30 minutes to minimize the effects of knee joint loading experienced during activities of daily living prior to the assessment.[7,8] Ultrasound images of anterior femoral articular cartilage were captured in both knees with a Vivid iQ ultrasound machine and 12L-RS linear probe (GE Healthcare, Boston, MA) with a valid[6,186] and reliable[7-9,183] assessment technique. After unloading, participants were instructed to sit with their backs flat against the wall and bend their contralateral knee to 140° of knee flexion as determined by a manual goniometer. The position of the posterior aspect of the calcaneus of the flexed knee was recorded using a tape measure affixed to the table to ensure similar knee positioning between the 4-month and 6-month session (Figure 2A & 2B). To image the resting femoral articular cartilage, the ultrasound probe was placed perpendicular to the anterior surface of the femoral condyles and aligned with the most anterior aspects of the medial and lateral femoral condyles, superior to the patella.[8] A transparency grid placed over the monitor display of the image was used to record the position of the medial femoral condyle, lateral femoral condyle, and intercondylar notch to improve reliability of knee images post exercise and between sessions.[8] A total of three images were collected on the contralateral limb followed by the involved limb of participants recovering from ACLR.

6 MONTH POST-ACLR ASSESSMENT

The hydration screening process and resting ultrasound imaging assessment described at the 4-month assessment were repeated at the 6-month assessment.

IMAGE PROCESSING

Ultrasound images were processed using open source Image J software (National Institute of Health, Bethesda, MD). Resting femoral articular cartilage images were randomized by a study team member and processed by 1 blinded rater (C.L.) using built-in measurement tools. Total femoral articular cartilage cross-sectional area (CSA) was measured as the space between the outer borders of the medial and lateral condyles in addition to the superior synovial-cartilage border and the inferior cartilage-bone border (Figure 3A).[7-9,183] The central point of the femoral articular cartilage was manually identified by the rater as the middle of the synovial-cartilage border of the articular cartilage separating the medial and lateral upslopes (Figure 3A). After identifying the total CSA and the central point of the femoral articular cartilage, the images were processed through a custom MATLAB code (Version 9.2, Mathworks, Natik, MA) to segment the total femoral articular cartilage CSA into medial, intercondylar, and lateral compartments. The intercondylar compartment length was defined as the middle 25% of the femoral articular cartilage extending from the manually identified central point (Figure 3B). The medial compartment length was defined from the medial border of the intercondylar compartment to the outer border of the medial condyle and the lateral compartment length was defined from the lateral border of the intercondylar compartment to the outer border. Medial, intercondylar, and lateral femoral articular cartilage compartmental CSA (mm^2) were normalized to individual compartment length to calculate femoral articular compartmental thickness (mm) for each image (Figure 3B).

STATISTICAL ANALYSIS

Participant characteristic differences between 4- and 6-months post-ACLR were assessed with paired t-test. A previous study reported no differences of resting medial and lateral femoral articular cartilage compartmental thickness between the contralateral limb of individuals with a history of ACLR compared to dominant limb of healthy controls.[9] Therefore, the contralateral limb of the participants with ACLR was used as a control limb for statistical analysis in this study. Main effects and interactions for between limb (involved limb and contralateral limb) and time (4 and 6 months post-ACLR) differences were assessed using a 2-way repeated measure analysis of variance (ANOVA). Significant interactions were further investigated using a paired sample t-test to identify differences between limbs at each time point and within limbs across time. Alpha was set to 0.05 *a priori*.

RESULTS

A total of 20 participants (10 Male/10 Female, age range = 16-33 years old, 10 hamstring graft/9 bone-patellar tendon-bone grafts/1 Allograft) participated in this longitudinal study and 100% of participants completed both visit assessments (days between testing sessions = 58.5 ± 10.4). There were no differences in BMI of adult participants (p=0.20) and BMI percentile of adolescent participants (p=0.68) between 4- and 6-months post-ACLR (Table 10). There was a significant difference in months since surgery (p <0.001) between the 4- and 6-month visit. There were no significant limb main effects, time main time effects, or interactions for any of the femoral articular cartilage thickness outcomes (Table 11). Average between limb and time femoral articular cartilage compartmental thickness differences for all compartments are reported in Table 12. Medial, intercondylar, and lateral femoral articular cartilage compartmental thickness in the involved and contralateral limbs over time are presented in Figure 8. Medial, intercondylar and lateral femoral articular cartilage compartmental thickness between limbs at each individual time point are represented by boxplots in Figure 9.

Table 10. Participant and study session characteristics (Mean ± Standard Deviation)

	4 Month Visit	6 Month Visit	*p*-value
BMI for Adults (kg/m^2, N=10)	28.6 [20.8, 39.6]	28.9 [21.3, 40.1]	0.20
BMI for Adolescents (Percentile, N=10)	70.5% [21.0%, 83.0%]	71.5% [23.0%, 82.0%]	0.68
Months Since Surgery (N=20)	4.0 ± 0.3 [3.5, 4.4][a]	6.1 ± 0.3 [5.6, 6.6][a]	<0.001[a]

[a] = $p<0.05$

Table 11. Resting femoral articular cartilage compartmental thickness (mm) at 4- and 6-months post-ACLR (Mean ± SD)

Compartment	4 Month Visit		6 Month Visit		Main Limb Effect p-values	Main Time Effect p-values	Interaction Effect p-values
	Involved Limb	Contralateral limb	Involved Limb	Contralateral limb			
Medial Thickness (mm)	2.04 ± 0.59	2.01 ± 0.39	2.13 ± 0.56	2.05 ± 0.37	0.50	0.22	0.44
Intercondylar Thickness (mm)	2.53 ± 0.52	2.54 ± 0.48	2.61 ± 0.78	2.52 ± 0.44	0.78	0.72	0.49
Lateral Thickness (mm)	2.05 ± 0.29	2.08 ± 0.32	2.12 ± 0.35	2.07 ± 0.32	0.92	0.30	0.24

All main limb and time effects, and interactions are not significant (p>0.05)

Table 12. Resting femoral articular cartilage compartmental thickness (mm) differences between limbs and over time (Mean ± Standard Deviation)

Compartment	Between Limb Differences		Between Time Differences	
	4 Months	6 Months	Involved Limb	Contralateral Limb
Medial Thickness (mm)	0.02 ± 0.32	0.09 ± 0.47	0.09 ± 0.41	0.03 ± 0.15
Intercondylar Thickness (mm)	-0.01 ± 0.49	0.09 ± 0.84	0.08 ± 0.60	-0.02 ± 0.17
Lateral Thickness (mm)	-0.03 ± 0.35	0.05 ± 0.37	0.07 ± 0.27	-0.01 ± 0.07

Larger values indicate greater involved limb cartilage outcomes compared to the contralateral limb or greater cartilage outcome at 6 months compared to 4 months

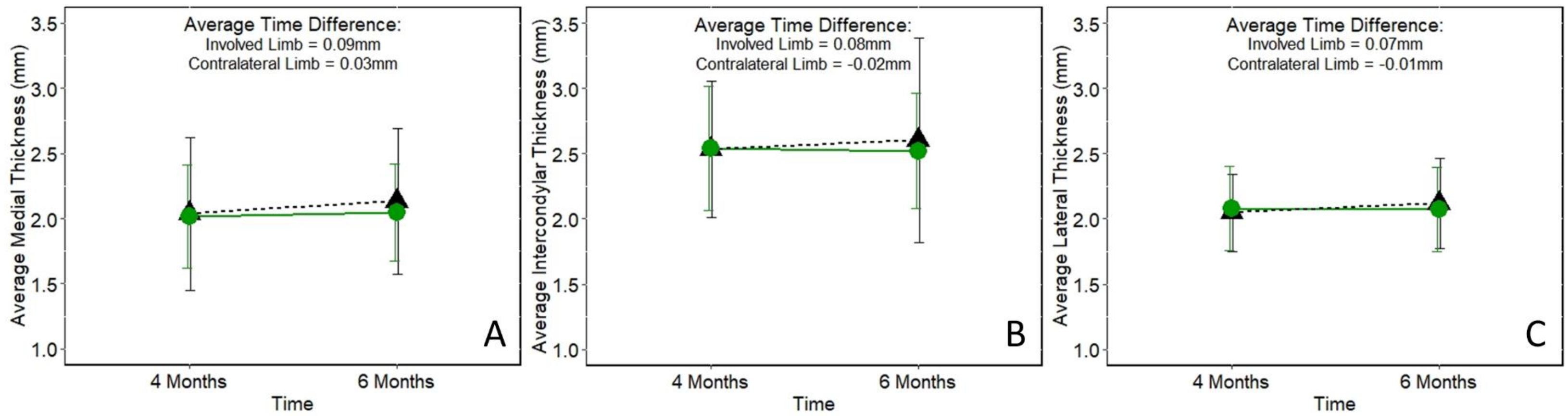

Figure 8. Medial (A), intercondylar (B), and lateral (C) femoral articular cartilage compartmental thickness in the involved and contralateral limbs from 4 to 6 months post ACLR. Green circles and the solid lines represent the contralateral limb and black circles and dotted lines represent the involved limb

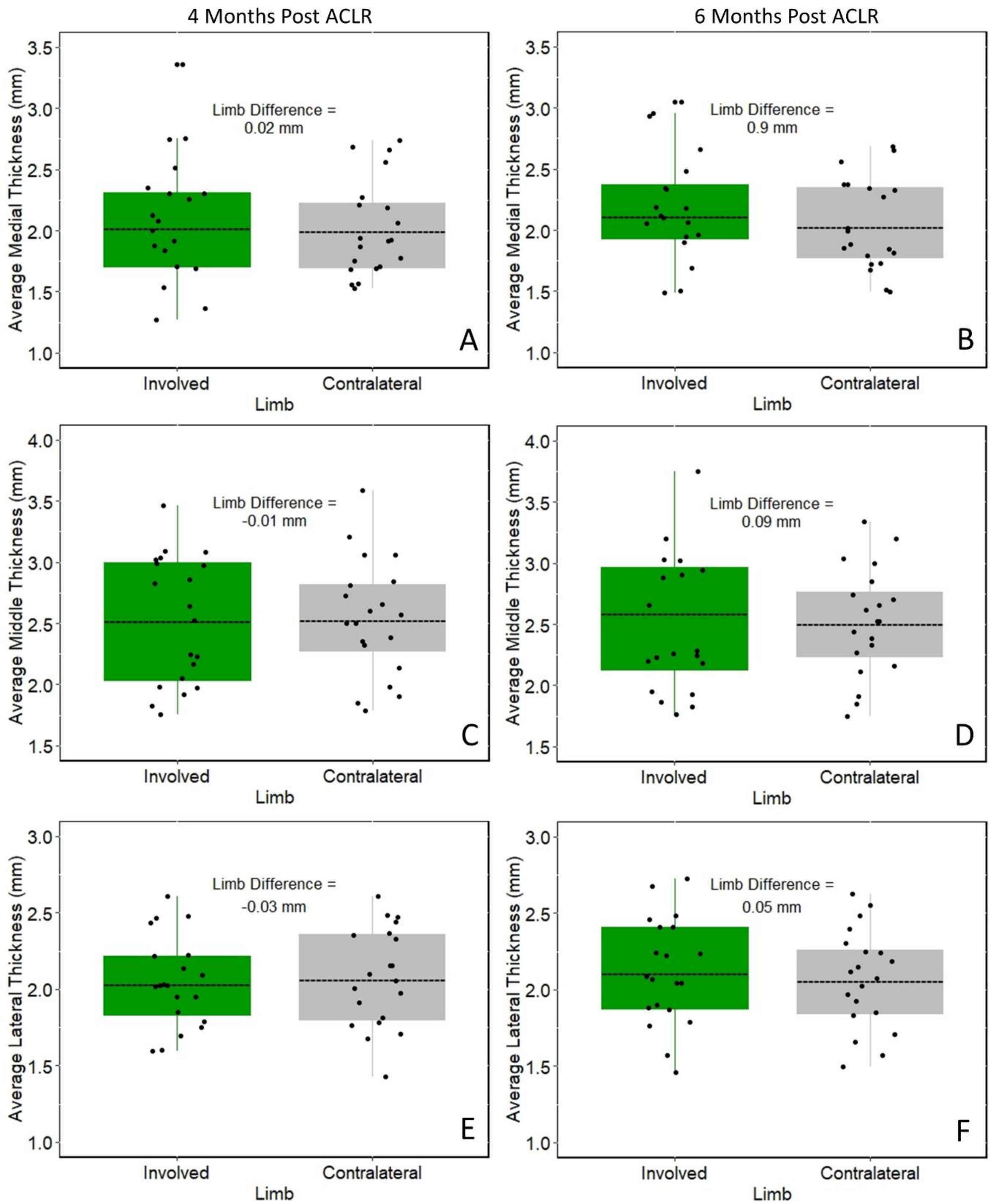

Figure 9. Medial (A & B), intercondylar (C & D), and lateral (E & F) femoral articular cartilage thickness between limbs at 4- and 6-months post-ACLR. The bottom and top of the vertical line represent the minimum and maximum of the cartilage thickness outcome. The bottom and top of the box indicate the first and third quartile of the cartilage thickness outcomes. The line

Figure 9 (cont'd)

represents the average cartilage thickness outcome. Green boxes represent the involved limb and gray boxes represent the contralateral limb

DISCUSSION

Individuals with a history of ACLR have a 4-6 times elevated risk of developing radiographic presence of knee joint structural changes indicative of osteoarthritis more than 10 years after surgery compared to individuals without a history of knee injury.[203] Biochemical and histological knee joint changes may be present within the first 6 months post-ACLR,[13,150] but it is unclear if structural changes in the knee joint articular cartilage are present while individuals remain in rehabilitative care. Accessible ultrasound machines that can perform assessments of resting femoral articular cartilage thickness may help identify structural changes or limb differences in individuals with a history of ACLR.[9,148] In this longitudinal study, participants recovering from ACLR did not demonstrate significant difference in cartilage thickness between limbs or over time within 6 months of surgery. The results of our study suggest that 1.) structural cartilage thickness changes or limb differences may not occur 4 to 6 months after ACLR, 2.) 2 months between longitudinal ultrasound assessment may not be a long enough time to capture meaningful structural changes in femoral articular cartilage, and 3.) ultrasound assessment may not be a sensitive measure to detect articular cartilage changes or limb differences before 6 months post-ACLR while patients remain in rehabilitative care.

We hypothesized that the involved limb would demonstrate greater between limb differences compared to the contralateral limb at 6-months post-ACLR based on an extensive body of literature indicating that individuals consistently experience risk factors for knee PTOA. The development of PTOA involves complex relationships between metabolic, mechanical, structural pathways.[18] Overall, many wet biomarkers (i.e. serum, urinary, synovial fluid) indicative of cartilage breakdown and pro-inflammatory processes are elevated within the first few months post-ACLR providing evidence of a catabolic knee joint environment.[150]

Additionally, patients demonstrated altered knee joint biomechanics[123,204] during the first 4 to 6 months post-ACLR indicating mechanical changes. Therefore, metabolic and mechanical pathways precede pre-radiographic, structural changes in the knee within the first 6 months post-ACLR.[148] The presence asymmetrical knee joint loading, and or elevated pro-inflammatory and cartilage breakdown are mechanical and metabolic biomarkers that may identify individuals who are at risk for develop PTOA earlier than ultrasound post-ACLR.

A systematic review suggests that structural changes of the tibiofemoral articular cartilage (i.e. changes in cartilage thickness) are not detectable on MRI imaging until 2 years after surgery.[157] The results of the current study suggest that the same may be true of resting ultrasound imaging assessment of femoral articular cartilage thickness within the first 6 months post-ACLR. Other MRI imaging associated with articular cartilage compositional changes[155,156] as opposed to structural changes, indicate that femoral articular cartilage proteoglycans may decrease and water content may increase between pre-operative to 6 months post-ACLR, but not 6 months to 12 months after surgery.[13,16,159] In further support of these conclusions, a cross-sectional study assessing ultrasound measures of femoral articular cartilage in individuals, on average 3 years post-ACLR, reported greater involved limb medial thickness compared to the contralateral limb.[9] Ultrasound may not be a clinically relevant tool to identify between limb femoral articular cartilage structural differences or changes over time during the first 6 months after ACLR. However based on evidence from the ultrasound-based cross-sectional study,[9] future research should utilize longer longitudinal assessments post-ACLR (i.e. 2-3 years) to determine when structural changes in femoral articular cartilage are apparent with accessible ultrasound assessments and the length of time necessary to detect changes between assessments. Diagnostic ultrasound machines are accessible at most orthopedic clinics and hospitals and can be used in examination rooms without referral to outside facilities for other imaging modalities. Any type of ultrasound assessment reduces exposure to radiation when compared to traditional, diagnostic radiographic imaging, and are easier to use to complete longitudinal assessments compared to MRIs. While the standard ultrasound assessment technique involves extensive image processing time by the assessor, it still provides a promising valid and reliable image screening of femoral articular cartilage structure that should be continued to be researched for appropriate clinical application.

A limitation of this study is that the presence of concomitant meniscal surgical procedures or articular cartilage pathologies at time of ACLR surgery were not controlled for despite the fact that they may impact synovial knee joint health. Individuals undergoing ACLRs and meniscectomies are 3.54 (95% Confidence Interval = 2.56-4.91) more likely to develop knee OA compared to individuals undergoing an isolated ACLR[50] and damage to the articular cartilage such as bone contusions are also associated with the development of tibiofemoral OA after ACLR.[54] Surgical information could only be extracted from 80% (n=16) of participants in this study, but approximately 56% (n=9) of participants also received a meniscectomy or meniscal repair surgery at the time of ACLR. We were unable to determine who had articular cartilage damage at the time of injury, but previous study report as many as 80% of participants suffer a bone contusion along with the ACL injury. Meniscal and articular pathologies impact a large percentage of patient and could not be controlled for in our analyses. While our sample size was appropriately powered to assess differences between limbs and across time points in this population, a larger sample size may be necessary to determine the effects of meniscal surgical procedures or articular cartilage pathologies impact resting femoral articular cartilage thickness.

CONCLUSION

Individuals within 6 months of ACLR did not demonstrate significant differences in femoral articular cartilage outcomes assessed with ultrasonography between limbs or from 4 to 6 months post-ACLR. Ultrasound may not be a clinically relevant assessment tool to identify early synovial joint changes during a time when individuals recovering from ACLR consistently undergo rehabilitative care. Future longitudinal research studies should assess when ultrasound assessment of resting articular cartilage compartmental thickness can detect between limb differences or changes over time post-ACLR.

CHAPTER 5: CUMULATIVE KNEE JOINT LOADING AND FEMORAL ARTICULAR CARTILAGE THICKNESS 4 TO 6 MONTHS POST-ACLR

ABSTRACT

Altered mechanical knee joint loading is a contributor to the development of knee post-traumatic osteoarthritis in individuals with a history of anterior cruciate ligament reconstruction (ACLR). Poor knee joint health after ACLR is associated with greater knee abduction moment, lesser knee extension moment, and lesser vertical ground reaction force (vGRF) during the first 50% of stance phase, but a gap exists in the literature understanding its relationship to cumulative knee joint loading (steps/day). The purpose of this longitudinal study was to assess the associations among gait biomechanics, steps per day at 4 months post-ACLR, and involved limb femoral articular cartilage compartmental thickness at 6 months post-ACLR. A total of 19 participants (9 Male/10 Female, age range=16-33 years old) recovering from ACLR completed mechanical knee joint loading assessment at 4 months and ultrasound assessment of involved limb femoral articular cartilage imaging at 6 months. Participants' gait biomechanics were captured with 3D motion capture and 2 embedded force plates while walking at a self-selected pace. Participants also wore a physical activity monitor on their hip for seven days during all waking hours to measure steps per day. Femoral articular cartilage images were captured in participants' involved knee. A semi-automated segmentation processing technique was used to measure medial femoral articular cartilage thickness (mm). The biomechanical outcome with the strongest relationship (r) to medial femoral articular cartilage compartmental thickness was entered into the linear regression model in addition to steps per day (6,028±1592) to predict medial femoral articular cartilage thickness. Peak knee extension moment had the strongest relationship with medial femoral articular cartilage thickness (r=0.45, p=0.047), while peak knee abduction moment

(*r*=0.22, *p*=0.35) and vGRF (*r*=0.05, *p*=0.85) did not demonstrate significant relationships. Knee extension moment and steps per day 4 months post-ACLR explained 39% of the variance in involved limb medial femoral articular cartilage thickness 6 months post-ACLR (*p*=0.003). Individuals who complete more steps per day with poor knee sagittal plane gait biomechanics demonstrate poorer structural medial femoral articular cartilage outcomes. At 4 months post-ACLR, individuals complete low accumulations of daily steps despite their ability to participate in approved moderate-intensity activities. Poor knee sagittal plane gait biomechanics should be addressed early during rehabilitation before integrating individuals into greater levels of activity.

INTRODUCTION

Post-traumatic knee osteoarthritis (PTOA) leads to disability in activities of daily living[1] and limits participation in physical activity.[205] As many as 50% of individuals undergoing anterior cruciate ligament reconstruction (ACLR) will rapidly develop radiographic PTOA within 20 years,[3] which is concerning considering the risk of ACL injury and the prevalence of ACLRs in individuals under the age of 20 is double that of individuals in any other decade of life.[44] Changes indicative of declining articular cartilage health include greater water content,[16] lesser proteoglycan content[11,16] and greater or lesser medial tibiofemoral articular cartilage structure (i.e. medial femoral articular cartilage thickness) [176] are present within the first 6 months post-ACLR and most often within the medial tibiofemoral compartment.[160] Specifically, diagnostic ultrasound is an emerging valid[6] and reliable[7] technique of assessing pre-radiographic changes in resting knee femoral articular cartilage thickness. Individuals post-ACLR demonstrate greater involved limb medial femoral articular cartilage compartmental thickness compared to their contralateral limb which is hypothesized to be representative of cartilage swelling.[9] Due to the high risk of knee PTOA in this clinical population, there is a critical need to understand which factors contribute to the initial development and accelerated progression of pre-radiographic articular cartilage structural changes after ACLR.

Cumulative mechanical loading of the knee joint which include gait biomechanics and daily volume of loading may have significant impact on knee articular cartilage structural changes after ACLR.[15] Greater peak knee abduction moments,[11] lesser knee extension moments,[120,121] and lesser vertical ground reaction forces (vGRFs)[16] during the stance phase of walking have been consistently identified after ACLR and are associated with biochemical, compositional, and structural changes of the tibiofemoral articular cartilage. These gait biomechanical alterations have the potential to change compression and shearing forces at the knee or shift joint contact forces from the lateral to medial tibiofemoral compartment leaving the articular cartilage unable to adapt to the different forces acting upon it.[15,202] Recent evidence also suggests that middle-aged individuals who are at high-risk for knee OA and participate in limited daily volume of loading may demonstrate MRI-imaging changes associated with lesser proteoglycan and greater water femoral articular cartilage content over 4 years.[17] This may impact individuals after ACLR when they are reported to complete less than 1,500 steps per day compared to those without a history of knee injury.[87] While existing evidence suggests gait biomechanics and volume of loading may individually play a role in PTOA development, a gap in the literature exists regarding the role of cumulative mechanical loading at the knee joint in individuals recovering from ACLR.

Mid-to-late rehabilitation post-ACLR marks a crucial period of time when biomechanical factors of mechanical loading may be associated with unhealthy knee articular cartilage structural changes. At 4 months post-ACLR, most patients are integrated back into activities such as running,[206] jumping and potentially cutting[14] which place greater biomechanical mechanical loading (shear forces and contact forces) on the knee.[202] At 6 months post-ACLR, greater than 50% of patients have been discharged from rehabilitation, and many health care professionals begin to consider clearing patients for unrestricted activity.[14] Therefore, interventions targeting mechanical loading risk factors should be incorporated before 6 months post-ACLR. The primary objective of this study is to determine how different characteristics of mechanical knee joint loading contribute to early femoral articular cartilage structure in individuals after ACLR. We hypothesize that greater peak knee abduction moment, lesser peak knee extension moment, and lesser peak vGRF 4 months post-ACLR will be associated with greater involved limb femoral articular cartilage compartmental thickness 6 months post-ACLR, but greater peak knee abduction moment will demonstrate the strongest relationship. We also hypothesize that greater peak knee abduction moment and lesser daily steps at 4 months post-ACLR will predict greater involved limb medial femoral articular cartilage compartmental thickness 6 months post-ACLR.

METHODS

This longitudinal study tracked individuals from 4 months (±2 weeks) to 6 months (±2 weeks) post-ACLR. Gait biomechanics and daily steps counts were collected at the 4-month assessment, and ultrasound assessed femoral articular cartilage thickness was collected at the 6-month assessment. This study was approved by the university's Institutional Review Board and all participants ≥ 18 years old provided written informed consent before participating in the study. Individuals under the age of 18 and their parents or guardians provided written informed assent and consent, respectively.

PARTICIPANTS

Participants were recruited from 4 fellowship trained orthopedic surgeons at the university orthopedic sports medicine clinic. Individuals were included in they were 16 and 30 years old with a primary unilateral ACLR. Participants were also required to be ambulatory and could achieve, full pain-free knee range of motion. Individuals were excluded from the study if they reported a history of lower extremity injury within the past 6 weeks, more than 1 ACLR, or rheumatoid arthritis. Participants were not excluded if they had concomitant pathologies or surgical procedures completed at the time of ACL injury or ACLR such as MCL tears, meniscal tears, meniscectomy, meniscal repair, bone marrow lesions, or microfracture surgery.

GAIT ASSESSMENT 4 MONTHS POST-ACLR

A 10-camera motion capture analysis system (Vicon Motion Systems Ltd., UK) and 2 embedded force plates (Advanced Mechanical Technology, Inc., Watertown, MA) were used to measure lower extremity walking gait kinematic and kinetic data at 240 and 1200 Hz respectively.[207] A total of 8 clusters of retroreflective markers each were placed on each participant's left and right foot, shank and thigh, lumbar spine and upper thoracic spine (Figure 10A & 10B).[207] Each cluster had 4 retroreflective markers for a total of 32 used in the biomechanical analysis. The proximal and distal joint segments and joint centers (Figure 10A) were identified using a stylus, and hip joint centers were calculated using the Bell method.[208] A right-handed Euler sequence was used to calculate ankle, knee and hip joint angles. Motion analysis software (Innovative Sports Training, Inc., Chicago, IL) was used to capture and process kinematic and kinetic data.[207] Data were filtered with a 4th order low pass Butterworth filter with a cut-off of 12 Hz for kinematic data and 120 Hz for kinetic data.

Prior to kinematic and kinetic assessment, participants were asked to walk along a 6-meter track between 4 TF100 timing gates (TracTronix, Belton, MO) to provide real-time measurements of a participant's gait speed.[201] Participants completed 10 practice trials to become familiar with the task and determine average habitual gait speed. Participants were asked to complete 5 successful gait trials on each leg collected by the motion capture system. A trial was considered successful if the participant's whole foot contacted the force plates, the participant did not stutter step or change stride length to load on the force plate and the participant walked within ± 5% of average habitual gait speed that was previously determined. Stance phase during gait was identified between initial contact (vGRF>10 N) and toe-off (vGRF<10 N) of the reconstructed limb. The primary outcomes extracted for analysis were peak internal knee abduction moment, peak internal knee extension moment and peak vGRF during the first 50% of the stance phase of the involved limb and averaged together for each participant. Greater negative values indicate greater internal knee extension moment, and greater positive values indicate greater knee abduction moment or vGRF.

Figure 10. A.) Clusters with 4 retroreflective markers each were placed on the thoracic and lumbar regions. The red circles over the medial and lateral knee joint line represent stylus placement used to identify the knee joint center. B.) Clusters with 4 retroreflective markers each were place on the outside of the right thigh and shank, and on top of the right foot. Cluster placement was identical on the left leg.

DAILY STEP COUNT MONITORING 4 MONTHS POST-ACLR

Participants were given an Actigraph Link activity monitor (Actigraph, LLC, Pensacola, FL) after the completion of the 4-month gait assessment. Participants were instructed to wear the monitor on their right hip for 7 days during all waking hours except during sleeping or during water activities. During the 7-day period, participants were asked to fill out a daily activity log recording time of day activity was performed, the type of activity, duration of the activity and perceived intensity of the activity. Raw tri-axial acceleration data were downloaded to Actilife software and processed through the software's proprietary filtering methods to calculate steps per day. Accelerometer data collection and analysis methods are described in detail in Table 13 based adequate reporting methods described by Montoye et al.[209]

Table 13. Accelerometer data collection and analysis methods

Items to Report	Methods
Model of Accelerometer	Actigraph Link
Data Collection Sampling Rate	30 Hz[87]
Data Analysis Epoch Length	60s epoch[74]
Place of Accelerometer	Right Hip[74]
Number of participants receiving accelerometer	19
Accelerometer distribution method	Received in-person, returned in-person or in the mail
Days of data collection at each time point	7 days
Criteria for defining non-wear of accelerometer	Minimum Length: 90 minutes Small Window Length: 30 minutes Spike Tolerance: 2 minutes[76]
Number of valid days and number of minutes per day of accelerometer data needed to be included in analysis	≥ 4 days with 480 minutes per day[76]
Accelerometer data PA outcome of interest and interpretation method	Steps per day
Number of participants non-compliant or had accelerometer malfunction issues	1

ULTRASOUND ASSESSMENT OF FEMORAL ARTICULAR CARTILAGE THICKENSS 6 MONTHS POST-ACLR

Participants returned for a second study session 6 months post-ACLR. Before the ultrasound assessment, a participant's hydration status via urine specific gravity (USG) was assessed with an Atago 3730 digital refractometer (ATAGO U.S.A., Inc., Bellevue, WA). Dehydration may impact articular cartilage imaging assessment and should be eliminated as a potential confounding factor.[154] If participants' USG exceed 1.025 then they were rescheduled until their USG was below 1.025.[185] Ultrasound assessment was completed by a single assessor using the Vivd iQ System (General Electric Company, Boston, MA) with 12L-RS linear probe with a sampling rate of 12 MHz. Upon arrival, participants were asked to sit in a long sitting position for 30 minutes to neutralize any effects from prior activity.[8] After 30 minutes, the participant was asked to align his or her back against the wall and place his or her surgical knee into 140° of knee flexion determined by a manual goniometer.[8]

The linear probe was placed perpendicular to the surface of the anterior femoral articular cartilage aligned horizontally between the inner most portions of the medial and lateral condyles and superior to the patella.[8] A horizontal grid over the real-time image of the ultrasound cartilage was used to center the intercondylar notch in relation to the grid and record the positions of the medial condyle and lateral condyle based on the grid coordinates.[8] Once correct placement was achieved, a screenshot of the articular cartilage was recorded. The coordinates of the three landmarks were used for all imaging assessments of the involved knee.

IMAGE PROCESSING

The methods described have high intra-session reliability (ICC = 0.98-0.99).[8] Randomized images will be processed using open source Image J (National Institute of Health, Bethesda, MD) to calculate average medial femoral articular cartilage thickness and medial cross-sectional area.[8] Total cross-sectional area (CSA) was measured by 1 blinded rater (C.L.) using built-in measurement tools. Total CSA was defined as the space between the outer borders of the medial and lateral condyles in addition to the superior synovial-cartilage border and the inferior cartilage-bone border (Figure 3A).[7-9,183] The central point of the femoral articular cartilage was manually identified by the blinded rater as the middle of the synovial-cartilage border of the articular cartilage separating the medial and lateral upslopes (Figure 3A). After identifying the total CSA and the central point of the femoral articular cartilage, the images were processed through a custom MATLAB code (Version 9.2, Mathworks, Natik, MA) to segment the total femoral articular cartilage CSA into medial and middle sections. The middle segment length was defined as the inner 25% of the femoral articular cartilage extending from the manually identified central point (Figure 3B). The medial segment length was defined from the medial border of the middle segment to the outer border of the medial. Average medial thickness (mm) normalized to segment length and CSA (mm^2) were calculated for each image of the femoral articular cartilage (Figure 3B).

STATISTICAL ANALYSIS

Pearson's *r* product moment correlation coefficients were used to evaluate and select the kinetic gait parameter (peak internal knee abduction moment, peak internal knee extension moment, or peak vGRF within the 50% of the stance phase) with the strongest relationship to average medial femoral articular cartilage thickness deformation. This was done to reduce the number of potential predictors and to prevent multicollinearity between the predictor variables. A total of 2 predictor cumulative loading outcomes were used to predict each femoral articular cartilage deformation explanatory outcomes, separately. A linear regression model with forward entry was used to assess the ability of daily step counts and the selected gait outcome to predict involved limb medial femoral articular cartilage compartmental thickness.

SAMPLE SIZE ESTIMATION

An *a priori* sample size estimations was determined using open-source software, G*Power (Version 3.1.9.2, Henrich Heine Universität Düsseldorf, Brunsbuttel, Germany). An effect size of 0.44 was calculated from a previous study reporting that knee extension moment during gait explained 31.8% of the variance in ultrasound-defined femoral articular cartilage CSA.[186] Based on this calculation, a sample of 29 participants is necessary to achieve a power of 80% and alpha level of 0.05 using 2 predictor variables.

RESULTS

A total of 20 participants (10 Male/10 Female, age range = 16-33 years old) participated in this longitudinal study, and 95% (N=19) of participants completed all components of both visit assessments (days between testing sessions = 58.9 ± 10.4). A single participant (male, age=25 years old, 4-month BMI=39.6 kg/m^2, 6-month BMI=40.1 kg/m^2) was removed from the analysis because the participant did not meet minimum requirements for physical activity monitor wear time. There were significant differences between months since surgery, but not BMI or BMI percentile between the 4- and 6-month visit (Table 14). Average mechanical loading assessed 4 months post-ACLR and medial femoral articular cartilage compartmental thickness assessed 6 months post-ACLR are reported in Table 15.

Correlation matrix relationship results between gait biomechanical outcomes 4-months post-ACLR and medial femoral articular cartilage compartmental thickness 6-months post-ACLR are reported in Table 16. Lesser involved limb knee extension moment assessed 4 months post-ACLR was significantly correlated with greater involved limb medial femoral articular cartilage compartmental thickness ($r=0.45$, $p=0.047$) assessed 6 months post-ACLR. Involved limb knee abduction moment ($r=0.22$, $p=0.35$) and vGRF ($r=0.05$, $p=0.85$) assessed 4 months post-ACLR were not significantly correlated with involved limb medial femoral articular cartilage compartmental thickness assessed 6 months post-ACLR (Figure 11). Based on this analysis, involved limb knee extension moment and steps per day were entered as predictor variables in the linear regression analysis for involved limb medial femoral articular cartilage compartmental thickness. Lesser peak knee extension moment ($\Delta R^2=0.20$, $p=0.02$) and greater average steps per day ($\Delta R^2=0.19$, $p=0.04$) assessed 4 months post-ACLR significantly predicted greater involved limb medial femoral articular cartilage compartmental thickness assessed 6 months post-ACLR ($R^2=0.39$, $p=0.03$). Figure 12 illustrates the relationship between observed medial femoral articular cartilage thickness and medial femoral articular cartilage thickness values predicted by lesser knee extension moment and greater steps per day.

Table 14. Participant and study session characteristics (N=19)

	4 Month Visit	6 Month Visit	*p*-value
BMI for Adults (kg/m^2)	29.1 ± 6.1 [20.8, 38.9][a,d]	29.4 ± 6.2 [21.3, 39.4][a,d]	0.27
BMI for Adolescents (Percentile)	70.5 [21.0, 83.0][b,c]	71.5 [23.0, 82.0][b,c]	0.68
Months Since Surgery	4.0 ± 0.2 [3.5, 4.3][a]	6.1 ± [5.6, 6.6][a]	<0.001
Days Monitor Worn	6.2±1.5 [4,10][a]	-	-
Time Monitor Worn	5,304.6±2,165.3 [2578.0, 11,781.0][a]	-	-

[a] = reported as Mean ± Standard Deviation [Minimum, Maximum]; [b] = reported as Median [Minimum, Maximum]; [c] = based on N=9; [d] = based on N=10; [e] = p<0.05

Table 15. Mechanical loading outcomes 4 months post-ACLR and medial femoral articular cartilage compartmental thickness 6 months post-ACLR

Predictor and Explanatory Outcomes	(Mean ± Standard Deviation)
vGRF (Nm/kg)	1.12 ± 0.08
Internal Knee Extension Moment (Nm/kg)	-0.24 ± 0.11
Internal Knee Abduction Moment (Nm/kg)	0.09 ± 0.04
Steps/Day	6085 ± 1592
Medial Thickness (mm)	2.13 ± 0.57

Table 16. Correlation matrix between gait biomechanical outcomes 4-months post-ACLR and medial femoral articular cartilage compartmental thickness 6-months post-ACLR

	Knee Extension Moment (Nm/kg)	Knee Abduction Moment (Nm/kg)	vGRF (Nm/kg)	Medial Thickness (mm)
Knee Extension Moment (Nm/kg)	-	-	-	-
Knee Abduction Moment (Nm/kg)	r = -0.08, p = 0.75	-	-	-
vGRF (Nm/kg)	r = -0.19, p = 0.41	r = 0.56, p = 0.01*	-	-
Medial Thickness (mm)	r = 0.45, p = 0.047*	r = 0.22, p = 0.35	r = -0.05, p = 0.85	-

*=relationship is statistically significant, $p<0.05$

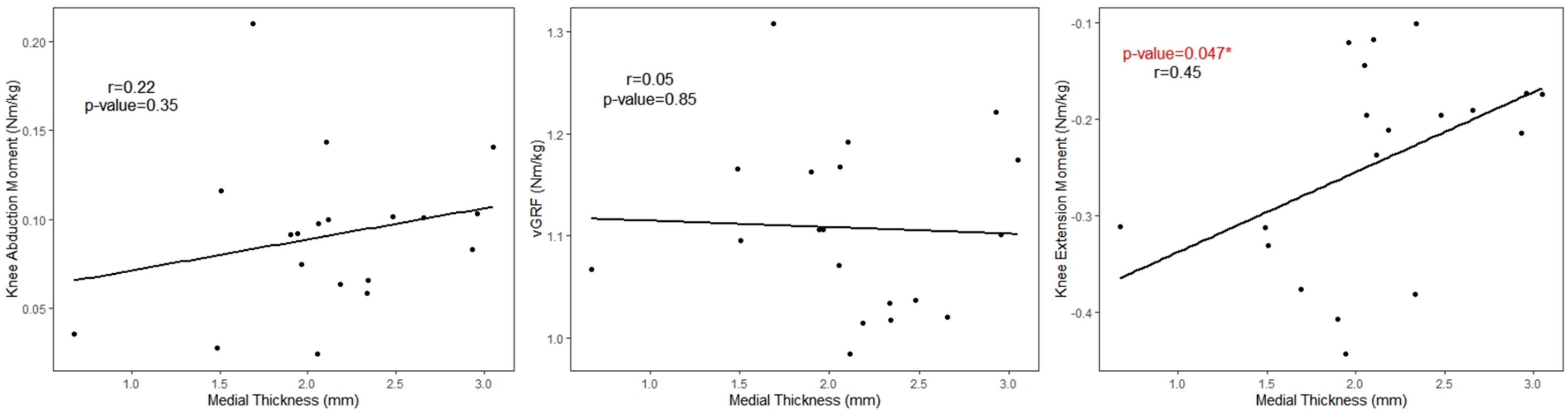

Figure 11. Relationships between mechanical knee joint loading outcomes during gait at 4 months post-ACLR and average medial femoral articular cartilage thickness at 6 months post-ACLR. Negative values indicate greater knee extension moment, but positive values indicate greater knee abduction moment and vGRF.

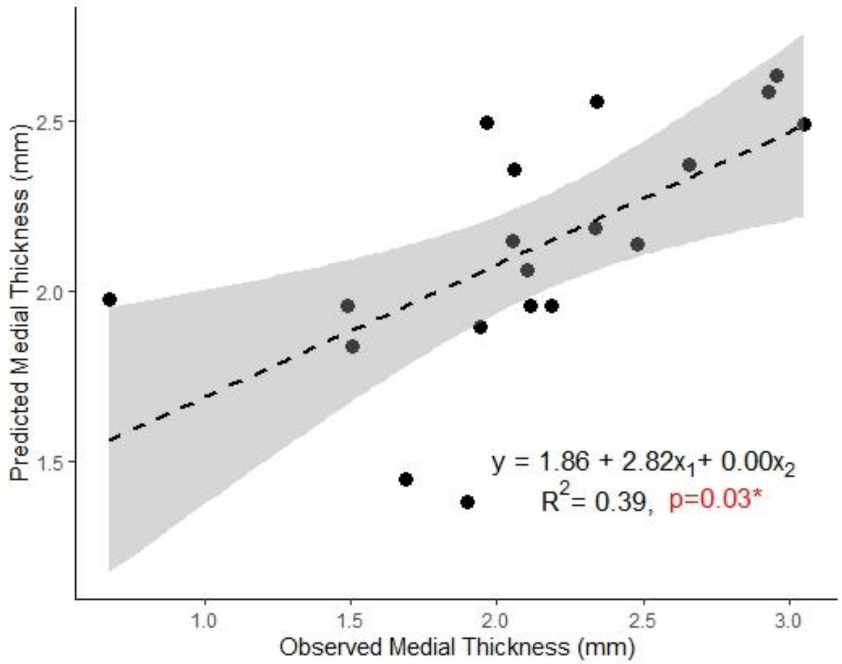

Figure 12. Association between observed and predicted involved limb medial femoral articular cartilage thickness

DISCUSSION

Altered mechanical knee joint loading is a modifiable risk factor in the accelerated development of PTOA in individuals recovering from ACLR.[18] Poor patterns of mechanical loading identified through greater peak knee abduction moment, lesser peak knee extension moment, and greater peak vGRF during gait are related to biochemical, histological, and structural changes indicative of PTOA development.[11,16,159,175] However, the relationship between knee joint health mechanical knee joint loading that takes into account the cyclical nature of loading or how often the knee is loaded or not loaded throughout the day has not been explored in individuals with a history of ACLR. In our study, lesser knee extension moment and greater steps per day at 4 months post-ACLR were associated with greater medial femoral articular cartilage thickness 6 months post-ACLR. These findings indicate that individuals who return to greater volumes of ambulatory activity with unresolved sagittal plane kinetic alterations may demonstrate greater involved limb changes to medial articular cartilage structure within 6 months of ACLR.

Articular cartilage thinning is a fundamental signs of radiographic knee OA in later phases of disease progression.[147] Conversely, early in the development of knee joint OA, medial femoral articular cartilage may thicken as a precursor to later degenerative changes. Greater thickness of tibiofemoral articular cartilage during the initiation phase of knee OA development may result from greater water content and cartilage swelling.[210] Interestingly, this pattern has been reported on MRIs approximately 2 years after ACLR.[211,212] These findings are also consistent with ultrasound assessment of femoral articular cartilage which identified cartilage thickening in the involved limb compared to the contralateral limb on average 3 years after ACLR.[9] In our study, individuals with altered gait biomechanics who took more average daily steps demonstrated greater medial femoral articular cartilage thickness which may be an indicator of poor structural changes in the knee joint. Compared to previous literature, this longitudinal study took a unique and comprehensive approach to understanding cumulative mechanical knee joint loading by including gait biomechanics and the amount of cyclical loading together. Individuals in our study demonstrate a complex relationship between knee sagittal plane kinetics indicative of limb underloading and daily cyclical loading activity which would be considered ideal if evaluated in isolation. These results highlight the multifactorial and multidirectional aspects of mechanical knee joint loading that interact to influence articular cartilage structure which has not been previously reported. Understanding these interactive relationships may help better guide impairment-based rehabilitation approaches since individuals with a history of ACLR may demonstrate different alterations in gait kinetics and volumes of loading.

Contrary to our findings, Teng et al. reported that greater peak external knee flexion moment during gait assessed 6 months post-ACLR is associated with declining proteoglycan content (i.e. greater T1 rho relaxation times) in the medial femoral condyle assessed via MRI at 1 and 2 years post-ACLR.[16] Participants in the Teng et al. study were older (30.6±8.6 years old) and had lower BMI (23.9±2.7)[16] which are both risk factors that negatively impact knee joint health[10,213] which may account for the differences in these results compared to the current study. Volume of activity may also impact this relationship. While gait biomechanics were the primary predictor outcomes, the amount of activity participants completed at 6 months post-ACLR (i.e. self-reported or accelerometer-based) were not reported in the Teng. et al. study.[16] Based on our findings, we speculate that the relationships between gait kinetics and knee articular cartilage health may be moderated by the participants' volume of activity. Future research should assess the relationships between gait kinetics, volume of loading, and MRI-based tibiofemoral T1 rho relaxations time assessments in individuals within the first 6 months to a year post-ACLR.

In our study, lesser knee extension moment during the stance phase of gait was moderately related to thicker medial femoral articular cartilage. "Stiffened knee" gait is described as adopting lesser internal knee extension moment and knee flexion excursion during the stance phase of gait. This gait strategy is prevalent in the involved limbs of individuals at 6 and 12 months post-ACLR[214] and is associated in lesser medial tibiofemoral contact forces.[215] In this case, lesser knee extension moment may reduce medial tibiofemoral contact forces and underload the femoral articular cartilage. It is hypothesized that lesser knee extension moment may contribute to the development of PTOA.[15] The framework suggests that compartments of the articular cartilage that were once acclimated to a certain degree of shear and compressive forces of loading may experience lesser mechanical load due to gait pattern alterations during the initiation phase of PTOA after ACLR.[4,15,18] As a result, the medial femoral articular cartilage compartment may not adapt and may be unprepared to accept greater accumulations of cyclical mechanical loading (i.e. greater steps per day). The concept of underloading is supported by previous research reporting that individuals who developed radiographic medial knee PTOA 5 years after ACLR, demonstrated lesser tibiofemoral contact forces at 6 months after surgery compared to individuals who did not develop PTOA.[216] Therefore, addressing knee extension moment underloading using promising real-time biofeedback gait retraining interventions which improve "stiffened knee" gait through modulation of vGRF during walking are successful after a single session[217] and may have implications for improving biochemical indicators of cartilage breakdown.[178]

In our study, volume of activity was a significant predictor of knee articular cartilage structure. In addition to knee articular cartilage health, low volume of loading may impact other aspects of their health. For example, physical activity participation has various impacts on an individual's health including improvements in mental health[64] and reducing the risk of developing a myriad of chronic, non-communicable diseases.[65] Adults who meet national physical activity guideline recommendations (≥150 minutes of moderate-vigorous physical activity) achieve approximately 7,000 steps per day.[218] Regardless of injury history, men and women over the age of 20 (n=3,725) and adolescents between 12 and 19 years old (n=2,610) who participated in the NHANES study completed an average of 9,685[219] and 8,225-11,660 steps per day, respectively.[220] In comparison, individuals 4 months post-ACLR in our study only completed an average of 6,085 daily steps and 74% (n=14) of those individuals did not achieve 7,000 steps per day. During mid to late phases of ACLR rehabilitation, many individuals are completing lesser daily steps compared to the general population and may not be accumulating adequate steps per day necessary to achieve recommended levels of physical activity. A cross-sectional study also reported that individuals complete less steps per day compared to healthy controls as long as 5 years after ACLR[87] indicating that this trend may continue even once patients receive physician clearance for return to unrestricted activity. Recovery from ACLR may promote physical inactivity behavior during rehabilitation which may continue even after individuals no longer have physical activity restrictions. While individuals recovering from ACLR have sports activity or even jogging restrictions at 4 months, this does not reduce their ability to participate in approved ambulatory activities that increase step accumulation throughout the day or achieve recommended weekly physical activity guidelines. Once high-risk gait biomechanics have been addressed in rehabilitation, health care providers should encourage increasing moderate-intensity physical activity participation to meet national recommendations and educate patients on how to achieve these goals through approved ambulatory activities. Future research should determine if specific daily step accumulation recommendations or step-based goals can help maintain knee joint health and reduce the risk of developing functional impairments.

BMI is a significant predictor of knee OA in middle-aged individuals with and without a history of knee injury.[142] A limitation of this study is that BMI was not controlled for in the statistical analysis. BMI is a challenging outcome to control when including adolescents and adults because BMI is measured differently in these populations. Appropriate BMI reporting in adolescents is calculated as a percentile compared to national percentiles based on age and sex.[102] In order to limit the number of predictors and covariates in our linear regression, biomechanical outcomes were normalized to an individual's body weight. A strength of this study is its use of accelerometers to measure daily activity in free-living settings compared to self-reported activity. However, in order to fully understand the amount of underloading that occurs daily, sedentary behavior may be a better outcome. Actigraph monitors worn at the hip are adequate measures of activity, but other wearable accelerometers (i.e. Activpals attached to the thigh) may better capture length of time in positions of sedentary behavior such as lying down or sitting.[221] Future research should assess how underloading measured through sedentary behavior relates to knee joint health after ACLR.

CONCLUSION

Greater volume of loading with high-risk biomechanics in individuals recovering from ACLR is associated with poor structural changes in the medial femoral articular cartilage. Individuals who walk with lesser knee extension moment may underload the knee joint reducing the ability of the medial femoral articular cartilage compartment to adapt to greater steps per day. During mid-late phases of rehabilitation, individuals recovering from surgery are completing low amounts of steps per day that may reduce their ability to achieve national physical activity recommendations. Once high-risk gait biomechanics are addressed, health care providers should encourage greater daily activity and educate patients on how they can increase their activity through approved ambulatory activities.

CHAPTER 6: SUMMARY AND CONCLUSIONS

SUMMARY

The purpose of this dissertation was to 1.) establish intra-rater and inter-rater reliability of a standard resting and novel post-loading after 3,000 steps ultrasound assessment of femoral articular cartilage structure in healthy individuals, 2.) assess between limb and time differences of medial, intercondylar and lateral femoral articular cartilage structure in individuals 4 and 6 months post-ACLR, 3.) determine the ability of cumulative mechanical knee joint loading assessed 4 months post-ACLR to predict medial femoral articular cartilage structure assess 6 months post-ACLR in individuals recovering from surgery.

RELIABILITY OF RESTING CARTILAGE AND CARTILAGE RESPONSE TO LOADING ULTRASOUND ASSESSMENTS OF FEMORAL ARTICULAR CARTILAGE

A total of 30 participants (age=21.8 ± 3.8 years, gait speed = 1.3 ± 0.2 m/s) completed both sessions of the observational laboratory study. Participants completed on average 3,009 steps in 28.4 minutes during the loading protocol in the first visit. During the loading protocol in the second visit, participants completed on average 3008 steps in 28.4 minutes. Resting cartilage assessment of femoral articular cartilage structure demonstrated excellent intra-rater ($ICC_{2.k}$=0.99) and test-retest $ICC_{2.k}$=0.97-0.99) reliability. Cartilage response to loading ultrasound assessment demonstrated good to excellent intra-rater reliability ($ICC_{2.k}$=0.84-0.95), but poor test-retest reliability ($ICC_{2.k}$=-0.36-0.46). Resting cartilage ultrasound assessment can be reliably used to assess between processing sessions and over multiple study visits, but the cartilage response to loading ultrasound assessment should not be used when assessing outcomes over multiple study visits. Femoral articular cartilage may not consistently deform in healthy participants due to step-tracking equipment with poor reliability resulting in underloading or overloading beyond the recommended optimal range (3,00 steps). The pattern or cyclical nature of articular cartilage deformation response at less than 1,000 steps or greater than 5,000 steps is unclear and should be assessed in future research. Regardless with the current knowledge, reliable step-tracking devices should be used in future studies to ensure optimal loading during the loading protocol for consistent cartilage deformation.

LONGITUDINAL ULTRASOUND ASSESSMENT OF FEMORAL ARTICULAR CARTILAGE THICKNESS 4 TO 6 MONTHS POST-ACLR

A total of 20 participants recovering from ACLR completed the second longitudinal study (10 Males/10 Females, age range=16-33 years old, 10 hamstring graft/10 bone-patellar tendon-bone graft/1 allograft) at 4- and 6-months post-ACLR (days between testing sessions = 58.5±20.4). There were no significant limb main effects (p range=0.50-0.92), time main effects (p range=0.22-0.72), or interactions (p range=0.24-0.49) for any of the femoral articular cartilage thickness outcomes. Individuals may not have femoral articular cartilage structural limb differences or changes within the first 6 months post-ACLR. Standard ultrasound assessment of femoral articular cartilage structure may not be able to detect limb differences or changes across time during mid-late periods of rehabilitation.

ASSOCIATIONS BETWEEN CUMULATIVE KNEE JOINT LOADING AND MEDIAL FEMORAL ARTICULAR CARTILAGE THICKNESS WITHIN 6 MONTHS POST-ACLR

A total of 19 participants with a history of ACLR (9 Male/10 Female, age range =16-33 years old) completed the longitudinal study at 4 and 6 months post-ACLR including knee joint mechanical loading assessments at 4 months post-ACLR. Participants walked approximately 6085 steps per day (standard deviation = 1592) wore the physical activity monitor for an average of 6.2 days (standard deviation = 1.5) and 5304.6 minutes per day (standard deviation=11,781.0).

Involved limb medial femoral articular cartilage compartmental thickness at 6 months post-ACLR was significantly associated with involved limb knee extension moment (r=0.45, p=0.047), but not knee abduction moment (r=0.05, p=0.85) and vGRF during the first 50% of stance phase (r=0.22, p=0.35) during gait at 4 months-post ACLR. Greater involved limb knee extension moment (p=0.02) and greater steps per day (p=0.04) assessed 4 months-post ACLR predicted greater involved limb medial femoral articular cartilage compartmental thickness at 6 months post-ACLR (R^2=0.39, p=0.03). Cumulative knee joint loading is associated with femoral articular cartilage structure and volume of activity may moderate the relationship between gait biomechanics and knee joint health. After ACLR, individuals with poor knee gait alterations that underload the knee joint may be unable to adapt to greater volume of loading resulting in femoral articular cartilage swelling. Health care providers should use evidence-based interventions early during rehabilitation to target individuals with lesser knee extension moment as individuals are integrated back into weight-bearing activities.

LIMITATIONS

A limitation of both studies is that we did not consider how stage of skeletal maturity may impact femoral articular cartilage changes in our adolescent participants. Most individuals undergoing ACLR are less than 20 years old.[44] Therefore, it was imperative to include adolescents in our first and second study to include a sample reflective of the general population. Individuals under the age of 20 may not have reached the highest stage of skeletal maturity. Adolescents without a history of knee injury who haven't achieved skeletal maturity demonstrate MRI-assessed tibiofemoral articular cartilage thickness increases over a year (boys = 0.8%, girls = 1.4%).[135] It is unclear if these changes can be detected over a 2-month period using standard ultrasound assessment or if synovial joint injury such as ACL tears impact articular cartilage growth. A total of 5 adolescents without a history of knee injury and 10 adolescents with a history of ACLR were included in the first and second study, respectively. We did not assess skeletal maturity in our participants in either study, but this may be an important consideration for future research. Future studies should determine if changes in skeletal maturity affect longitudinal standard ultrasound assessment of femoral articular cartilage structure.

A second limitation of the second study is that concomitant injuries or surgical procedures were not included as a covariate in the statistical analyses. Concomitant injuries and surgical procedures consistently accompany ACL tears and ACLRs.[45] Meniscal or chondral damage (i.e. bone bruises, bone marrow lesions) are relevant pathologies that increase the risk of PTOA in individuals with a history of ACLR.[50,54] Meniscal injuries occur in 60% of individuals with ACL tears and may be surgically treated with meniscectomy or meniscal repairs.[45] Individuals undergoing meniscectomies have a 3.5 times increased risk of PTOA within 10 to 25 years post-ACLR.[50] Furthermore, greater chondral damage demonstrates a moderate association with greater radiographic presence of tibiofemoral PTOA within 6 years post-ACLR.[54] We were only able to retrieve the surgical records of (N=16) participants which decreases our sample size. At the time of surgery, approximately 63% of the participants (N=10) had a meniscal injury, 19% of participants (N=3) had a meniscectomy, and 35% of participants (N=7) had a meniscal repair. Of the 16 participants, we were unable to access any of the patients initial imaging records to determine the presences of bone bruises or bone marrow lesions. However, all surgical records reported that the tibiofemoral articular cartilage was intact for all 16 patients at the time of surgery. Future studies should assess the effects of meniscal injury, meniscal surgical procedures, and chondral damage (i.e. bone bruises or bone marrow lesions) on femoral articular cartilage thickness assessed with the standard resting ultrasound technique.

A third limitation of the second study is that we did not control for body mass index (BMI) for adults or BMI percentile for adolescents in the linear regression analysis. BMI is a risk factor for the development of knee OA.[142] However, this is primarily reported in middle-aged individuals regardless of injury history.[142] Individuals with a history of ACLR represent a unique population the develops accelerated knee OA, because the ACL tears are most likely to occur in individuals below the age of 20.[44] It is unclear if BMI percentile in adolescents increases the risk of knee OA development. Performing separate linear regression analysis for adolescents and adults to control for BMI percentile versus BMI would reduce our sample size to 10 participants for each regression. Regardless, knee joint biomechanics were normalized to body mass. Future research should determine if BMI or BMI percentile affect standard resting ultrasound femoral articular cartilage thickness in individuals with a history of ACLR.

STRENGTHS

ACCESSIBLE ASSESSMENT TOOLS FOR HEALTH CARE PROFESSIONALS

The second study incorporated the use of accessible ultrasound machines and clinically relevant volume of activity assessments. Early identification of articular cartilage degeneration is key in secondary prevention efforts, but radiographic and MRI-based assessments may not identify pre-radiographic articular cartilage changes or are difficult to utilize for longitudinal assessments which limits their feasibility for early screenings for individuals at risk for PTOA development. Diagnostic ultrasound machines are ubiquitously available in orthopedic clinics and hospitals. With more research, the standard ultrasound assessment of femoral articular cartilage structure has the potential to be integrated into healthcare clinics to identify individuals at risk for developing knee OA. The ultrasound technique utilized in the second study has not been applied in this population during early periods of recovery after ACLR making the study novel. Additionally, we utilized research-grade activity tracking technology for valid data collection to provide better context about volume of activity. However, consumer-grade activity tracking technology is widespread in today's culture and outcomes like steps per day can easily be assessed and modified by clinicians and patients through consumer-grade devices such as FitBit monitors or smart watches.

INTEGRATIVE APPROACH TO UNDERSTANDING KNEE JOINT MECHANICAL LOADING

The second study takes a multifaceted approach to addressing the effects of loading on knee articular cartilage health early after ACLR. Traditionally, knee joint gait biomechanics and volume of loading have been assessed individually after ACLR,[87,118] but considering these factors conjointly provides a more comprehensive assessment of contributors to knee loading during activity. The results of this study provide the first step in a line of research which characterizes the effects of under- or over-loading behavior on articular cartilage health during critical points of the recovery process following ACLR to slow or mitigate the rapid development of PTOA commonly observed in this at-risk population. Both volume of loading and magnitude of loading (gait biomechanics) are associated with poor knee articular cartilage structure within the first 6 months post-ACLR. Both facets of mechanical knee joint loading should be considered in clinical treatment and future research as a more comprehensive risk factor. By identifying which load-related factors are associated with poor knee articular cartilage structure, we may be able to develop and implement safe, progressive walking-based protocols during recovery with the goal of promoting physical activity related behaviors and maintaining healthy knee articular cartilage.

CLINICAL IMPLICATIONS AND FUTURE RESEARCH

The utility of ultrasound assessment for knee joint health is promising but requires future research before health care providers adopt the technique in clinical practice. First, the resting cartilage assessment semi-automated processing remains time-consuming. This is despite the reduction in processing that only requires manual segmentation of the total CSA and improvements in calculating thickness across the entire articular cartilage compartments.[182] Advancing to a fully-automated processing technique would enhance the clinical feasibility of the technique considering the ubiquitous prevalence of ultrasound machines in orthopedic clinics. Secondly, ultrasound assessments of pre-radiographic knee joint health may be more impactful when utilizing a grading system of multiple outcomes similar to radiographic assessments of knee osteoarthritis (i.e. Kellgren-Lawrence Classification).[147,222] In addition to articular cartilage thickness, ultrasound may be used to assess synovitis, meniscal extrusion, joint effusion, and cartilage echo-intensity.[8,222] Outcome measure scoring systems assessed via ultrasound have been recommended to screen individuals with radiographic evidence of knee osteoarthritis,[222] but multifaceted ultrasound assessments of pre-radiographic changes early in knee osteoarthritis development have not been established. Future research should incorporate the resting cartilage assessment along with other valid and reliable ultrasound-based knee joint health outcomes (i.e. meniscal extrusion and cartilage echo-intensity) to potentially establish an adequate screening to identify individuals at risk for knee osteoarthritis development.

To our knowledge, this is the first study to longitudinally assess femoral articular cartilage using the standard resting ultrasound technique after ACLR. Rehabilitation after ACLR is may completed within this 6-month period.[14] This study has a longitudinal design to better understand the relationship between factors of loading during rehabilitation and knee articular cartilage joint health when many individuals have returned to activity. Gait biomechanics have been the focus of mechanical knee joint loading and PTOA research post-ACLR, but the linear regression results of the third manuscript suggest that volume of loading is also a key contributing factor for researchers and clinicians to consider moving forward.

Extensive literature indicates that there are consistent relationships between gait biomechanics and biochemical, compositional, and radiographic changes in knee joint health post-ACLR.[11,120,175] Future research assessing knee joint health and volume of loading should also consider incorporating wet inflammatory and cartilage metabolism biomarkers, MRI of T1 rho and T2 relaxation times, and x-ray assessments to better understand the strength of these relationships. While novel, these results are focused on a specific period of time post-ACLR (< 6 months). Extensive longitudinal assessments may help clarify if the relationship between volume or loading and knee joint health are time dependent post-surgery, and if the direction of the relationships change based on time. For example, individuals with worse symptoms post-ACLR demonstrate lesser vGRF at 6 months, but greater vGRF at 12 months during walking compared to individuals with fewer symptoms.[126] Longer longitudinal assessments may help determine if relationships between volume of loading and knee joint health change in a similar way to gait biomechanics. Additionally, researchers assessing mechanical knee joint loading should consider the multifactorial nature of this pathway in PTOA development. Future research should incorporate consistent assessments of cyclical loading and expand their assessment to understand other loading aspects such as intensity of loading (i.e. moderate to vigorous physical activity) or rate of loading (i.e. cadence or gait speed) post-ACLR.

The results of the third manuscript also suggest that volume of loading is an important clinical assessment. Clinicians should consider including volume of loading assessments in day-to-day practice to understand how much cyclical loading is occurring daily and how this relates to achieving or not achieving physical activity guidelines. Volume of loading can be measured clinically through consumer grade technology (i.e. pedometers, Fitbits, smart watches). At 4-months post-ACLR, patients demonstrate low step counts post-ACLR which may impact their ability to meet national physical activity guidelines. Clinicians should consider educating patients about the impact of physical inactivity on long-term health and encourage individuals to increasing their physical activity participation within the context of their restrictions post-surgery. Future research should explore interventions aimed at increasing step counts using consumer grade technology such as accelerometers and goal-setting phone apps for clinicians to provide as evidence-based interventions for their patients

CONCLUSIONS

Resting cartilage ultrasound assessment of femoral articular cartilage structure demonstrates better measurement properties when assessing cartilage structural across multiple study visits when compared to the cartilage response to loading ultrasound assessment technique in individuals without a history of knee injury. Resting cartilage ultrasound assessment of femoral articular cartilage structure cannot detect involved and contralateral limb differences or changes across time within the first 6 months after ACLR indicating that knee articular cartilage structural changes may not occur while patients typically engage in knee rehabilitation. Therefore, this may be a beneficial time to address modifiable risk factors such as altered mechanical knee joint loading that are associated with the accelerated development of individuals with a history of ACLR before structural changes begin to occur. Volume of mechanical knee joint loading may moderate the relationships between knee gait biomechanics and poor knee articular cartilage structure. Specifically, individuals demonstrating lesser knee extension moment who walk greater steps per day demonstrate greater medial femoral articular cartilage thickness which is associated with cartilage swelling. Individuals with lesser knee extension moment gait biomechanics should undergo evidence-based interventions to address poor gait biomechanics during the early phases of rehabilitation before they are introduced to greater levels of daily activity.

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