Identifying and Targeting Modifiable Risk Factors Linked with Knee Joint Health after Anterior Cruciate Ligament Reconstruction

by

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Dedication

This dissertation is dedicated to my family –

To my mom Valerie, my sister Anastasia, my grandparents Diana, Diane, Armando, and Louis, and to my dad looking after me from up above.

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Lastly, **to my family**, this dissertation is for you all. You are all the strongest people I know, and I cannot express how much your love and support has meant to me as I have completed my PhD. We did it.

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List of Abbreviations

ACL	anterior cruciate ligament
ACLR	anterior cruciate ligament reconstruction
ANOVA	analysis of variance
AU	arbitrary unit
BMI	body mass index
BPTB	bone patellar tendon bone
BW	body weight+
CI	confidence interval
cm	centimeter
COM	center of mass
CT	computerized tomography
dB	decibel
DEXA	dual emission x-ray absorptiometry
ECM	extracellular matrix
EI	echo-intensity
GAG	glycosaminoglycan
GRF	ground reaction force
HSD	honestly significant difference
Ht	height

HT	hamstring tendon
Hz	hertz
ICC	intraclass correlation coefficient
IL	interleukin
ILD	interlimb difference
IQR	interquartile range
JSW	joint space width
KAM	knee adduction moment
KFM	knee flexion moment
KFA	knee flexion angle
KFE	knee flexion excursion
KEE	knee extension excursion
Kg	kilogram
LCL	lateral collateral ligament
MCID	minimally clinically important difference
MCL	medial collateral ligament
MDC	minimal detectable change
MHz	mega hertz
mm	millimeter
m	meter
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
N	newton

Nm	newton-meter
OA	osteoarthritis
PA	physical activity
PFJ	patellofemoral joint
PG	proteoglycan
ROI	region of interest
ROM	range of motion
s	second
SD	standard deviation
SEM	standard error of mean
SPM	statistical parametric mapping
SS	self-selected
TNF-α	tumor necrosis factor – alpha
US	ultrasonography or ultrasound
vGRF	vertical ground reaction force
3D	three dimension

Abstract

High body mass index (BMI) is consistently, and independently, linked with elevated OA risk in uninjured and anterior cruciate ligament reconstruction (ACLR) populations. High BMI is a national health concern and nearly 40% of patients with ACLR are categorized as overweight or obese. Nonetheless, a lack of data has directly assessed the potential mechanisms driving elevated risks of post-traumatic osteoarthritis (OA) in high BMI patients with ACLR and it is plausible these patients experience differential recovery trajectories – necessitating unique rehabilitation strategies. Altered walking patterns are ubiquitous following ACLR which if left unresolved, may perpetuate cartilage degradation and influence OA onset. Unfortunately, typical rehabilitation lacks gait-specific rehabilitation tools to adequately restore walking patterns. Identifying clinically feasible strategies to facilitate gait recovery is essential to improving patient function and minimizing post-traumatic OA risk. Therefore, the overarching aims of this dissertation represents a series of cross-sectional investigations aimed at 1) assessing modifiable risk factors (i.e., high BMI and walking biomechanics) linked with post-traumatic OA following ACLR and 2) examining gait retraining strategies and monitoring strategies to improve and better detect biomechanical risk factors for post-traumatic OA after ACLR. **Aim one** of this dissertation explored the effects of BMI on ultrasound-based measures of trochlear cartilage thickness and gait biomechanics after ACLR. We found high BMI uniquely influenced cartilage thickness differences and moderated the

relationship between walking mechanics and trochlear cartilage thickness. **Aim two** further explored the impact of high BMI on joint health after ACLR by evaluating surrogate measures of cartilage mechanical integrity. We found those with high BMI after ACLR exhibited greater cartilage strain, larger echo-intensity (EI) changes post-exercise and larger between-limb differences in cartilage outcomes compared to normal BMI counterparts with ACLR. Further, individuals who habitually walked with greater knee loads, and joint range of motions exhibited lesser strains and EI changes. For **aim three**, we assessed the feasibility of manipulating walking cadence to improve knee motions and load outcomes using auditory biofeedback. We found cueing individuals with ACLR to walk at slower cadences acutely facilitated sagittal knee motions and moments. Modifying cadence is highly translatable to the clinic given the need for minimal equipment, but future longitudinal investigations are needed to confirm the long-term efficacy of this biofeedback approach. Lastly, **aim four** investigated how acutely manipulating walking speeds altered interlimb symmetry in gait mechanics between ACLR individuals and uninjured controls. We observed those with ACLR experienced differential responses to speed manipulations compared to uninjured individuals where gait asymmetries became magnified at fast speeds and reduced at slow speeds, but only in ACLR patients. The use of fast walking speeds could be advantageous when assessing gait function clinically as it may aid in characterizing an individual's functional competence. Further, increasing speed could be used as a gait retraining strategy to increase knee loads and motions after ACLR. Overall, data from this dissertation suggests that those with high BMI after ACLR may present with earlier OA-related disease features and may require more aggressive rehabilitation and implementation of

disease-modifying treatments. Further, we provide initial evidence that cadence manipulation could be an avenue for future gait intervention programs while manipulating walking speeds may be a useful task-specific constraint that can increase (or decrease) musculoskeletal demands – such knowledge may be of use when designing or modifying intervention approaches or when assessing gait recovery throughout post-operative rehabilitation.

Chapter 1 Introduction

1.1 Statement of the problem

Anterior cruciate ligament (ACL) injuries are among the most common lower extremity joint injuries occurring in over 300,000 individuals annually with the majority opting for reconstructive surgery (ACLR).[1] Recovery from ACL rupture typically requires surgical reconstruction, as well as strenuous and extensive rehabilitation efforts to combat the host of impairments that accompany injury and to ensure individuals are capable of returning to safe physical activity. Unfortunately, traditional standard-of-care rehabilitation is unsuccessful in ameliorating key impairments and protecting individuals from serious long-term health consequences, as over 50% of ACL-injured/reconstructed individuals will go on to develop post-traumatic osteoarthritis (OA) 5-14 years after injury [2, 3]. OA is a substantially debilitating disease causing poor health-related quality of life and increasing the risk of developing comorbidities. The rapid time-course of post-traumatic OA development after ACL-injury/reconstruction is further troubling given that youth and adolescents are considered most prone at risk for ACL injuries. Thus, many individuals are susceptible to experiencing debilitating disease symptoms as early as the second or third decade of life.[3] Furthermore, the high rates and rapid progression of post-traumatic OA are a major public health concern as the disease is an economic burden incurring over $11 billion in annual health care costs[4], and to date has no cure available.

Prevention strategies in those at risk for idiopathic OA are difficult to implement given the disease often develops slowly with minimal symptomatic presentation, however

post-traumatic OA has a known “start-point” (i.e., injurious event like an ACL-tear) and thus researchers and clinicians are uniquely positioned to implement targeted post-traumatic OA prevention strategies. Nonetheless, the time-course of post-traumatic OA development leading up to disease diagnosis is not fully understood and preventative strategies have been seldom identified. Therefore, there is a critical need to expand our knowledge on the risk factors associated with, and the underlying mechanisms for post-traumatic OA development in order to help inform the development of intervention targets to combat its incidence and mitigate its substantial burdens.

1.2 Justification of Research

The contributors to post-traumatic OA risk after ACL injury and reconstruction (ACLR) are multifaceted, but a host of modifiable and non-modifiable factors have been connected with higher odds of developing this debilitating disease. Of the numerous factors impacting post-traumatic OA development, joint loading during walking is modifiable factor shown to influence cartilage health [5-9] after ACLR. For example, a wealth of research has established that impairments in gait mechanics plague those with ACLR, and these persist despite the completion of rehabilitation. Thus, it is important to elucidate how gait/loading characteristics may impact cartilage health and to identify strategies that may permit the normalization of gait during rehabilitation[10, 11].

Compelling evidence suggests high BMI is linked to a three times greater odds of developing post-traumatic OA in patients with ACLR.[12, 13] Further, recent studies support high BMI as one of the strongest predictors of accelerated post-traumatic OA within five years post-ACLR.[14] Unfortunately, there is considerable lack of research investigating the mechanisms underlying the considerably elevated risks of post-

traumatic OA in high BMI patients with ACLR. Given that BMI is highly modifiable, elucidating which factors may be contributing to post-traumatic OA risk is critically needed to adequately identify targets for early intervention strategies.

Given this, the overarching aims of this dissertation were to globally evaluate how modifiable risk factors for post-traumatic OA such as altered gait mechanics and high BMI acutely influence knee cartilage health outcomes in those with ACLR and evaluate potential strategies to improve gait mechanics post-ACLR. We examined these questions via four separate investigations completed between 2020-2023. Highlighted below are the specific aims and hypotheses for each of the dedicated investigations:

1.3 Specific Aims

The Specific Aims of this dissertation are comprised of the following:

AIM 1A: To compare gait biomechanics and cartilage thickness between individuals with high and normal BMI after ACLR.

AIM 1B: To evaluate the moderating effect of BMI on the associations between walking biomechanics, and US-based measures of femoral trochlear cartilage thickness (medial, lateral and medial: lateral thickness ratio).

Hypothesis 1A: We hypothesized that those with high BMI (i.e., BMI > 27.0 kg/m^2) would exhibit smaller normalized peak KFM and vertical GRFs but larger KAM and cumulative knee load indices. Further, we hypothesized that high BMI would be associated with thinner medial and lateral femoral trochlear cartilage bilaterally.

Hypothesis 1B: We also hypothesized that BMI would moderate the association between knee loading mechanics and cartilage thickness in the ACLR limb, wherein a positive association would be observed between loading and cartilage outcomes (e.g., higher KFM/GRF/KAM linked with thicker cartilage), but this relationship would only be present in the normal BMI group.

Significance of AIM 1: Previous research has connected high BMI with elevated risk of post-traumatic OA after ACLR, but few studies have directly compared functional and/or joint health outcomes between BMI groups in this population. High BMI is associated with altered gait biomechanics and poor cartilage structural and functional properties

irrespective of ACLR and it is possible these factors are disproportionately altered when combined with ACLR. Findings generated from this first investigation will provide critical data comparing gait and patellofemoral cartilage structure in those with ACLR and help determine if those with high BMI exhibit poorer knee outcomes. Such knowledge will help fill fundamental gaps in our overall understanding of the factors contributing to the disproportionately higher risk of post-traumatic OA after ACLR.

AIM 2: To evaluate the associations between BMI and knee biomechanics on the acute changes in trochlear cartilage thickness (i.e., strain) and echogenicity (EI) after a 30-minute incline treadmill walk in those with ACLR.

Hypothesis 2A: We hypothesized those with high BMI after ACLR would exhibit greater cartilage strains and changes in cartilage EI following an acute walking stimulus compared to normal BMI individuals with ACLR after controlling for sex and time post-ACLR.

Hypothesis 2B: We also hypothesized that greater GRF loading rates, knee moments and angles would be associated with greater and medial and lateral femoral trochlear cartilage strain and changes in cartilage echogenicity following our walking stimulus.

Significance of AIM 2: High BMI patients with ACLR may represent a subset of patients that are at elevated risk for a more accelerated onset of post-traumatic OA. Early signs of OA may manifest as reduced ability to withstand mechanical loading and thus, *in vivo* assessments of cartilage functional properties may help us understand if those with high BMI after ACLR may be exhibiting poorer cartilage health outcomes. Leveraging an exercise stress-test design, data from this second investigation will provide novel data on cartilage strain assessments after ACLR and help identify how BMI may be impacting cartilage health in this patient population.

AIM 3: The purpose of this investigation was to evaluate the acute effects of modifying step length via cadence manipulations during treadmill walking (i.e., 90%, 100%, and 110% preferred cadence) on knee joint biomechanics bilaterally in individuals 9-12 months after ACLR.

Hypothesis 3A: We hypothesized that knee kinematics and kinetics would increase with step-lengths (i.e., peak moments, angles and excursions would be smallest at shorter step-conditions and greatest at longer step-conditions).

Hypothesis 3B: We also hypothesized that the magnitude of changes in biomechanical outcomes would be similar between both ACLR and the contralateral limb.

Significance of AIM 3: Although post-traumatic OA genesis is complex, aberrant knee mechanics, such as reduced knee moments and angles, is a risk factor for post-traumatic OA and is a persistent finding for upwards of 10-years post-ACLR [10, 15-20]. Unfortunately, few if any gait retraining strategies have been identified to ameliorate knee loading deficits and are seldom included in standard-of-care post-operative ACLR rehabilitation programs[21, 22]. Therefore, identifying strategies that can improve knee loading patterns in ACLR patients may have tremendous potential for maintaining cartilage health and thereby preventing or mitigating early cartilage degeneration. Gait retraining strategies that offer low-cost solutions with minimal equipment, such as metronome biofeedback, could offer substantial clinical utility and excellent potential for applications outside a lab setting [23, 24]. As such, data generated from this study provides initial pilot data on potentially clinically feasible gait retraining options that can target knee mechanics post-ACLR.

AIM 4: The primary purpose of this study was to comprehensively evaluate the effects of manipulating walking speed (i.e., 20% above and below self-selected speeds) on between-limb differences in limb (vertical and anterior-posterior GRF) and joint (knee flexion moment, angle, and excursions) gait biomechanics in individuals who were between 9-12 months post-ACLR and uninjured controls. A secondary purpose of the study was to evaluate gait biomechanical differences between ACLR participants and matched controls.

Hypothesis 4A: Our primary hypothesis was faster walking speeds would induce larger between-limb differences in gait mechanics in those with ACLR but not healthy controls.

Hypothesis 4B: We also hypothesized that those with ACLR would walk with lesser GRFs, and knee flexion moment and angles bilaterally compared to uninjured control participants.

Significance of AIM 4: Quantifying the restoration of normal gait after ACLR can be an important benchmark for researchers and clinicians as it offers a goal to strive towards throughout rehabilitation. Nonetheless, some difficulties exist in gait assessments given that bilateral impairments are observed in those with ACLR compared to controls and thus, between-limb symmetries may overestimate knee function. We have previously shown that manipulating walking speed is a simple, task-specific strategy that can elucidate larger asymmetries during walking in persons early post-op (within 2-3 months), but our findings were limited to ground reaction forces which do not fully characterize knee-specific loads [25]. Further, it is not clear if using simple speed manipulations can similarly magnify gait biomechanical asymmetries in individuals closer to the time of

return-to-activity, as our previous work examined patients who were approximately 9 weeks post-ACL reconstruction. Therefore, findings from this study will extend our previous work while also providing data that can help improve gait assessment and rehabilitation options in clinical settings.

1.4 Organization of Dissertation

The following sections of this dissertation have been structured to include a review of the literature **(Chapter 2)** that provides the scientific background, rationale and justifications for overarching dissertation aims. The core chapters of the dissertation **(Chapters 3-6)** detail the four separate research aims that were conducted at the University of Michigan between 2020-2023. We are currently submitting manuscripts for each aim for publication in peer-reviewed journal – **Chapters 5 and 6** have been accepted for publication while **Chapters 3 and 4** are in the process of submission. Finally, **Chapter 7** details a summary of the dissertation, limitations of our work and future directions for related research.

Chapter 2 Literature Review

2.1 Overview

The purpose of this literature review is to: 1) provide background on ACL injuries, their common treatment strategies and the burdens associated with this trauma, 2) examine the prevalence of OA after ACL rupture and reconstruction, 3) provide an overview of the anatomy, and function of articular cartilage while highlighting the impact of OA on cartilage integrity 4) describe the risk factors thought to contribute to OA risk after ACL injury, with a focus on gait biomechanics and BMI 5) examine imaging modalities used to diagnose and monitor cartilage and OA outcomes, and 6) highlight potential opportunities for clinically feasible rehabilitation strategies to mitigate OA risk after ACL injury.

2.2 Anterior Cruciate Ligament Injury and Surgery: Background and Burdens

Rupture of the anterior cruciate ligament (ACL) is one of the most treated lower-extremity traumas with approximately 250,000 ACL ruptures occurring annually in the United States [1]. While ACL ruptures can occur in isolation, many patients present with concomitant injuries to other knee structures such as tears to the menisci and neighboring ligaments, as well as damage to articular cartilage and subchondral bone [26-28]. A variety of risk factors influence one's susceptibility for an ACL rupture, but generally, it appears that youth and adolescents are most prone to this injury [3]. Indeed, recent work shows increasing trends of pediatric and adolescent ACL ruptures (e.g., ages 10-19) [29,

30] which, in part, may be attributed to the increased participation in competitive youth sports and earlier sport-specialization of young athletes [31, 32]. As will be discussed in following sections, the disproportionate number of ACL ruptures in this younger age group is problematic given the lasting and profound impact of this injury on health-related quality of life, overall physical functioning, and more serious long-term consequences such as the accelerated onset of OA.

In those suffering an ACL rupture, non-operative and operative treatment options exist, but surgical reconstruction is most often chosen to restore mechanical stability of the joint and reduce the risk for subsequent meniscal injury, particularly in patients hoping to regain a physically active lifestyle [33, 34]. While ACL reconstruction is generally considered superior to conservative treatment due to the resolution of joint instability and preservation of the menisci, it is important to recognize that current surgical techniques are largely unable to restore normal joint kinematics [35-39] and do not appear to reduce the risk of OA when compared to non-surgical management [2]. Regardless of treatment option chosen, patients must undergo rigorous post-injury and/or post-operative rehabilitation aimed at ameliorating the plethora of impairments that present after injury/reconstruction [22, 40, 41]. For instance, patients with ACLR frequently present with significant anterior knee pain, joint effusion, reduce joint range of motion and quadriceps dysfunction amongst other functional impairments [18, 22, 40, 42-47]. Thus, rehabilitation is generally focused on remediating these knee signs and symptoms in order to improve patient function, facilitate safe return to physical activity, and hopefully reduce the risk for re-injury.

Unfortunately, current standard-of-care rehabilitation is not adequate to facilitate optimal objective and-subjective measures of patient function after ACLR. Further, return-to-activity criteria varies significantly and thus, the rates of passing clinical criteria are largely variable and depend on the outcomes used clear patients (i.e., quad strength and knee ROM symmetry, absence of effusion, symmetrical performance on hop tests etc.,) For example, Overwhelming evidence suggests typical standard-of-care rehabilitation does not sufficiently restore important outcomes like quadriceps function as significant alterations in muscle (i.e., atrophy, fat infiltration, fiber type changes) [48-53] and neural pathways (e.g., spinal and supra-spinal inhibition) [45, 51, 54-57] continue to be uncovered in cohorts upwards of 5 years post-surgery. Further, a myriad of functional deficiencies persist after ACL-reconstruction such as altered movement biomechanics and poor neuromuscular control during numerous dynamic tasks (i.e., walking, running, hopping, jump landing) [11, 16, 20, 58-69]. The inability of traditional rehabilitation to ameliorate the wide array of impairments after surgery is troubling as these lingering deficits carry significant consequences for the individual. Insufficient muscle strength and poor neuromuscular control have been previously linked to increased re-injury risk [3, 70, 71] and joint-space width narrowing [72] while altered walking mechanics are thought to influence the early development of OA after ACL-reconstruction [73-76]. Given these lasting consequences of ACL injury and reconstruction, it is imperative that research is conducted to help understand and identify strategies to improve these suboptimal short (i.e., poor strength, altered walking mechanics) and long-term patient outcomes (i.e., OA).

2.3 Osteoarthritis after Anterior Cruciate Ligament Injury and Surgery: An Overview

The Osteoarthritis Research Society defines OA as a disorder commonly affecting load-bearing joints, such as the knee, that manifests as a culmination of anatomic and physiological derangements in nearly all structures within the joint (i.e., cartilage, ligaments, and bone) [77]. OA is one of the leading causes of disability globally [78-82] and it has been estimated that over 10 million individuals are diagnosed with symptomatic knee OA in the United States alone [83, 84]. The burdens associated with developing OA are substantial, impacting both society and the individual themselves. The direct financial costs of all OA cases exceed $100 billion annually, while at the individual level many who suffer from OA experience substantial wages lost due to the severe physical limitations associated with the disease [79, 80, 85, 86]. Persons with OA also experience significant reductions in quality of life and are generally at risk of developing additional co-morbidities, such as cardiovascular disease, due to the common OA-related barriers to physical activity (i.e., pain, stiffness) [87, 88]. Given that OA rates continue to rise, it is imperative that avenues to mitigate the risk of developing OA are identified so that treatment options can be established, and the burdens of the disease can be reduced.

A wide array of risk factors are linked to knee OA development such as high BMI, sex, and age as well anatomic factors like lower-extremity alignment [12, 80, 82, 89, 90]. It is also well recognized that prior knee joint injury (e.g., ACL rupture; meniscal injury) is one of the strongest predisposing factors for OA, increasing one's risk for the developing the disorder four-fold [90-92]. Of all knee OA cases in the United States, approximately 10% develop secondary to joint injuries and are referred to as post-traumatic [4]. Post-traumatic OA is highly prevalent after ACL injury [12], occurring rapidly in both

patellofemoral and tibiofemoral compartments [14, 93-95]. Further, post-traumatic OA is considered a more accelerated disease process compared to typical idiopathic OA phenotypes as approximately 12% of patients are diagnosed with post-traumatic OA within the first 5 years post-operatively [14], a number that increases to around 50% of patients around 10-20 years post-injury/surgery [12, 91]. The premature development of OA after knee joint injury is, thus, concerning given a high number of ACL ruptures occur in persons between the ages of 15-25, meaning many young individuals may develop OA symptoms as early as their third or fourth decade of life. Comparatively, the reported prevalence of idiopathic OA does not exceed 30% until approximately 60-70 years of age [91, 92, 96, 97]. Thus, those suffering from post-traumatic OA may experience a greater number of years lived with disability and potentially an earlier need for joint replacement compared to their idiopathic OA counterparts [3, 91, 98]. Unfortunately, end-stage treatment for OA (i.e., joint arthroplasty) in younger patients leads to poorer post-surgical outcomes and a higher likelihood of requiring subsequent revision surgeries [99, 100]; a fact that further highlights the unique challenges and consequences associated with the accelerated disease process of post-traumatic OA.

It is well understood that OA is a highly complex and multifactorial disorder carrying serious lifelong consequences. Currently, there is no known cure for OA and once the disorder presents clinically, the changes occurring to the joint are often considered irreversible [6-8, 101, 102]; underscoring the need for early disease detection and aggressive interventions that are capable of delaying or preventing OA. However, in order to characterize early disease alterations and adequately develop targeted interventions, a detailed understanding of how structures within the joint (i.e., cartilage) maintain their

function in both health and disease is paramount to fully appreciate the many factors implicated in the OA process.

2.4 Articular Cartilage: Function, Pathology and Assessment Techniques

2.4.1 Basic Anatomy of Cartilage

Articular (hyaline) cartilage is a highly complex tissue that wraps along the articulating surface of diarthrodial joints. The main functions of the tissue are to allow near frictionless articulation between bones and to absorb, redistribute and dissipate forces [103, 104]. Despite the tissue's limited ability for self-repair, due to a lack of vascularity, cartilage can withstand millions of loading cycles without failure. The remarkable capacity of cartilage to maintain its vital load-bearing functions for decades can be attributed to the tissue's unique ultrastructure and composition [103, 105, 106]. Therefore, gaining a better understanding of the contributions of cartilage structure and composition to the tissues unique load-bearing abilities in a healthy state is important to fully appreciate the breakdown of tissue health and function that occurs with disease such as osteoarthritis.

Cartilage is best described as a multiphasic tissue, containing a porous-permeable solid matrix, a fluid phase of primarily water (approximately 80% of the tissues wet weight), and an ion phase consisting of dissolved electrolytes [105, 106]. The tissue is comprised of a highly organized extracellular matrix (ECM), housing a variety of macromolecules such as type II collagen, proteoglycans (PG) along with other non-collagenous proteins and the interstitial fluid [102, 105-107]. Type II collagen is the most abundant collagen type within the tissues ECM, but several other collagen types also exist to a lesser extent [103]. The collagen fibrils are important components of the cartilage ECM due to their strength in tension and because the unique meshwork-like

arrangement of fibers help restrain PG within the matrix. Together, the type II collagen-PG interactions form the solid matrix of articular cartilage [106-108]. Several types of PGs also exist within cartilage with aggrecan being the most common and largest aggregating PG. Proteoglycans consist of a core protein along with glycosaminoglycan (GAG) sidechains (such as chondroitin and keratan sulfate) that are covalently bonded to the protein core. Because GAG sidechains possess a high negative charge, PGs within cartilage are primarily responsible for maintaining tissue hydration as well as the production of swelling pressures that strongly resist compression [102, 105, 106].

The only metabolically active cell within articular cartilage is the chondrocyte which is tasked with orchestrating the maintenance, assembly and normal turnover of the articular cartilage's complex ECM [103, 109]. Chondrocytes are sparsely populated throughout the depths of articular cartilage (accounting for only 1-10% of the tissue volume) and are surrounded by a pericellular matrix consisting of type VI collagen and high concentrations of PG. Together, a chondrocyte and its surrounding pericellular matrix form a chondron which generally consists of a single chondrocyte, except in the deep zone of cartilage where several chondrocytes may inhabit a single chondron [110-112]. Functionally, the chondron and its pericellular matrix plays an important role in modulating the numerous biomechanical and biochemical signals occurring within the tissue (e.g., cell deformation, changes in fixed-charge density, hydrostatic pressures, growth factors, cytokines etc.) which in turn influences chondrocyte function [111, 113]. Overall, the ability of chondrocytes to regulate its biosynthetic activity in response to these numerous signals is crucial to maintaining homeostasis of the tissues ECM and preserving the normal functioning of cartilage over time [103, 109].

2.4.2 Articular Cartilage Zonal Properties

Although macromolecules within articular cartilage such as PG, type II collagen and the interstitial fluid are present throughout the entire tissue, their concentration and organization are inhomogeneous and are unique to specific "zones". For example, structurally, cartilage can be partitioned into four distinct regions: the superficial, transitional, deep, and calcified cartilage zones [103, 114]. Within each zone, water content, solid matrix structure, macromolecule content (i.e., PG and type II collagen) and even the number and morphology (i.e., shape) of chondrocyte cells differ drastically [103, 114]. Given this inhomogeneity of ECM components, it can be understood that each zone possesses distinct functional properties which may uniquely contribute to the overall load-bearing function of the tissue.

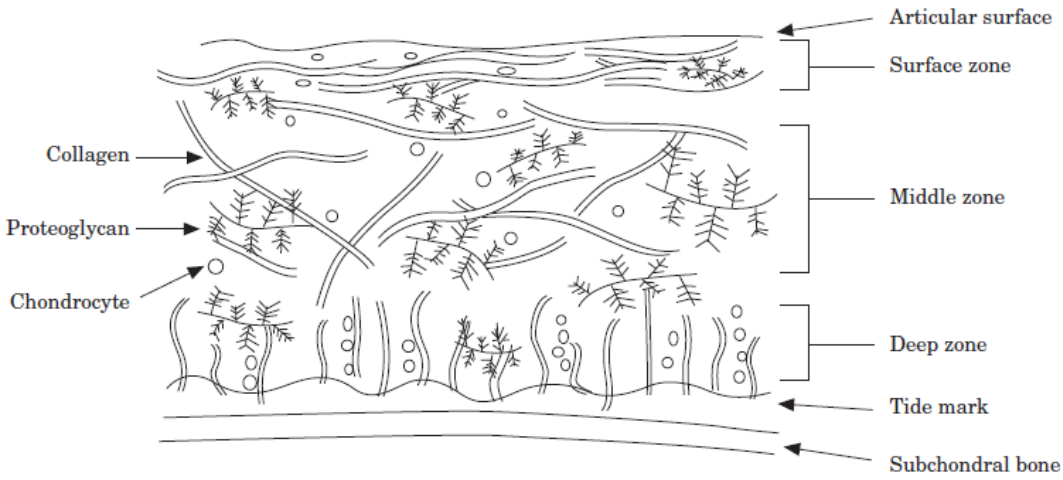

Figure 2.1 Representation of the major structural and compositional components of articular cartilage. Depicted are the large type II collagen fibers, proteoglycans, and the chondrocyte which his the only metabollically active cell within the tissue. Beneath the three cartilage layers (i.e., surface or superficial, middle and deep zones) is the unerlying subchondral bone delineated by the tide mark where collagen anchors the tissue. Figure from Setton et al, 1999. Reprinted with permissions (License #5139500797881)

The superficial zone of cartilage is the thinnest region of the tissue (comprising the first 10-20% of the tissue depth), containing the highest concentrations of chondrocytes, a dense network of type II collagen (with fibrils orientated parallel to the articular surface),

as well as the highest water content of all cartilage zones [109, 115, 116]. Chondrocytes in this region are also flatter than in other tissue zones, while PG content is generally at its lowest. The dense parallel arrangement of type II collagen in the superficial zone serves an important role in resisting high shear and tensile stresses occurring about the tissue surface given the high strength of collagen in tension. In addition to providing the tissue with tensile stiffness, the type II collagen network in this zone also partly contributes to the compressive properties of cartilage [107, 109, 115]. For example, although much of the compressive stiffness of the tissue is attributed to fluid pressurization facilitated by PG interactions [117], the superficial collagen meshwork effectively helps “confine” the fluid flow/pressurization that occurs when loads are applied to the joint. Therefore, degradation of the superficial zone (i.e., fibrillation, type II collagen disorganization), as is seen in the very early stages of OA, may have substantial effects on the functional capacity of the tissue (i.e., reduced tensile and compressive stiffness) and place undue stresses about deeper regions of cartilage [118].

In the transitional (or middle) zone, chondrocyte cellularity, water and type II collagen content are lesser when compared to the superficial zone [114, 116]. PGs are most abundant in this area and chondrocytes in this region display a more rounded morphology relative to superficial zone chondrocytes [109, 115]. Because of the high concentrations of PG in this zone, the middle zone of cartilage possesses greater compressive stiffness than superficial cartilage and thus undergoes less intratissue strain when compressed (e.g., superficial cartilage is more compliant than middle and deep cartilage). Type II collagen orientation and density also differ in the transitional zone as cartilage here is arranged somewhat randomly and is less dense than the superficial

zone. Thus, the intrinsic tensile stiffness of this zone is lesser than superficial cartilage which, in general, tends to decrease with increasing tissue depth [108, 114]. However, the randomly oriented collagen fibrils of the transitional zone provide an improved resistance to shear stresses than the superficial zone which could be partly attributed to the oblique arrangement of some fibrils in the collagen network.

Lastly, in deep cartilage, water content is minimal, PG concentrations are high and chondrocyte cellularity is further reduced. Chondrocyte orientation is also distinct from other zones as cells here are aligned vertically in columns. Type II collagen fibrils in this region possess the largest diameters within the tissue and exist in long bundles that are oriented perpendicular to the cartilage surface. The fibrils also cross the tide mark of the calcified cartilage region which acts to anchor the tissue to the subchondral bone. Functionally, deep cartilage also possesses strong compressive properties like the transitional zone, which is attributed to the high concentration of PG in this region. As a result, deep zone cartilage experiences the least amount of intratissue strain compared to middle or superficial zones [119, 120]. However, while the deep layer of cartilage possesses superior compressive properties relative to the regions above, the intrinsic tensile and shear properties of the matrix are reduced given the perpendicular orientation of type II collagen fibrils [121-124].

2.4.3 Articular Cartilage Biomechanical Function

Knowledge of the functional characteristics of healthy articular cartilage and the underlying factors contributing to its unique load-bearing abilities is critical to understanding the deleterious consequences that result with disease such as OA. During normal motion of the joints (such as the knee) cartilage undergoes compressive, shear and tensile stresses but the tissue is primarily loaded in compression. Under load, cartilage displays time-dependent viscoelastic behavior, such as creep and stress-relaxation, and undergoes measurable deformations (i.e., reduces in thickness) that is reversible upon unloading [105, 106, 118]. Functionally, the deformational response of articular cartilage to loading helps to increase joint congruence, resulting in an increased contact area and an overall reduction in stress about the tissue. The main load-support mechanism underlying this deformation behavior in compression is predominantly attributed to the flow of interstitial fluid through the porous-permeable solid matrix [103, 117, 125]. Accordingly, these flow-dependent mechanisms also underlie the time-dependent creep and stress-relaxation behaviors observed when the tissue is subjected either to a constant stress (i.e., creep behavior), or strain (i.e., stress-relaxation response).

Under a constant load, cartilage deformation occurs in a non-linear fashion (i.e., creep), characterized by a rapid initial deformation that gradually declines until reaching an equilibrium position where no further deformation occurs [103, 105, 125]. At equilibrium, the tissue stress is fully borne by the collagen-PG solid matrix given that interstitial fluid flow ceases. This time-dependent deformational behavior is attributed to the tissues strain-dependent permeability wherein initially, fluid loss is rapid but gradually

diminishes over time with increasing strain as solid-matrix compaction serves to limit the rate of fluid exudation from the tissue [126-129]. When the tissue is held to a constant strain, the stress-response of articular cartilage also exhibits time-dependent variability. For example, initially, the stress observed within cartilage is high but gradually diminishes over time which is due to large pressures and fluid exudation occurring near the articular surface and the severe compaction of the superficial cartilage layer [128]. As time progresses, significant redistribution of interstitial fluid occurs, and compaction of the solid matrix diffuses depth-wise throughout cartilage. As a result, the contact stress needed to maintain the magnitude of tissue strain subsequently declines until reaching an equilibrium (i.e., stress-relaxation phenomena).

Overall, the viscoelastic properties and flow-dependent mechanisms underlying these deformational behaviors in compression provide articular cartilage with a strong ability to dissipate energy during load-bearing while also limiting the load magnitudes borne by the solid-matrix. Several factors such as PG concentration, water content, and tissue permeability, amongst other load-specific factors (e.g., rate and load duration) influence cartilage viscoelasticity and its ability to resist deformation in response to compression [105, 106, 118]. For instance, the immediate pressurization and flow of the interstitial fluid in response to an applied load is facilitated by PGs due to their net-negative charge [130-133]. As fluid flows throughout the porous-permeable solid matrix, significant interstitial drag forces are produced within the tissue that contribute to both load support and compaction of the solid matrix. As noted above, this compaction of the solid matrix is responsible for articular cartilage's strain-dependent permeability, which is critical to help regulate the rate at which fluid is exuded from the tissue [126-129]. Water

content is an additional factor that may impact deformational behavior of cartilage given the inverse relationship between water content, tissue permeability, and the intrinsic compressive modulus of the solid matrix [134]. Thus, it is plausible that increases in water content like that seen in degenerated cartilage (i.e., OA) may contribute to increased deformation under load relative to “healthier” cartilage [134]. Similarly, PG depletion and disorganization of the collagen matrix which are additional early signs of OA can significantly impact the biomechanical properties of cartilage due to their impact of fluid pressurization mechanisms and integrity of the solid matrix (i.e., collagen orientations).

While flow-dependent mechanisms are the predominate source of load-support for cartilage in compression, interstitial fluid flow plays substantially less of a role in resisting tensile and shear loads [108, 135, 136]. Thus, the intrinsic stiffness of solid-matrix components and PG-collagen interactions are considered the main contributors to tensile and shear stiffness of cartilage. In response to tensile loads, collagen fibrils realign, and are stretched through the PG gel of the matrix about the axis of loading. The density, arrangement, and number of cross-linking of type II collagen fibrils as well as the frictional resistance produced between the collagen-PG molecules contribute to the intrinsic stiffness and viscoelasticity of cartilage in tension.

Similarly, the intrinsic stiffness of cartilage in shear is mainly attributed to the content of collagen in the tissue as is evidenced by the strong relationship between type II collagen and the shear modulus [137]. Even though PG molecules themselves do not provide direct resistant to shear loads, they indirectly contribute to the overall shear stiffness of articular cartilage via their role in “inflating” the PG-type II collagen solid-matrix which places collagen fibrils in a state of pre-stress [137]. Indeed, experimental depletion

of PG led to significantly reduced compressive and shear moduli in cartilage explants which may suggest that fluid pressurization mechanisms play a supportive role in the ability for collagen fibrils to resist shear stresses [137].

Collectively, articular cartilage biomechanical function under load can be attributed to both flow-dependent and flow-independent factors that together may serve as indicators of tissue health. For example, significant alterations in macromolecule content (i.e., PG, water, type II collagen) and disorganization of the ECM that occur in early stages of OA can lead to impaired articular cartilage biomechanical function (i.e., compressive stiffness) and a reduced ability to absorb and dissipate loads. Consequently, cartilage may be subjected to abnormal deformations under load via reduced fluid support mechanisms which may require the solid matrix to support a greater proportion of applied loads. Over time, this shift in load support between fluid and solid phases may promote matrix breakdown that further impacts the tissues functional capacity. Therefore, a decline in articular cartilage biomechanical function (i.e., changes deformational behavior) may be a useful indicator of overall tissue health and may offer unique insight into identifying potentially early signs of OA in populations at-risk for developing the disease.

2.4.4 Topographical Variations in Knee Cartilage Structure and Function:

The depth-dependent nonuniformities of ECM component concentrations and organizations noted above endows cartilage with biomechanical properties well equipped to handle a combination of compressive, tensile and shear loads during joint motion. Similarly, tissue composition, morphology and biomechanical properties also vary topographically throughout the entire joint. For instance, regions of tibial and femoral cartilage can be categorized into weightbearing and non-weightbearing regions and

further, into tibial regions uncovered and covered by the menisci. Because of the high stresses and strains placed upon weight-bearing regions of cartilage, it has been shown that these regions possess superior structural and mechanical properties compared to less frequently loaded regions in order to adequately accommodate high mechanical demands [138, 139]. Indeed, cartilage displays greater concentrations of PG in areas of high weight-bearing whereas non-weightbearing regions and cartilage near the periphery generally possess greater collagen content [139, 140]. Cartilage regions covered by the menisci in the tibia also exhibit unique structural and biomechanical properties as previous works have shown meniscal covered cartilage to be stiffer and thinner than regions uncovered by menisci [138]. It has been posited that these adaptations may be attributed to the shock absorbing role of the meniscus in transferring loads between soft-tissue structures [138, 141].

Overall, many have suggested these observations reflect that articular cartilage is a mechanically habituated tissue and these topographical variations in cartilage properties are necessary adaptations to meet the load-bearing demands of the knee joint [5, 6, 142]. These findings detailing the apparent adaptability of articular cartilage to localized loading has driven much research to characterize under what conditions articular cartilage health may be maintained, enhanced, or disrupted. Developing an improved understanding of articular cartilage “adaptability” is critical as it may help illuminate the potential contributors to OA pathogenesis which may have far reaching implications for mitigating the numerous and severe burdens of this complex disorder.

2.4.5 Maintenance of Cartilage Health and Function

In a healthy state, articular cartilage is capable of withstanding high magnitudes of joint loading over decades of use without failure despite limited self-repair capabilities. To preserve cartilage health and sustain the tissue's unique biomechanical properties, chondrocytes are tasked with maintaining homeostatic turnover of the tissue's complex ECM (i.e., a balance between breakdown and synthesis of ECM components). Mechanical loading is considered a strong contributor to the regulation of cartilage health and function but its role on tissue homeostasis depends on the type (i.e., static vs. dynamic), magnitude, and rate of applied loads [6, 9, 143-149]. Numerous *in vitro* studies have demonstrated that moderate levels of dynamic (cyclic) loads facilitate an anabolic response by chondrocytes, which results in increased synthesis of key ECM components like PGs and type II collagen [6, 8, 9, 144, 147]. Conversely, static or sustained compressive loads lead to diminished synthesis rates of ECM components and blunt impact loads (i.e., high rate) can promote cartilage breakdown and induce chondrocyte apoptosis [9, 143, 147, 150].

Although there are less data detailing the effects of loading on regulating cartilage health *in vivo,* studies in both animals and humans have shown that moderate magnitudes of loading (i.e., moderate physical activity) are beneficial for cartilage maintenance and can even promote enhanced load-bearing properties such as increased cartilage thickness and proteoglycan content [8, 142, 151-154]. Further, in healthy populations, many studies have shown that individuals who walk with increased joint loads (i.e., joint moments or contact pressures) exhibit thicker cartilage and lower T1ρ/T2 relaxation times (indicative of better cartilage composition) [139, 155, 156]. Additional data from full or

partial joint immobilization models have also underscored the importance of moderate magnitudes of mechanical loading for maintaining joint health [157-159]. For example, insufficient joint loading induced by immobilization partly mimics the deleterious alterations seen in early OA stages as reduced loading promotes rapid cartilage atrophy (i.e., thinning) and proteoglycan depletion [6, 157, 159]. Thus, the results of these investigations provide support for the notion that routine loading of cartilage is necessary to maintain tissue homeostasis and preservation of the ECM, whereas insufficient or excessive/injurious loading magnitudes could be detrimental for tissue health [6].

In addition to these aforementioned mechanical factors, biochemical mediators (i.e., pro-inflammatory cytokines) are also considered strong regulators of articular cartilage homeostasis as the presence of a heightened pro-inflammatory environment can lead to disordered chondrocyte function. In normal healthy conditions, the presence of biochemical factors like pro-inflammatory cytokines are not widely present in the joint. However, their concentrations have been shown to be upregulated with OA, associate with disease severity, and are predictive of cartilage loss and disease progression [160-167]. Classically, the role of inflammatory factors in OA were not strongly considered given that the degrees of inflammation did not rival those seen with rheumatoid arthritis. Nonetheless, evidence continues to cement the strong role of inflammation in both OA incidence and progression given the ability of these biological factors to precipitate derangement of multiple tissues in the joint. Pro-inflammatory cytokines like TNF-α and members of the interleukin family, for example, stimulate production of matrix degrading proteases (i.e., metalloproteinases, aggrecanases) while simultaneously inhibiting synthesis of ECM macromolecules (i.e., PG, type II collagen) [101, 168-170]. As a result,

the increased involvement of cytokine activity on chondrocytes is thought to tip the normally slow, balanced turnover of cartilage towards catabolism. Therefore, in order to fully appreciate the complex mechanisms contributing to OA development, improving our understanding of the factors that may trigger these inflammatory processes is paramount; knowledge that may also help identify potential disease-modifying targets for intervention.

2.4.6 Early Cartilage Degeneration: Signs and Symptoms

Osteoarthritis is a relatively slow developing disorder that involves a progressive remodeling and degradation of cartilage structure and composition [114, 115]. Although overt cartilage loss, osteophytes, pain and joint stiffness are hallmark characteristics of more advanced stage-OA, the initial stages of the disorder generally occur without much "disturbance" [171, 172]. Rather, subtle alterations in cartilage composition and ultrastructure progress and ultimately disrupt the normal function of the tissue. For example, cartilage of the superficial zone is thought to experience the earliest changes with disease; characterized by PG loss, increased water content and disorganization of the type II collagen matrix (Figure 2, Pane B) [7, 107, 173]. Additional features of early OA can also be visualized at the surface of the tissue wherein cartilage may become fibrillated and rough [103]. Eventually as the disease progresses, these subtle surface fibrillations may develop into large fissures which accompany loss of the cartilage matrix, sclerosis of subchondral bone, osteophyte formation and chondrocyte death in later stages of OA (Figure 2). Interestingly, chondrocyte metabolism in early OA cartilage is characterized by increased synthesis rates of PG and collagen, but these accompany upregulation of degradative enzymes such as aggrecan- and collagen-ases [163, 164, 171, 174]. Thus, despite the attempt of chondrocytes to "repair" the tissue by increasing

synthesis rates, the concomitant increase in enzymatic degradation generally leads to a net loss of tissue components like PG.

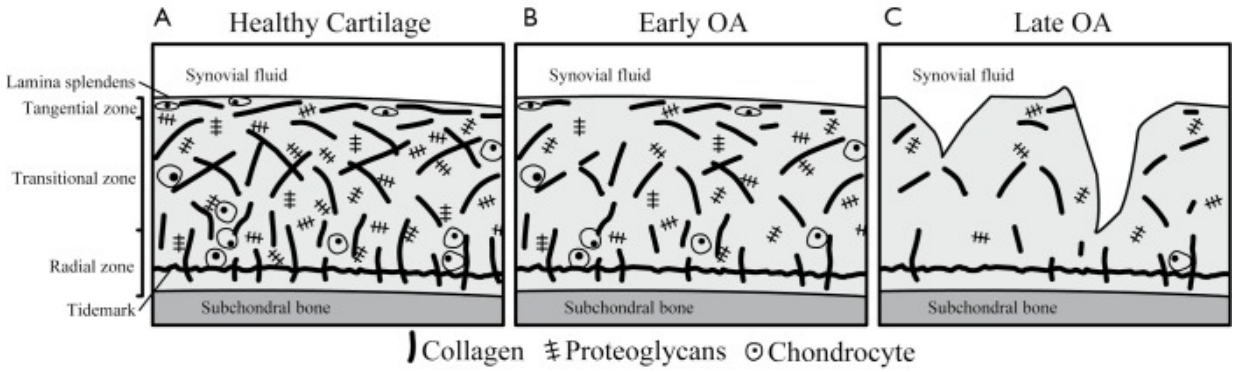

Figure 2.2. Representation of the changes in cartilage structure and composition with advancement of OA. The extracellular matrix of healthy cartilage is rich in proteoglycan, type II collagen and the chondrocytes that maintain the tissues unique arrangement. As OA initializes, proteoglycan concentrations become reduced, while the type II collagen meshwork becomes disorganized. The hallmark signs of late-stage OA are cartilage loss, depletion of matrix components, and cell death. Figure from Matzat et al, 2013. Reprinted with permissions.

Although much of these initial OA-related changes are generally compositional alterations (i.e., decreased macromolecule/water content), cartilage structure has also been observed to undergo a relative thickening in this disease stage [175-178]. However, the increases in cartilage thickness in early OA is often described as a pathological swelling partly attributed to increased tissue hydration. The mechanisms driving observations of increased water content with early OA, however, are not fully understood. It has been suggested that damage to the collagen fibrillar network reduces the matrix ability to restrain PGs and internal fluid pressures which may partly influence swelling behavior in the tissue, even in the presence of PG depletion [131, 132, 179]. Regardless, despite the increased tissue thickness, early OA cartilage possess significantly poorer load-bearing capacity (i.e., reduced cartilage stiffness) which can be attributed to the

combination of altered PG/water content and disorganization of the type II collagen network. Together, these early compositional (i.e., PG, water) and structural alterations (i.e., collagen matrix disorganization) have drastic consequences on cartilage biomechanical function as early-OA cartilage has an impaired ability to pressurize fluid (i.e., reduced PG) and the PG-collagen solid matrix is less able to retain water due to an increased permeability [107, 180]. Therefore, early OA pathological alterations to cartilage may manifest as increased deformation under load and it is plausible the solid matrix may be subjected to increased stress given the reduction in fluid support mechanisms [107, 173, 180-182].

Early OA is a difficult stage of the disease to detect given that structural changes have not yet occurred and symptoms rarely present. However, significant metabolic, compositional, and structural disorganizations lead to a biomechanical weakening of the tissue as noted above. It has been posited that this weakened functional state of cartilage in early OA may act to further exacerbate the vicious cycle of cartilage catabolism as the increased mechanical strains may promote a disruption in normal chondrocyte remodeling processes by increasing production of matrix degrading proteases. If these degenerative biological processes continue unabated, the shift in chondrocyte metabolism towards catabolism may become amplified as degraded matrix fragments can also serve as secondary contributors to the inflammatory cascade that ultimately leads to destruction of the tissue [102, 183, 184]. Given that articular cartilage has limited ability for self-repair, it is imperative to understand if these initial changes in cartilage metabolism, and early signs of tissue degeneration may be mitigated or reversible.

2.4.7 Is Early OA Reversible: Implications for Disease-Modifying Interventions

When cartilage is lost, and the disease presents radiographically (i.e., in late-stage OA), the severe damage to the tissue and surrounding structures are largely considered permanent as cartilage lacks the ability to regenerate. As a result, treatment options for patients presenting in this stage of OA become limited to managing symptoms and slowing disease progression (i.e., delaying inevitable joint replacements). However, it has been posited that initial OA-related changes to cartilage may be partly reversible and amenable to interventions. Specifically, it is suggested that the initial loss of PGs in the early stages of OA does not reflect permanent degradation and chondrocytes may be capable of eliciting a repair response and recover PG content [185]. For example, limb immobilization models in animals have demonstrated that the resulting loss of PG and thinning of cartilage with disuse is almost completely reversible upon periods of remobilization (i.e., weeks to months) [158, 186-189]. Similarly, *ex vivo* data from Karsdal et al., suggests that aggrecanase-mediated degradation of PGs via short-term catabolic stimulation is not permanent and PG content can be replenished when chondrocytes are treated with anabolic growth factors [190]. However, authors also observed that when the tissue experienced MMP-mediated degradation of aggrecan and type II collagen, chondrocytes were not capable of producing a similar anabolic response and the recovery potential, particularly of type II collagen, was lost.

Although these data lend support for the potential reversibility of initial OA-related tissue alterations, a dearth or research has translated such findings to human cohorts. Thus, it remains unclear what methods may be useful to help facilitate the reversal of early cartilage degeneration in those at high risk for OA development. Nonetheless, if

early deleterious tissue alterations with OA are indeed reversible, it becomes paramount to identify methods capable of detecting the earliest signs of tissue pathology so that interventions could be implemented and the risk of developing the disorder can be at least prolonged if not completely prevented.

2.4.8 Imaging Modalities to Assess Cartilage Health and Structure

A range of imaging modalities have been used to evaluate cartilage and joint health to help characterize the OA pathology and diagnose the disease [191-195]. The current gold-standard OA diagnostic tool is radiographs wherein bony abnormalities and joint space width (JSW) are graded to determine the presence and severity of OA [194, 196, 197]. Most commonly, the Kellgren-Lawrence scale is used and a grade of 2, defined as the presence of osteophytes and possible JSW narrowing, signifies OA [197]. Unfortunately, radiographs expose patients to ionizing radiation, do not allow for visualization of important related OA pathologies of the soft tissues, and only allows for a 2-dimensional view of the joint. As such, radiographs lack the ability to track pre-clinical OA features such as subtle changes in cartilage composition and morphology (i.e., surface fibrillation, fissures etc.). The ability to evaluate these initial soft-tissue abnormalities is crucial given that they precede structural features of OA that generally don't surface until late disease stages when significant degeneration is already established (i.e., overt cartilage loss) [198, 199].

Advanced imaging approaches like magnetic resonance imaging (MRI) offers significant advantages over standard radiographs, allowing for both compositional and structural evaluation of articular cartilage amongst other soft-tissue structures (i.e., menisci, ligaments etc.) [191, 199, 200]. MRI is currently the gold-standard for direct

evaluation of cartilage structure like thickness and volume [201]. Thus, MRI may have improved sensitivity to detect structural changes with OA compared to radiographs given that factors like meniscal extrusion, which confound traditional JSW measures measurements do not impact MRI-based thickness measures [201-203]. Composition of the cartilage ECM can also be assessed via MRI, most commonly using T1ρ and T2 relaxation times [200]. The use of compositional MRI metrics is valuable to evaluate early OA-related alterations to cartilage given that these markers are sensitive to changes in PG (T1ρ), water content and organization of the type II collagen network (T2 relaxation times) [199, 204-210]. As such, many consider MRI as a powerful imaging tool in OA given that it provides researchers and clinicians the ability to track OA throughout its entire disease process (i.e., from pre-clinical to end-stage OA) [192, 194, 198, 211]. Nonetheless, despite these numerous benefits, MRI scans are extremely costly, not widely accessible, and require long scan times that can be uncomfortable for patients which severely limit the overall practicality of using MRI routinely in standard clinical care.

Ultrasonography (US) is an additional imaging modality that can directly image soft tissue structures in the knee like cartilage, meniscus, amongst other structures (i.e., fat pads, bursa etc.). US continues to be recognized as a promising imaging tool for OA both in a diagnostic and disease monitoring role given its numerous benefits over traditional radiographs and more advanced modalities like MRI [211-213]. For example, US offers low operating costs, is extremely portable, does not subject patients to ionizing radiation while scan times can be performed bedside relatively quickly (i.e., approx. 5 minutes) [192, 193]. Therefore, the use of US imaging as a supplementary OA imaging tool may solve critical cost barriers and accessibility issues that prohibit the widespread and routine

monitoring of cartilage health in both the clinical and research settings. Furthermore, if US imaging is indeed capable of detecting early features of OA, this imaging tool could help better identify at-risk patients, facilitate more effective monitoring of disease progression and aid in evaluating the efficacy of interventions. As this dissertation will utilize US imaging to assess femoral cartilage outcomes after ACLR, the following sections will detail US-based assessment of articular cartilage, its use to evaluate articular cartilage outcomes after ACLR, as well as some technical aspects of US and image acquisition.

2.4.9 Ultrasonographic Assessments of Knee Articular Cartilage

US imaging has been established as an accurate, noninvasive tool able to visualize articular cartilage in numerous weightbearing and non-weightbearing joints. In the knee, a significant portion of the femoral trochlea, a region of cartilage encompassing the patellofemoral joint, can be easily visualized via US by placing the knee at or near maximal flexion. Qualitatively, US exhibits high specific and sensitivity to assessing trochlear cartilage lesions and semi-quantitative measures have been moderately correlated with histologic and arthroscopic gradings [214, 215]. Femoral trochlear cartilage structure (i.e., thickness) has also been routinely assessed quantitatively via US. US-based thickness measures have been validated against anatomic measures from cadavers, are repeatable (ICCs Range: 0.76-0.96) [216-220] and are strongly correlated to gold-standard MRI-based assessments of cartilage thickness ($\rho > 0.80$) [217, 218]. Tibiofemoral cartilage is not generally accessible to US imaging due to the inability of the transducer beam to penetrate bony structures, which limits some applicability of this imaging tool. However, it has become increasingly clear that OA in the patellofemoral

compartment (isolated and concurrent with tibiofemoral OA) is highly prevalent in both general and injured populations like those post-ACLR [94, 221-223]. Further patellofemoral OA may be a stronger source of OA symptoms than tibiofemoral OA [224, 225]. Thus, the relative low-cost and clinic accessibility of US may offer considerable clinical value as a diagnostic or disease monitoring tool to evaluate patellofemoral OA outcomes in at risk populations.

In addition to structural evaluation of cartilage, US imaging may be capable of evaluating cartilage composition, albeit indirectly. For example, it has been hypothesized that evaluating the *in vivo* change in cartilage thickness/deformation in response to loading bouts may serve as a useful surrogate of cartilage composition, given that the biomechanical properties of cartilage (i.e., compressive stiffness) are influenced by the composition of its ECM (i.e., PG, water, type II collagen) [219, 226-233]. Typically, MRI has been used to characterize cartilage deformational behavior in response to varying activities *in vivo* (i.e., walking, running etc.) [227-237]. However, inherent limitations of MRI such as long scan times, cost, and accessibility issues limit the utility of this modality for routine serial evaluation of cartilage deformation characteristics. As such, US-based assessments of cartilage deformation have become increasingly common in recent research given the devices portability and relatively quick acquisition times [219, 238-240]. Unfortunately, few studies have evaluated the sensitivity of assessing deformation via US to differentiate between individuals with and without pathology; data critical to help establish the overall sensitivity of US to detect OA features and strengthen its potential usefulness as a clinical imaging tool.

Overall, US may have value as an OA imaging tool both clinically and in the research setting where modalities like MRI may not be easily accessed or afforded. However, it is important to recognize some of the limitations inherent with US imaging. Compared to other imaging modalities (i.e., MRI, CT, X-ray etc.) US is operator dependent and thus, careful attention must be taken when acquiring images to limit image acquisition errors and optimize image repeatability. Despite this source of variability, previous work has shown generally good agreement between US operators and good-excellent intra-rater reliability for femoral cartilage thickness measures. Several technical factors can also impact the representation of soft-tissue structures on US images, such as probe frequency, angle of insonation, device focus position, and image gain [193, 241, 242]. For imaging deep structures (i.e., the hip joint), lower probe frequencies (e.g., 8-12MHz) are indicated so that the US beam can sufficiently travel the depth of the tissue of interest. Alternatively, the use of higher frequencies (i.e., 12 MHz or greater) can improve clarity when imaging superficial tissues like knee cartilage [243]. Insonation angle, defined as the angle of the ultrasound beam relative to the tissue/structure of interest, is also a vital component to consider when acquiring images. It is recommended that the ultrasound probe is placed orthogonal to the tissue of interest to limit refraction of the ultrasound beam [242]. Generally, misalignment in the insonation angle can lead to overestimation errors when calculating metrics like cartilage thickness [241, 242]. Lastly, device-specific factors like focus position and image gain (i.e., brightness) can also impact the overall clarity of the imaged tissue and should be adjusted according to the type of tissues being imaged (e.g., skeletal muscle, cartilage).

2.4.10 Ultrasonographic Assessments of Knee Articular Cartilage after ACLR

An increasing number of studies have utilized US imaging when aiming to evaluate trochlear cartilage characteristics in both healthy and clinical populations (ACLR, OA individuals) [212, 213, 219, 238, 239, 244-252]. After ACLR, trochlear cartilage thickness assessed via US has been shown to be both thinner, thicker, or not different in ACLR knees compared to contralateral and/or healthy control knees [220, 247]. In cohorts around 3-5 years post-operatively, Pamukoff et al., observed 25% thinner cartilage in ACLR relative to contralateral knees whereas Harkey et al., showed that ACLR knees had 10% thicker cartilage compared to the contralateral. At more acute post-operative phases, Lisee et al., assessed trochlear cartilage thickness bilaterally at 4- and 6-months after ACLR, but observed no differences between limbs or changes in thickness between time-points [248]. Interestingly, authors noticed that a portion of patients in their cohort exhibited opposite changes in cartilage between time-points that exceeded minimal detectable change [248]. For example, 45% of participants exhibited thickening in at least one trochlear region while 35% of participants exhibited thinning. Although it is not clear from these data why certain patients exhibited longitudinal thickening as opposed to thinning, increasing evidence has highlighted the heterogeneity of cartilage structural changes with OA [253]. As such, it is plausible these observations of unique patterns of trochlear cartilage changes after ACLR both signify post-traumatic OA-related pathology.

To date, few investigations have utilized US imaging to evaluate trochlear cartilage outcomes in ACLR populations and results are largely conflicting. It is difficult to contextualize these findings with observations from studies using MRI, however, as most have focused on tibiofemoral cartilage outcomes. Of the few studies directly evaluating

trochlear regions after ACLR, findings generally align with results seen with US and are similarly inconclusive [254-256]. For example, cross-sectional observations have shown that trochlear cartilage was not different in ACLR knees relative to uninjured controls [254]. Conversely, Frobell et al., observed that rapid thinning occurs in the femoral trochlea in the first two years after ACLR whereas central femur cartilage displays thickening [257]. Interestingly, recent work by Pius et al., observed that regional thickening and thinning can occur throughout subregions of the femoral trochlea over a four-year follow up period [95]. Authors also observed that females exhibited larger areas of thickening in the femoral trochlea and throughout the entire cohort, cartilage thickening was more common in all cartilage regions than thinning. As such, there is no consensus on whether cartilage in ACLR knees follows similar patterns of thickening or thinning, but it appears this may be region specific (i.e., patellofemoral vs. tibiofemoral).

Overall, paucity of data has sufficiently tracked cartilage structural changes following ACLR, particularly in the patellofemoral joint. Future work is thus needed to better characterize the trajectories of patellofemoral cartilage structural changes after ACLR which are currently poorly understood. It is likely the limited evidence on cartilage structural changes following ACLR can be partly attributed to the cost-prohibitive nature of MRI. Utilizing low-cost imaging approaches like US may have some merit to solve this critical cost-barrier of MRI and may permit more studies to serially track trochlear cartilage outcomes in research or clinical settings in populations with ACLR. However, additional studies, both longitudinal and cross-sectional, are needed to confirm the usefulness of US to evaluate trochlear cartilage outcomes after ACLR; data that is currently scarce.

While US-evaluations of trochlear cartilage thickness after ACLR have become more common, no studies to date have evaluated indirect measures of cartilage composition using US in ACLR populations (i.e., assessments of cartilage deformation behavior). Strong evidence suggests alterations in cartilage composition (e.g., increased T1ρ and T2 times) occur rapidly in ACLR knees, surfacing as early as 6-months post-operatively [258-260]. Previous work has also linked higher T1ρ and T2 times to reductions in cartilage stiffness which may manifest as increased deformation in response to walking [226, 261]. Thus, it is plausible ACLR knees may exhibit increased deformation relative to their uninjured limb which may occur prior to any structural changes like thinning or thickening. Although evaluating cartilage thickness is a simple metric important for tracking OA progression, structural assessments are often not sensitive to detecting initial disease stages which are generally characterized by alterations in the content and organization of macromolecules within the ECM. As such, future work should consider evaluating if US can indirectly detect changes in cartilage deformation behavior as this may be a more sensitive metric to detect the early deleterious alterations in cartilage health that ensues after ACLR.

2.5 Risk Factors for Post-traumatic Osteoarthritis after ACL-Injury and Surgery

Currently, the direct cause of post-traumatic OA after ACL injury is not well understood. However, a variety of factors are thought to contribute to this heightened risk for disease development such as demographic factors (i.e., age, sex, BMI), those occurring during the initial injury (i.e., concomitant injuries to adjacent structures) and those developing secondarily during the recovery process (i.e., quadriceps muscle weakness, abnormal gait biomechanics, weight gain etc.). Meniscal, chondral and

subchondral bone lesions are frequently observed with acute ACL-rupture and have been associated with poorer patient outcomes and in some cases increased risk for post-traumatic OA [262, 263]. Injury to the meniscus for instance may influence future post-traumatic OA risk by directly altering the load-distribution and contact patterns within the knee. Importantly, these mechanical alterations in the knee may be independent of those that occur with ACL-injury and persist after ACLR (i.e., change in normal contact locations) [264, 265]. Although the link between chondral and subchondral injuries and post-traumatic OA is not entirely clear, the areas surrounding subchondral bone damage and chondral lesions show significant chondrocyte degeneration, osteocyte necrosis, and PG loss which may impact the load-bearing capacity of the surrounding tissues [266-268]. Given that these concomitant injuries present uniquely with each individual injury, treatment options may be limited to surgical management (i.e., meniscectomy or repair) and may not be modifiable (i.e., concomitant injury severity). Conversely, risk factors for post-traumatic OA that develop secondary to injury and/or surgery (i.e., muscle weakness, gait abnormalities, weight gain) may offers researchers and clinicians the opportunity to implement preventative strategies almost immediately following clinical presentation. As such, if rehabilitation can be optimized to effectively ameliorate these sequalae, it is possible that an individual's risk for developing post-traumatic OA can be drastically reduced. While it is recognized that a host of risk factors may ultimately contribute to post-traumatic OA after ACL injury, the following section will be focused on detailing modifiable risk factors relevant to this dissertation - gait dysfunction and high body mass index.

2.5.1 Gait Biomechanical Abnormalities after ACL-Injury and Reconstruction

It is well known that habitual, cyclic mechanical loads are a potent stimulus necessary for maintaining cartilage homeostasis [6, 9]. As walking is the most common source of cyclic activity, a wealth of research has aimed at identifying potential impairments in lower-extremity gait patterns after ACL injury and ACLR as these changes may influence one's risk for post-traumatic OA. Anatomically, loss of the ACL results in abnormal arthrokinematics between the femoral and tibial articular surfaces wherein the tibia remains internally rotated with significantly increased anterior translation [35, 75, 269-271]. Such kinematic alterations have been shown to impact both tibiofemoral and patellofemoral articular contact points by shifting locations more posteriorly and laterally in the medial tibia [74, 75, 272] while shifting patellofemoral contacts more laterally on the femur [273, 274]. Reconstructive techniques aim to restore normal arthrokinematics but often fail to address rotational alterations as ACLR knees tend to display an external rotation offset during gait that does not appear to resolve with time, persisting for several years post-surgery [35-38].

Numerous gait alterations have been observed in those with ACLR during walking. For instance, previous authors have observed that individuals with ACLR walk with smaller ground reaction forces in the ACLR limb relative to contralateral and control limbs [15, 16, 275] and may walk with greater loading rates compared to uninjured populations [276]. Temporal-spatial characteristics have also been shown to be altered in the first months after surgery, as smaller steps and reduced stance times have been observed in ACLR limbs relative to the contralateral [277-279]. For example, substantial step-length asymmetries are present within 6-12 weeks post-ACLR, but some studies have shown

that patients are able to achieve similar step-lengths within 6-months of surgery [277, 280]. Nonetheless, it remains unclear how long these spatiotemporal alterations last after ACLR as few investigations have examined temporal-spatial characteristics post-ACLR.

Deficits in sagittal plane knee kinematics and kinetics are also common after ACLR particularly in early phases post-operatively. In the first year after surgery, a wealth of studies have shown that ACLR individuals walk with reduced external knee flexion moments (KFM), increased knee flexion angle (KFA) at heel strike, and reduced knee flexion excursions in the first 50% of stance compared to contralateral and control limbs [10, 16, 19, 20, 64, 281-285]. Persistent alterations in sagittal plane kinematics and kinetics have been thought to contribute to post-traumatic OA risk after ACLR. For example, ACLR knees often display increased KFA at heel-strike, which may shift the contact location in the ACLR-knee towards a more posterior (and thinner) region of the femoral cartilage [5, 286]. As a result, cartilage regions unaccustomed to the magnitude and frequency of loads may become overloaded while previously loaded regions may become underloaded [74]. Coupled with evidence of truncated knee flexion and extension excursions in the ACLR-knee [64, 282, 283, 287], it is also possible that load distribution within the joint becomes more concentrated to smaller areas of cartilage, which may impact contact pressures and influence tissue breakdown.

Although the extent of altered sagittal plane knee mechanics have been well defined in individuals with ACLR, less data is available detailing deficits in frontal plane gait mechanics. During gait, knee joint loads are disproportionately greater in the medial compared to the lateral tibiofemoral compartment and thus, it is not surprising that tibiofemoral OA is substantially more common in the medial knee [12, 288, 289].

Therefore, evaluating if medial knee loading characteristics are impacted after ACLR may help better understand the role of altered gait on post-traumatic OA risk in this population. The external knee adduction moment (KAM) is a widely used surrogate of medial knee loading, reflecting the relative load distribution between medial and lateral knee compartments [290-296]. A higher KAM is generally interpreted as higher medial knee compartment joint loading. Butler et al., produced some of the earliest findings of altered frontal plane knee mechanics in this population, as the authors observed that at 5 years post-surgery, individuals with ACLR walked with an increased KAM compared to healthy controls. More recent data in a cohort 10-years post-ACLR also showed an elevated KAM in ACLR-knees versus the contralateral limb [20]. Conversely, studies in earlier time periods post-surgery suggest ACLR individuals may walk with reduced KAM's in the ACLR relative to contralateral knees and controls [69, 275, 297-299]. Several studies have also employed EMG-driven musculoskeletal models to better approximate medial knee loads after ACLR and have produced similar findings; medial contact forces are lower in ACLR-knees compared to the contralateral knee, in some instances for upwards of two to three years after surgery [58, 298, 300, 301]. As such, these findings in conjunction with consistent observations of reduced sagittal plane knee moments, at least in the early phases after ACLR (i.e., within the first year of surgery), may suggest that joint underloading in the involved limb could precipitate early cartilage deterioration in the injured knee.

While it has been established that early gait changes after ACLR are generally characterized by reduced loading in the reconstructed limb, gait alterations at later time-points after surgery (i.e., >24 months post-ACLR) are less clear. For example, previous

studies have shown kinetic outcomes (i.e., vGRF, KFM, KAM) are greater, lesser, or not different between limbs [19, 67, 69, 302-304]. Several investigations have shown that individuals with ACLR may normalize sagittal plane angles and joint excursions between 12-24 months post-surgery [58, 282, 297, 304]. However, these results must be interpreted with caution given that comparisons to the uninvolved limb may lead to an overestimation of gait recovery [16]. Thus, as few studies have serially evaluated gait mechanics throughout and beyond rehabilitation, our understanding of how individuals with ACLR evolve their gait over time is limited in part because most evaluations are cross-sectional or do not include comparisons to control groups. As a result, it is unclear if gait rehabilitation targets may differ depending on the time phase post-ACLR. Given that articular cartilage may respond differently to these altered joint mechanics depending on OA status (i.e., pre-clinical, early or end-stage OA), a clear understanding of how gait alterations evolve depending on time frame post-surgery and how these changes may influence cartilage health would help facilitate the development and individualization of gait intervention strategies.

2.5.2 Altered Gait Mechanics Influence Knee Cartilage Health after ACLR:

Collectively, ACLR leads to substantial and persistent changes in the joint mechanical environment that lead to the early onset of post-traumatic OA. These abnormal mechanics do not appear to fully resolve with time and traditional rehabilitation efforts are insufficient to adequately restore normal gait in a timely manner (i.e., prior to return-to-sport). Many studies have observed that compositional alterations in articular cartilage seem to progress rapidly in the first years after ACLR, presenting as elevated T1ρ and T2 relaxation times in both tibiofemoral and patellofemoral cartilage relative to

the uninvolved limb [260, 305-308]. Therefore, understanding if these early gait abnormalities are implicated in the initial degenerative signs of post-traumatic OA is critical given that walking mechanics are a modifiable factor that if resolved, may help slow or reverse disease progression.

Some of the first longitudinal evidence linking gait and post-traumatic OA outcomes was reported by Wellsandt and colleagues who assessed differences between knee loading characteristics in ACLR patients who developed post-traumatic OA at five years compared to those who did not [298]. Authors found the post-traumatic OA group walked with lesser medial knee contact forces, KAM, and KFM in the ACLR relative to the contralateral knee at six months post-ACLR. Conversely, those who did not develop post-traumatic OA achieved symmetrical contact forces and moments at six months post-ACLR. Authors speculated early knee unloading post-ACLR may have contributed to early onset post-traumatic OA. Unfortunately, the post-traumatic OA group in this cohort was small (n=9) and the use of radiographs prohibits the ability to link knee loading after ACLR to early compositional alterations preceding structural loss [191, 200]. More recent cross-sectional evidence has provided support for the link between joint underloading and early cartilage degeneration after ACLR. Pfeiffer et al., demonstrated lesser KAM and vertical ground reaction force (GRF) loading rates were linked to reduced proteoglycan content assessed via T1ρ MRI in patients between six to 12 months after ACLR [258].

These findings provide initial evidence suggesting early underloading of the ACLR-knee may promote changes in knee joint cartilage. While gait deficits at later time points after ACLR are slightly less clear, it is consistently shown GRF, KFM, KAM, and medial contact forces are reduced in the ACLR relative to both contralateral and control limbs for

at least the first year post-ACLR [10, 16, 64, 298]. Taken together, reduced knee loading likely alters cartilage homeostasis by evoking a “deconditioning” tissue response (i.e. decreased proteoglycan synthesis) as cartilage experiences lesser mechanical stimuli [73, 158]. Gradual proteoglycan depletion can have drastic impact on cartilage load-bearing properties after ACLR by decreasing tissue resiliency and increasing permeability of the ECM [105-107, 134]. Consequently, the cartilage solid matrix may experience elevated stresses as the main load-bearing mechanism facilitated by proteoglycan becomes impaired [105, 107]. However, such early changes may not reflect permanent reductions in tissue properties as proteoglycan loss, at least induced via joint immobilization, is partly reversible [159]. Therefore, if gait impairments can be corrected early on during post-operative rehabilitation, early degenerative changes after ACLR may be diminished or ameliorated.

Altered gait mechanics have long been theorized as a driver of post-traumatic OA development ACLR, and recent animal model data has provided a mechanistic link between aberrant sagittal plane gait characteristics and disease development [309]. However, it remains poorly understood how gait mechanics influence cartilage outcomes (i.e., compositional/thickness changes) in humans after ACLR as evidence mostly conflicts; particularly across the disease time-course (i.e., from pre- to post-OA diagnosis). For instance, *in vivo* data from humans have shown that both excessive and insufficient loading have been linked with deleterious cartilage outcomes. [246, 258, 298, 310-312]. Unfortunately, this lack of clear findings severely limits the ability of researchers and clinicians to design and optimize gait retraining strategies because there is no consensus if joint underloading, overloading or kinematic changes are main drivers of

post-traumatic OA after ACLR. As such, further work is needed to clarify which gait variables are most strongly implicated in post-traumatic OA after ACLR and if increasing or decreasing load early during rehabilitation is beneficial.

2.5.3 High Body Mass Index after ACL-Injury and ACLR Prevalence and Associations with Post-Traumatic OA Risk

High body mass index (i.e., overweight BMI 25.0-29.9 kg/m^2 or obese BMI$\geq$ 30.0 kg/m^2 individuals) is a continually growing national health problem as the number of persons who are overweight or obese has tripled in the United States over the last four decades [313]. In those undergoing ACLR, recent estimates have shown that nearly 40% and 25% of patients were classified as either overweight (BMI 25.0-29.9 kg/m^2) or obese (BMI$\geq$ 30.0 kg/m^2), respectively [314, 315]. Given that overweight and obesity rates are expected to increase in the next decade, it is reasonable that the number of patients presenting with injury will similarly increase. Overall, the observed increased prevalence of high BMI in the both the general population and in those with ACL injury presents is problematic given that high BMI is considered a major risk factor for OA [316]. High BMI irrespective of joint injury increases the odds of developing the disorder 2-4x [317, 318]. In patients with ACLR, previous work has also linked high BMI with increased odds of developing post-traumatic OA [12, 13], and high BMI was one of the strongest predictors of post-traumatic OA incidence at 5 years post-surgery [14]. As such, research understanding the potentially negative consequences of high BMI concurrent with ACL injury is imperative as this subset of patients may present with unique challenges throughout the ACL-recovery process and be at risk for a more accelerated onset of post-traumatic OA relative to their normal BMI counterparts.

Independent of ACL-injury, extensive work has shown that gait is altered in individuals with high BMI which may contribute to OA [291, 319-324]. Most commonly, these individuals exhibit reduced habitual walking speeds, and walk with increased double limb-support times, wider step widths and greater absolute joint moments, knee contact forces, and loading rates [319-321, 323, 325, 326]. Increased joint loads and in particular, medial compartment loading (i.e., KAM) have been shown to predict tibial cartilage loss in knee OA individuals with high BMI [292]. Further, some authors have observed interactions between high BMI and gait metrics like the KAM. Astephen-Wilson et al., observed that radiographic disease severity was better predicted by the combination of BMI and the KAM compared to either factor alone [327]. Brisson et al., also observed that in healthy individuals the relationship between the KAM and cartilage thickness differed between high and low BMI groups [292]. Thus, it has been hypothesized that BMI may have a moderating role on gait markers relevant to OA (i.e., the KAM) and the detrimental effect of increased joint loads may be exacerbated in those with higher BMI.

Altered gait biomechanics are a common sequalae after ACLR [10, 11]. As highlighted in previous sections, individuals with ACLR may adopt a joint underloading strategy early after surgery, and joint contact locations may be shifted to regions of cartilage that may not be habituated to the frequency and magnitude of joint loads [15, 16, 20, 328, 329]. These relatively abrupt changes in the mechanical load environment of the knee have been posited to predispose the joint to the rapid onset of post-traumatic OA [5, 74, 298]. Given that ACLR and high BMI independently impact knee loading characteristics, it can be hypothesized that the combination of high BMI and ACLR may

lead to significantly worse gait impairments. While direct support for this hypothesis is scarce, recent data has aimed to better understand the associations between high BMI and gait mechanics in persons with ACLR. For example, Pamukoff et al., observed that obese individuals with ACLR walked with disproportionately larger increased external KAMs and vertical GRFs in the involved limb while Davis-Wilson et al., showed those with high BMI after ACLR walked slower compared to their uninjured high BMI counterparts [330, 331]. An increased KAM in high BMI individuals with ACLR coupled with the known alterations in normal knee contact locations resulting from injury may contribute to the increased risk for post-traumatic OA in this subset of patients. For instance, the excess loads attributed to high BMI may exacerbate the negative effects of altered knee contact patterns in ACLR knees, leading to a more accelerated degenerative pathway. In fact, data from animal models have shown that the combination of high BMI and joint injury lead to more severe OA that progressed more rapidly than either factor in isolation [332]. Nonetheless, current evidence on the effects of high BMI after ACLR is unclear and conflicting. Thus, future work is needed to confirm or refute these findings and further, there is a critical need to understand if markers of early post-traumatic OA (i.e., decline in cartilage composition) may present early in high BMI individuals with ACLR relative to their normal BMI counterparts.

Although considerable studies have implicated high BMI as a strong risk factor for OA in multiple joints, the mechanisms underlying obesity-induced OA are complex and have not been fully elucidated. Originally, it was thought that the increased joint loads associated with high body mass was the primary driver of OA in these individuals given consistent findings of increased joint moments and contact forces observed when

comparing high and low BMI groups. However, it has become increasingly understood that altered biomechanics are not solely responsible for obesity-induced OA and the influence of metabolic factors such as inflammation play a major role in OA pathogenesis [101, 102, 169, 170, 333, 334]. For instance, high BMI has severe metabolic consequences, promoting a pro-inflammatory environment through a chronic elevation of systemic, low-grade inflammation [8, 316, 335, 336]. Increased adiposity associated with high BMI leads to heightened production of both adipocytokine's (i.e., leptin, adiponectin etc.) and other pro-inflammatory mediators (i.e., TNF-α, IL-6 etc.,) that are present systemically and may be produced locally in the joint (i.e., infrapatellar fat pad)[168, 170, 337]. Previous work has shown that adipocytokines can directly and synergistically facilitate the upregulation of degradative enzymes known to disrupt normal cartilage turnover and accelerate articular cartilage catabolism [168-170]. As such, many authors consider the increased risk of OA in those with high BMI is likely attributed to this unique combination of altered biomechanical loading in the presence of a robust, pro-inflammatory environment.

Overall, the interaction between high BMI and ACLR likely influences multiple pathways at which cartilage degeneration is known to be facilitated. For example, it has been well documented that a robust transient inflammatory response accompanies acute ACL-rupture leading to increased concentrations of both pro- and anti-inflammatory cytokines in synovial fluid [305, 338-341]. With surgery, these inflammatory mediators may be reinvigorated, and the presence of this altered inflammatory environment may perpetuate rapid breakdown of the cartilage ECM [338]. Given that elevated inflammation is characteristic of high BMI, joint trauma in those with high BMI may induce a

disproportionately greater inflammatory response at the joint level. Thus, it is plausible post-traumatic OA may develop at an accelerated rate in patients undergoing ACLR with a high BMI. As post-traumatic OA ensuing after ACL-injury is an already rapidly progressing OA phenotype, this subset of patients may require significantly more aggressive rehabilitation and preventative efforts. Nonetheless, the dearth of work directly investigating these unique risk factors in tandem is a substantial barrier to identifying avenues for intervention and ultimately developing individualized treatment programs. Therefore, it is important that future research efforts aim at characterizing the potentially unique modifiable variables that may present in high BMI individuals with ACLR in order to improve our understanding of avenues to mitigate post-traumatic OA in this patient population.

2.6 Gait Intervention Strategies to Improve Knee Loading after ACLR

Gait retraining (i.e., a gait-specific intervention approach) involves targeting specific movement patterns through different modes of feedback to facilitate the adoption of new or the restoration of pre-pathological gait patterns. The goals of modifying gait via retraining vary by population but generally are aimed at reducing injury and/or fall risks, promoting improvements in mobility, and minimizing the risk of disease development or progression (i.e., knee OA). Previous work has shown gait retraining is effective at modifying knee mechanics and reducing injury risks in runners [342, 343], improving gait asymmetries and walking speeds post-stroke [344-349] and is capable of improving pain and knee loading characteristics related to disease progression in populations with knee OA [350-352]. Given that those with ACLR typically walk with marked gait asymmetries which are linked with post-traumatic OA risk [10, 11, 20], the inclusion of gait retraining

during post-operative rehabilitation may be integral to remediate abnormal knee mechanics and impede the development of post-traumatic OA. Unfortunately, few gait retraining strategies have been identified to ameliorate these abnormal knee patterns and seldom are included in standard-of-care post-operative ACLR programs [21, 22]. The lack of direct focus on restoring proper gait mechanics in current rehabilitation paradigms likely contributes to the suboptimal long-term post-traumatic OA outcomes that plague those undergoing ACLR. Therefore, an opportunity exists for future research to help identify, and develop strategies capable of facilitating the restoration of optimal gait mechanics after ACLR.

To date, most studies assessing potential gait retraining strategies in ACL populations have been generally limited to acute investigations (i.e., within session changes) with only a few studies including longitudinal follow-ups. For example, Moran et al., evaluated the efficacy of providing functional electrical stimulation to the quadriceps on gait and strength outcomes in the first month post-operatively [353]. The authors observed that after 3 weeks of training (10 min session, 3x/week), those randomized into the functional electrical stimulation group exhibited more symmetrical knee extensor strength than the typical neuromuscular electrical stimulation protocol. However, gait asymmetries (i.e., single-limb stance time) did not appear to be affected by this intervention approach. Authors reasoned that the application of electrical stimulation in a more functional manner could facilitate greater strength recovery after ACLR but whether this translates to improved gait mechanics is not clear because only temporal measures (i.e., stance times) were assessed in this investigation.

More recently, two investigations utilized a similar training approach (i.e., functional strength training) via the development of novel resistive knee braces [354, 355]. Though the type and scalability of resistance produced by the braces differed, both studies revealed positive gait adaptations in response to approximately 6-8 weeks of training with the resistive devices. For example, Rocchi et al., observed that compared to groups wearing a traditional brace, patients using the resistive brace walked with greater posterior GRF symmetry post-training, but knee extensor strength outcomes were similar between groups [354]. Brown et al., reported in a case study that 8-weeks of training with a novel bi-directional resistive brace led to improvements in ACLR limb knee flexion moments and knee flexion angles in midstance, resembling the mechanics of the contralateral uninjured limb [355]. Overall, these studies suggest that compared to traditional resistance/strengthening exercises, which are generally conducted in a non-specific manner (i.e., seated knee extensions), task-specific approaches may lead to a more optimal transfer of benefits [356-358]. Therefore, supplementing traditional strengthening in post-operative care with functional training devices (i.e., braces, neuromuscular electrical stimulation) may be an avenue to enhance the recovery of gait post-ACLR.

In the studies above, gait rehabilitation was approached by investigators using task-specific strengthening or neuromuscular training approaches during walking tasks to facilitate transfer of training. Task-specificity or task-specific practice is considered an important component of rehabilitation or training programs to improve motor learning, retention and to optimize changes in intended outcomes [357, 359]. Gait patterns can also be directly targeted via retraining approaches following similar specificity principles.

For example, recent efforts have demonstrated that peak sagittal plane knee flexion moments and knee excursions can be targeted by providing real-time biofeedback of the vertical GRF in those with ACLR [360, 361]. For instance, Luc-Harkey et al., and Evans-Pickett et al., observed that cueing an 5% increase in the first peak vertical GRF can elicit increases in the peak KFM and in knee flexion excursions during both weight-acceptance and mid-late stance [360, 361]. From these data, authors posited that cueing increased vertical GRFs may be a feasible approach to facilitate a less-stiff knee strategy and promote increased loading in the ACLR limb. Promoting greater knee flexion excursion could lead to a more effective distribution of joint loads across the articulating surface during the stance phase which may help preserve cartilage health after ACLR. Further, increased loading earlier after ACLR may provide an important stimulus to maintain cartilage health given the previous links between reduced loading characteristics and poor cartilage composition and biochemical markers of cartilage degradation [258, 362, 363].

While real-time biofeedback paradigms like cueing vertical GRF may be effective at changing relevant knee biomechanical variables, a current drawback of this approach and others (i.e., split-belt paradigms) [345, 349, 364, 365] is the need for expensive devices to provide feedback cues (i.e., force-sensing treadmill) which severely limits clinical utility. Ideally, gait retraining strategies that are low-cost would have the highest potential for widespread implementation in the clinical setting and thus have the greatest impact on patient outcomes. Recently, Milner et al., demonstrated that cueing changes in spatiotemporal parameters like step-length can effectively reduce or increase tibiofemoral contact forces during walking and may be an attractive gait retraining option with high

clinical applicability [366]. For example, step-lengths can be easily modified when walking on a standard treadmill using minimal additional equipment (only a metronome is required) [23, 342, 343, 367]. At constrained walking speeds, increasing step length can be achieved by cueing a slower cadence whereas smaller steps can be promoted by cueing a higher cadence. Importantly, previous work in healthy cohorts have shown that acutely manipulating step-lengths can directly affect knee biomechanical variables such as knee joint contact forces, knee sagittal plane moments and joint excursions [342, 366, 368, 369]. For example, generally, taking larger steps tends to increase joint loads (moments and contact forces) while facilitating increased knee flexion angles and excursions during stance phase [342, 368, 369]. Conversely, smaller steps may induce the opposite effect on gait outcomes [23, 342, 343].

In ACLR populations, Bowersock et al., showed that acutely modifying step-lengths during running is effective at acutely decreasing several relevant knee biomechanical outcomes in individuals approximately 4 years post-ACLR [23]. In this study, participants running at a 5% higher cadence (regulated via metronome) achieved step length reductions of approximately 6% compared to self-selected conditions. Concurrently, patellofemoral, and tibiofemoral joint contact forces also were reduced between 4-10% in the increased cadence condition compared to normal walking. While authors only included reduced step length conditions (105/110% self-selected cadence), these findings coupled with data from healthy cohorts [342, 366, 368, 369] may provide support for the use of step-length manipulations to acutely increase (or decrease) knee joint loading and kinematic outcomes (i.e., angles/excursions) after ACLR.

To our knowledge, only one study has evaluated the longitudinal effects of step/stride frequency as a gait biofeedback target in those with ACLR; however, stride rather than step frequency was used a biofeedback target. Decker et al., observed that providing metronome cues at a target stride frequency predicted via a force-driven harmonic oscillator model for 6-weeks (i.e., 3x/week, 20-30 minutes/session) could improve gait patterns in a group of patients with ACLR [17]. Specifically, the experimental group receiving stride frequency biofeedback experienced increases in stride length and stride frequency, and greater knee flexion angles and excursions after the 6-week training block (approximately 12 weeks post-ACLR). The force-driven harmonic oscillator model used by authors is predictive of preferred stride frequencies in healthy adults using anthropometric measures of the legs [370]. Given that ACLR patients walked at slower preferred stride frequencies compared to predicted values from the model, authors posited that training at a higher stride frequency was a sufficient stimulus to promote gait recovery compared to control groups who performed the walking program without any biofeedback cues. However, gait training in this study was performed in free-living over-ground conditions. If walking speeds are not constrained, step- or stride-frequency cues may lead to differential gait strategies to match the prescribed frequency (i.e., increased speed or cadence or both). Thus, it may be beneficial to understand how those with ACLR adapt to metronome biofeedback cues in a more constrained environment (i.e., fixed-speed walking).

Overall, targeting step-length to retrain gait may have substantial clinical value. Step length modifications can be performed easily in the clinic during treadmill training using freely available smartphone applications (metronome for audible feedback). This is

beneficial as it would allow clinicians the ability to iteratively track and adjust feedback targets as patients progress throughout rehabilitation. However, future work is needed to understand the biomechanical implications of manipulating step-lengths on walking biomechanics after ACLR. Further, understanding how cueing increased step-lengths via treadmill training transfers to gait mechanics over-ground or how lasting these gait changes may help better understand the potential usefulness of this gait retraining strategy to improve gait during rehabilitation.

Chapter 3 Associations between Body Composition, Gait Mechanics, and Ultrasonographic Measures of Femoral Trochlear Cartilage in Individuals with ACLR

3.1 Abstract:

Purpose: High body mass index (BMI) is a strong predictor of post-traumatic osteoarthritis (OA) risk after anterior cruciate ligament reconstruction (ACLR). Altered walking mechanics are independently affected by BMI and ACLR and are thought to influence OA risk. Yet, evidence directly assessing the impact of high BMI on walking patterns or cartilage outcomes after ACLR are limited. Here, we evaluated if high BMI moderates associations between gait and cartilage outcomes in individuals with ACLR.

Methods: Treadmill walking biomechanics were evaluated in forty normal BMI and twenty-four high BMI participants with ACLR at self-selected speeds. Normalized and absolute peak and cumulative loads were extracted for the peak knee flexion and adduction moment (KFM, and KAM) and vertical ground reaction force (GRF). Medial and lateral femoral cartilage thickness and thickness distributions (medial: lateral ratios) were assessed via ultrasound imaging.

Results: Those with ACLR and high BMI walked with reduced normalized peak vertical GRFs, and greater absolute peak and cumulative load outcomes compared to normal BMI individuals with ACLR. Those with ACLR and high BMI also exhibited *thinner* cartilage and greater medial: lateral ratios in the ACLR limb compared to their

contralateral limb whereas normal BMI individuals with ACLR exhibited *thicker* ACLR limb cartilage. Lastly, greater peak KAM and KAM cumulative load were associated with thicker lateral cartilage and lesser medial: lateral thickness ratios, but only in the high BMI group.

Conclusion: Having a high BMI along with an ACLR appears to lead to unique cartilage structural changes and impacts associations between loading outcomes and cartilage thickness in ACLR knees. Those with high BMI after ACLR may require different therapeutic strategies to optimize joint health in this subset of patients.

3.2 Introduction:

Individuals with anterior cruciate ligament reconstruction (ACLR) are at a dramatically elevated risk of developing post-traumatic osteoarthritis (OA), as between 30-50% of patients have OA within 10-20 years of surgery [2, 74, 80, 82]. Mounting evidence has demonstrated high body mass index (i.e., BMI ≥ 25.0 kg/m^2) is one of the strongest predictors of post-traumatic OA after ACLR [12-14]. High BMI is hazardous for articular cartilage health as increased mass and adiposity are linked to aberrant knee loading and elevated pro-inflammatory mediators (i.e., TNF-α, IL-6 etc.) that promote cartilage breakdown [8, 168-170, 320, 334]. Indeed, high BMI patients who undergo ACLR exhibit a higher risk of worsening patellofemoral OA features at 5-year follow-up than normal BMI patients who undergo ACLR,[221] providing evidence of a more accelerated development of post-traumatic OA after ACLR in this patient subset. Despite these compelling findings, few studies have directly evaluated how high BMI impacts relevant functional (i.e., walking mechanics, strength etc.) or cartilage outcomes after ACLR [371-374] and thus, fundamental gaps exist in our overall understanding of the factors contributing to the disproportionately higher risk of post-traumatic OA in this patient subset.

Knee joint mechanics during walking are widely considered a key regulator of articular cartilage health given the tissue's propensity to adapt its structure and composition in response to mechanical stimuli. Independently, those with ACLR or individuals with high BMI display altered walking mechanics, which are thought to strongly contribute to the elevated risk of knee OA observed in these populations [10, 11, 16, 20, 64, 309, 320, 321, 323, 325]. More recently, evidence has shown that patients with high

BMI who undergo ACLR exhibit slower strength recovery and disproportionately poorer walking mechanics than their normal BMI counterparts [13, 330, 372, 375] – data that may suggest this subset of patients exhibit unique post-operative recovery trajectories. For example, Pamukoff et al., observed that high BMI patients with ACLR exhibited larger knee adduction and smaller knee flexion moments (KAM and KFM, respectively) compared to both normal BMI patients with ACLR and uninjured control groups (both normal and high BMI) [376]. Irrespective of ACLR, those with high BMI already walk with elevated absolute peak joint loads compared to normal BMI individuals, even despite walking at preferentially slower speeds [322, 323, 325]. Reduced walking speeds are potentially hazardous for cartilage health independent of peak load magnitudes as slower walking increase the cumulative load demand about the knee and also promotes less dynamic (i.e., flat, or "static") loading profiles – factors that blunt cartilage matrix synthesis and promote tissue catabolism [6, 147]. Nonetheless, evidence depicting the effects of BMI on gait outcomes post-ACLR remains scarce, and it is unclear if cumulative load outcomes are also disproportionately impacted in high BMI ACLR patients – knowledge that may improve our understanding of the potentially unique biomechanical alterations apparent in this patient subset.

Typically, evidence has shown that higher magnitudes of joint loading during walking (both peak and cumulative loads) are positively associated with favorable articular cartilage properties such as increased tissue thickness and improved matrix qualities (i.e., denser type II collagen matrix, increased proteoglycans) [5, 155, 156, 377]. Conversely, immobilization models show that cartilage rapidly atrophies and undergoes compositional changes that mimic early OA [107]. Thus, these data together suggest that

habitual activity is a necessity to maintain cartilage homeostasis and may contribute to cartilage adaptation. In the acute phases of ACLR, promising data suggests that cartilage appears to maintain this normal positive/adaptive response to loading as those who walk with greater limb or knee loads magnitudes (i.e., GRF, knee moments) exhibit improved biomarkers of cartilage health (i.e., lower T1ρ/T2 relaxation times, reduced cartilage turnover biomarkers etc.) [258, 312, 362, 378-380]. Nonetheless, it remains unclear if this positive association between loading characteristics and cartilage health persists in more chronic time-periods after surgery. It is reasonable that a differential relationship could be expected between normal and high BMI ACLR patients given previous works in healthy populations [252, 291]. Understanding how BMI may influence associations between gait outcomes and cartilage health after ACLR is paramount as it is possible the time course of post-traumatic OA differs between these subsets of ACLR individuals, necessitating unique intervention strategies.

Currently, magnetic resonance imaging (MRI) is considered the gold-standard imaging biomarker to assess soft-tissue changes associated with OA [191, 192, 211, 257, 381, 382]. While MRI offers the ability to assess cartilage morphology throughout the entire joint, costs of imaging are substantial, and its use in standard clinical practice is limited. Alternatively, ultrasonography (US) has become more frequently utilized as an OA imaging modality given its low-cost and availability in most clinical settings [156, 193, 212, 220, 243-246, 249]. US is also considered to be a tool that can provide a valid and reliable assessment of femoral trochlear cartilage within the patellofemoral joint [244, 245], a region highly susceptible to OA-related degeneration within the first several years post-op after ACLR (i.e., thinning, compositional alterations, cartilage defects etc.) [221,

255-257]. Data from the Osteoarthritis Initiative has also suggested high BMI may be more preferentially associated with patellofemoral OA-features than tibiofemoral features [383]; substantiating findings of higher patellofemoral OA risks in overweight and obese patients after ACLR [12-14]. Therefore, US may be useful to evaluate the effect of BMI on patellofemoral cartilage status after ACLR. Unfortunately, most studies evaluating the associations between BMI and patellofemoral cartilage after ACLR are limited to semiquantitative outcomes [93, 221] and have not concurrently assessed walking biomechanics. As such, our current understanding of how these two independent risk factors interact remains incomplete.

The purposes of this cross-sectional study were to 1) compare gait biomechanics and cartilage thickness between individuals with high and normal BMI after ACLR and 2) to evaluate the moderating effect of BMI on the associations between walking biomechanics, and US-based measures of femoral trochlear cartilage thickness (medial, lateral and medial:lateral thickness ratio). We hypothesized that those with high BMI (i.e., BMI > 27.0 kg/m^2) would exhibit smaller normalized peak KFM and vertical GRFs but larger KAM and cumulative knee load indices. We also hypothesized that BMI would moderate the association between knee loading mechanics and cartilage thickness in the ACLR limb, wherein a positive association would be observed between loading and cartilage outcomes (e.g., higher KFM/GRF/KAM linked with thicker cartilage), but this relationship would only be present in the normal BMI group. Lastly, we hypothesized that high BMI would be associated with thinner medial and lateral femoral trochlear cartilage. For exploratory analyses, we also assessed body composition to better understand how

BMI and poor body composition (i.e., higher body fat) may be impacting gait and cartilage outcomes in these individuals.

3.3 Methods:

Data from this study are a part of a larger cross-sectional investigation consisting of several testing sessions. Gait and ultrasound data presented herein are from the first testing session while DEXA results are from the second testing session in which additional ultrasound procedures were also performed (not included here). The order of sessions was not randomized.

Participant Recruitment and Sample Size

We powered our primary analyses based on our primary outcome (KFM) and effect sizes obtained from previous work comparing the KFM between BMI groups in those with ACLR (f^2 = 0.22) [322]. We estimated a minimum of 24 individuals per group would be necessary to achieve 80% power (β=0.20, α=0.05) while accounting for predictor variables (sex, BMI). In total, we recruited sixty-four individuals with an ACLR who were categorized into normal BMI (N=40; BMI < 27.0 kg/m^2) and high BMI groups (N=24; BMI ≥ 27.0 kg/m^2). A BMI of 27.0 was chosen as our delineator of high BMI groups as this cut-off has been associated with a greater incidence and progression of knee osteoarthritis [384-387]. Participants were considered eligible for this study if they: 1) were between 14-45 years of age, 2) free from lower extremity injury in the past 6 months, 3) between 18-36 months post-ACLR, and 4) had no prior/current diagnosis of arthritis. Participants were excluded if they 1) had a history of a previous meniscal or ACL tear to either knee, 2) had an allograft reconstruction and 3) had any multi-ligament reconstructions. All participants meeting criteria provided written informed consent and minors recruited for this study

were required to provide informed assent and consent from a parent or guardian. All protocols in this study were reviewed and approved by the University of Michigan Medical School Institutional Review Board (IRBMED: HUM00169174).

Table 3.1. Participant demographics. Data are represented as Mean (SD) unless otherwise stated. BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction. MCL = Medial collateral ligament. LCL = Lateral collateral ligament. †Indicates significant difference

Demographics	Normal BMI (<27.0 kg/m^2)	High BMI (≥ 27.0 kg/m^2)
	Mean (SD)	Mean (SD)
Age (yrs.)	23.33 (5.74) †	27.28 (8.07)
Height (m)	1.70 (0.08) †	1.72 (0.10)
Weight (kg)	69.16 (7.82) †	91.4 (13.28)
BMI (kg/m^2)	23.85 (1.95) †	30.88 (3.66)
Body Fat (%)	27.79 (7.05) †	37.45 (8.16)
Lean Mass (%)	72.80 (7.20) †	60.85 (14.66)
Body Fat (kg)	17.84 (5.22) †	32.79 (9.90)
Lean Mass (kg)	46.63 (8.46) †	54.83 (10.38)
Sex (N)	24 F, 16 M	15 F, 9 M
Time-Post ACLR (mo.)	27.23 (7.09)	28.33 (8.40)
Preferred Walking Speed (m/s)	1.27 (0.15) †	1.20 (0.18)
Graft Type (N)	Patellar Tendon = 30 Hamstring Tendon = 10	Patellar Tendon = 20 Hamstring Tendon = 4
Meniscal Surgeries (N)	None = 29 Meniscectomy = 4 Repair = 8	None = 10 Meniscectomy = 3 Repair = 10
Collateral Ligament Injuries (N)	MCL Injury = 1 LCL Injury = 1 Neither = 38	MCL Injury = 0 LCL Injury = 1 Neither = 23

Gait Biomechanics and Walking Assessments:

Lower extremity knee kinematics and kinetics were collected using a 10-camera motion capture system (Qualisys, Gothenburg, Sweden) sampling at 200 Hz and a fully instrumented treadmill (Bertec, Columbus, Ohio) sampling at 2000 Hz. Each participant was outfitted with laboratory standard neutral cushion footwear (Nike Flex Run 9, Beaverton OR) and a total of 48 retroreflective markers. Static markers were placed bilaterally on the iliac crests, anterior superior iliac spine, greater trochanter, medial and lateral femoral epicondyles, malleoli, and the first and fifth metatarsal heads to determine joint centers as we have done previously [246, 322]. Dynamic markers were placed bilaterally on the calcaneus and rigid clusters of four non-collinear markers were affixed on the sacrum and bilaterally on thigh, shank, and foot segments to minimize soft-tissue artifact of single markers placed on skin. A standing calibration trial was captured, and static markers were removed leaving only rigid clusters and calcaneus markers for dynamic trials.

Afterwards, participants underwent approximately 15 minutes of treadmill walking across three separate walking conditions (self-selected, pre-determined [1.3 m/s], incline walking [5º, 1.3 m/s]), with only data from the self-selected speed condition reported herein. Self-selected walking speeds were tested overground and used to set treadmill speeds as done previously [388]. Briefly, each block of walking consisted of a two-minute acclimation period to allow participants to familiarize with the treadmill speed prior to initiating three 1 minute motion capture trials. During walking, participants were affixed with a chest harness as a safety precaution fastened over the shoulders and mid-chest (Petzl Chest'Air, West Valley City, UT). Instructions for walking were also standardized

and participants were instructed to maintain their body position in the center of the treadmill, to avoid cross-stepping as much as possible and to keep their eyes looking forward. Instructions were verbalized throughout the walking trials as reinforcement when needed.

Biomechanical Outcome Measures:

Biomechanical models were constructed from raw marker and force plate data via Visual 3D (C-Motion Inc., Germantown MD). We low-pass filtered raw marker position and force data using a fourth order zero-phase lag digital Butterworth Filter with cut-off frequencies set at 6 Hz and 10 Hz, respectively. The hip joint center was estimated using the Davis method [389]. Knee joint motions were defined as motion of the shank relative to the thigh using an XYZ Cardan rotation sequence (x= flexion/extension, y=ab/adduction, z=internal/external rotation) [390]. Filtered kinematic and kinetic data were combined for standard inverse dynamics procedures using inertial parameters estimated from Dempster [391] and Hanavan [392]. Stance phase for each condition was identified using a 50N threshold to define heel-strike and toe-off [388]. The peak KFM, KFA, and KFE were extracted from the first 50% of stance phase. Joint moments calculated from inverse dynamics were expressed as external and normalized to a product of body weight (N) and height (m) expressed hereafter as %BW*Ht. We calculated cumulative load outcomes (Nm*s or N*s) by integrating moment and force curves and included only positive portions of each stance waveforms [393]. For all biomechanical outcomes, only complete stance phases throughout each 1-minute trial were analyzed and the average value across all identified stance phases were used in subsequent statistical analyses.

Ultrasound Evaluation of Femoral Trochlear Cartilage:

Following gait biomechanical assessments, femoral trochlear articular cartilage thickness was assessed via US [219, 220, 246, 394]. All participants were asked to refrain from exercise for a minimum of 24 hours prior to testing sessions. Prior to US image acquisitions, participants completed a 45-minute non-weightbearing period in which they lied supine on a treatment table with their legs in full extension to allow adequate recovery of articular cartilage thickness from preceding weightbearing activity as previous research has shown cartilage can regain its resting thickness within 20-30 minutes post-exercise. To ensure measurement consistency, a single investigator acquired all US images using a GE LOGIQe device (General Electric, Fairfield, CT, USA) with standardized imaging parameters (B-Mode, Frequency=12MHz, Depth=3.5cm, Gain=50, Dynamic Range=75). US imaging of the femoral cartilage was conducted with participants knees placed in 140 degrees of flexion. The US transducer was placed transversely in line with the medial and lateral femoral condyles above the superior edge of the patella and tilted until the probe was perpendicular to the femoral cartilage surface. The intercondylar notch was centered on the screen and marked on a transparent grid for consistency and repeatability and a total of three images were acquired from each knee.

Femoral trochlear cartilage thickness was extracted from each image using an open-source app (SCOUT) created in MATLAB, which has excellent intra and inter-rater reliability (0.958-0.991) [395]. Images were cropped and filtered using a Butterworth filter within SCOUT to improve contrast and visibility of the cartilage echogenic borders. Following image optimization, the superior cartilage-synovium border and deep cartilage-

bone interface were manually traced to delineate regions of interest (ROI) and three, equally defined regions were assigned across the cartilage contour representing the medial, lateral, and intercondylar ROIs. The intercondylar ROI was centered on the deepest point of the intercondylar notch (identified via SCOUT) and spanned the middle 25% of the manually drawn cartilage ROI. In cases where the intercondylar notch were not correctly identified via SCOUT, manual adjustments were made to ensure anatomical correctness. Cartilage thickness was evaluated in the medial and lateral cartilage regions as the Euclidean distance between the cartilage-bone and cartilage-synovial space interface at every pixel in each respective ROI. Medial: lateral thickness ratios were defined as medial thickness divided by lateral thickness where ratios over 1 indicated greater medial thickness relative to lateral and ratios under 1 indicate greater lateral thickness relative to medial. To limit bias, a single investigator analyzed all images which were subsequently reviewed and confirmed by a separate investigator at the end of the study. Investigators were blinded from limb status (i.e., Injured vs. Uninjured) and all images were processed together after the completion of the study.

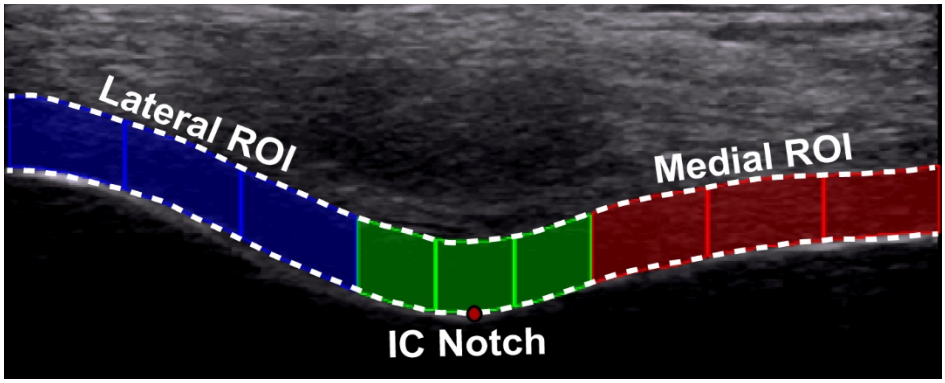

Figure 3.1. Femoral trochlea regions of interest. Red dot is the identified center of the trochlear groove which was used to segment regions. Dashed white lines depict the superficial and deep cartilage contours. Average thickness was extracted from Lateral (blue) and Medial (Red) ROIs and used for further statistical analyses.

Body Composition Assessments:

On a second day, body composition was assessed via dual-emission X-ray absorptiometry (DEXA: GE Lunar 2, GE Healthcare, Chicago IL) which determines body metrics like bone mineral density, lean body mass, fat mass, and segmental adiposity such as truncal and visceral fat mass. For this study, our outcomes of interests were the total amount of lean and fat mass (for descriptive purposes) and body fat % which will be used in statistical analyses.

Statistical Approach:

Descriptive statistics were computed for all relevant variables as well as demographic information for participants in each group (Tables 1-4). To compare gait biomechanics (normalized and absolute KAM, KFM, GRF, and cumulative load indices) and cartilage outcomes (medial, lateral and medial: lateral thickness ratios) between groups (high and normal BMI) and limbs (ACLR and non-ACLR), linear mixed models with a random factor of subject were completed via R Studio (lme4 package; R Core Team, Vienna, Austria). Significant interactions ($\alpha < 0.05$) were followed up with pairwise comparisons and significance values were corrected for multiplicity via Tukey's HSD. To evaluate if BMI impacted the relationship between gait and cartilage outcomes in those with ACLR, moderation analyses (regression) were conducted in SPSS via the PROCESS macro (version 3.1). Covarying for sex and time post-op, we evaluated the moderating effect of BMI group on the association between gait predictor variables (i.e., KAM, KFM, GRF, impulses etc.) and cartilage outcomes in the ACLR limb via the interaction term (BMI x predictor). All variables were mean centered prior to analysis and significant interactions were followed with post hoc probing of the conditional slopes within

each BMI group. Alpha level for significance was similarly set as $\alpha < 0.05$. Measurement reliability and precision for cartilage thickness were assessed via intraclass correlation coefficients ($ICC_{2,k}$: Two-way random effects, absolute agreement) and standard error of the measurement in a subset of participants (N=15; Appendix Table 3.6). Lastly, body composition outcomes (% fat mass, % lean mass, fat mass (kg), lean mass (kg)) were compared descriptively using independent t-tests (Table 3.1).

3.4 Results:

Gait Biomechanical Comparisons between BMI Groups

All means and standard deviations for each gait outcome were stratified by limb and group and depicted in Tables 6.2-6.3. Ensemble waveforms of all gait variables are also included in Figure 3.4. We did not observe any significant interactions for any limb/joint load outcomes of interest (i.e., KFM, KAM, GRF etc.). For normalized joint/limb load and cumulative load indices, we observed a significant main effect of group on peak vertical GRFs ($F_{1,61} = 6.41$, $p = 0.016$) and knee flexion moment cumulative loads ($F_{1,61} = 4.59$, $p = 0.036$), where those with high BMI after ACLR walked with lesser first peak vertical GRFs (Mean diff. = 0.042 BWs, $p = 0.016$) and greater normalized knee flexion moment cumulative loads (Mean diff. = 0.0016 Nm/kg*m/s, $t = 2.082$, $p = 0.041$). No other significant effects for group (F range: 0.31 – 1.31, $p > 0.05$) or limb (F range: 0.29 – 1.73, $p > 0.05$) were found for normalized load indices. For absolute load comparisons, we observed characteristic group effects showing that all peak moments and cumulative load indices were significantly larger in high BMI individuals with ACLR (t range: 3.450 - 13.0864, $p < 0.05$).

Table 3.2 Normalized Biomechanical Outcomes. Data are represented as Mean (SD). BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction.

Gait Outcomes	Normal BMI (<27.0 kg/m^2)		High BMI (≥ 27.0 kg/m^2)	
	ACLR Limb	Non-ACLR Limb	ACLR Limb	Non-ACLR Limb
Vertical GRF (%BW)	1.123 † (0.061)	1.133 † (0.064)	1.083 (0.075)	1.083 (0.075)
Vertical GRF Cumulative Load (% BW/s)	0.543 (0.038)	0.548 (0.038)	0.547 (0.043)	0.547 (0.047)
Peak KFM (%BW*Ht)	0.046 (0.012)	0.049 (0.12)	0.047 (0.015)	0.048 (0.014)
Peak KFM Cumulative Load (%BW*Ht/s)	0.0106 † (0.003)	0.0105 † (0.003)	0.0120 (0.004)	0.0125 (0.003)
Peak KAM (%BW*Ht)	0.038 (0.009)	0.038 (0.008)	0.036 (0.008)	0.036 (0.008)
Peak KAM Cumulative Load (%BW*Ht/s)	0.014 (0.004)	0.014 (0.003)	0.014 (0.002)	0.014 (0.003)

† = Indicates significant group main effect.

Table 3.3 Absolute Biomechanical Outcomes. Data are represented as Mean (SD). BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction

Gait Outcomes	Normal BMI (<27.0 kg/m^2)		High BMI (≥ 27.0 kg/m^2)	
	ACLR Limb	Non-ACLR Limb	ACLR Limb	Non-ACLR Limb
Vertical GRF (N)	762.20 † (93.44)	768.66 † (95.07)	974.21 (143.40)	969.65 (146.08)
Vertical GRF Cumulative Load (N/s)	369.04 † (56.16)	372.37 † (55.43)	491.49 (96.00)	491.30 (99.7)
Peak KFM (Nm)	53.73 † (15.98)	55.91 † (14.84)	71.22 (25.00)	73.05 (24.48)
Peak KFM Cumulative Load (Nm/s)	12.30 † (4.15)	12.09 † (3.66)	18.63 (7.57)	19.19 (6.55)
Peak KAM (Nm)	43.72 † (11.52)	44.20† (9.53)	54.26 (12.03)	54.75 (11.57)
Peak KAM Cumulative Load (Nm/s)	16.37 † (5.00)	16.60 † (4.54)	21.17 (5.60)	21.48 (6.14)

† = Indicates significant group main effect.

Femoral Trochlear Cartilage Comparisons Between BMI Groups:

All means and standard deviations for cartilage outcomes were stratified by limb and group and depicted in Table 3.4. For medial trochlear thickness, no limb x group interactions or main effects were observed (All $p > 0.05$).

For lateral trochlear thickness, we observed a limb x group interaction ($t = 2.01$, $p = 0.0487$). Post hoc comparisons indicated a significant difference in slopes between normal BMI and high BMI groups. In normal BMI ACLR patients, lateral cartilage tended to be *thicker* in the ACLR limb compared to contralateral limb (mean [95% CI]: 2.59 mm [2.42, 2.76] vs 2.51 mm [2.36, 2.67]) whereas high BMI individuals tended to exhibit *thinner* lateral cartilage in the ACLR compared to contralateral limb (mean [95% CI]: 2.32 mm [2.11, 2.54] vs 2.43 mm [2.23, 2.62]). Collapsed across limbs, normal BMI individuals with ACLR exhibited *thicker* lateral trochlear cartilage compared to high BMI individuals with ACLR (mean [95% CI]: 2.55 mm [2.44, 2.67] vs 2.38 mm [2.24, 2.52]).

Lastly, we observed a significant limb by group interaction for the medial: lateral trochlear cartilage thickness ratio ($t = 2.74$, $p < 0.01$). Post-hoc pairwise comparisons indicated that the ACLR limb exhibited greater medial: lateral cartilage thickness ratios (i.e., thicker medial cartilage relative to lateral) compared to the contralateral limb but only in the high BMI group (mean [95% CI]: 1.04 mm/mm [0.94, 1.14] vs 0.96 mm/mm [0.89, 1.03], $p = 0.014$). We also observed that high BMI patients with ACLR exhibited greater medial: lateral cartilage thickness ratios compared to normal BMI patients, but this was only significant in ACLR limbs (1.04 mm/mm [0.94, 1.14] vs 0.914 mm/mm [0.88, 0.5], $p < 0.01$).

Table 3.4 Trochlear Cartilage Outcomes via US. Data are represented as Mean [95% CI]. BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction.

Cartilage Outcomes	Normal BMI (<27.0 kg/m^2)		High BMI (≥ 27.0 kg/m^2)	
	ACLR Limb	Non-ACLR Limb	ACLR Limb	Non-ACLR Limb
Medial Thickness (mm)	2.35 [2.21, 2.49]	2.36 [2.21, 2.51]	2.28 [2.16, 2.40]	2.29 [2.11, 2.47]
Lateral Thickness (mm)	2.59 * [2.42, 2.76]	2.51 [2.36, 2.67]	2.32 * [2.11, 2.54]	2.43 [2.23, 2.62]
Medial: Lateral Thickness Ratio	0.91 [0.88, 0.95]	0.94 [0.91, 0.98]	1.04 † [0.94, 1.14]	0.96 † [0.89, 1.03]

* = Indicates interaction effect of limb x group. † = Indicates main effect of group.

Associations Between Walking Biomechanics and Cartilage Outcomes:

For medial cartilage thickness, covariates of age (t = -1.73, p = 0.09) and sex (t = -1.46, p = 0.15) were not predictive of trochlear thickness (total R^2 = 0.076, p = 0.10). Overall, neither peak load or cumulative load outcomes predicted medial trochlear thickness and BMI did not moderate any relationships between predictors and cartilage outcomes (All p > 0.05).

For lateral cartilage thickness, covariates were entered first together where sex predicted 8.2% of the variance in trochlear thickness (t = -2.30, p = 0.025) but age (t = -1.86, p = 0.069) did not have any predictive value (total R^2 = 0.134, p = 0.02). We observed that BMI moderated associations between peak KAM ($F_{5,58}$ = 4.423, total R^2 = 0.276, p < 0.01), KAM cumulative load ($F_{5,58}$ = 4.25, total R^2 = 0.27, p < 0.01) and lateral trochlear thickness (Figure 3.2). Post-hoc probing of the BMI group x KAM interaction (R^2 = 0.10 p < 0.01; Figure 3.2) indicated greater peak KAM was associated with thicker lateral trochlear cartilage in the high BMI group with ACLR (t = 2.58, p = 0.012) but not in the normal BMI group with ACLR (t = -1.05, p = 0.20). Similarly, for the BMI group x KAM

cumulative load interaction (R^2 = 0.083 p = 0.013; Figure 3.2), greater peak KAM cumulative load was associated with thicker lateral trochlear cartilage in the high BMI group (t = 2.72, p < 0.01) but not in the normal BMI group with ACLR (t = -0.34, p = 0.74).

Figure 2: Lateral Trochlear Thickness

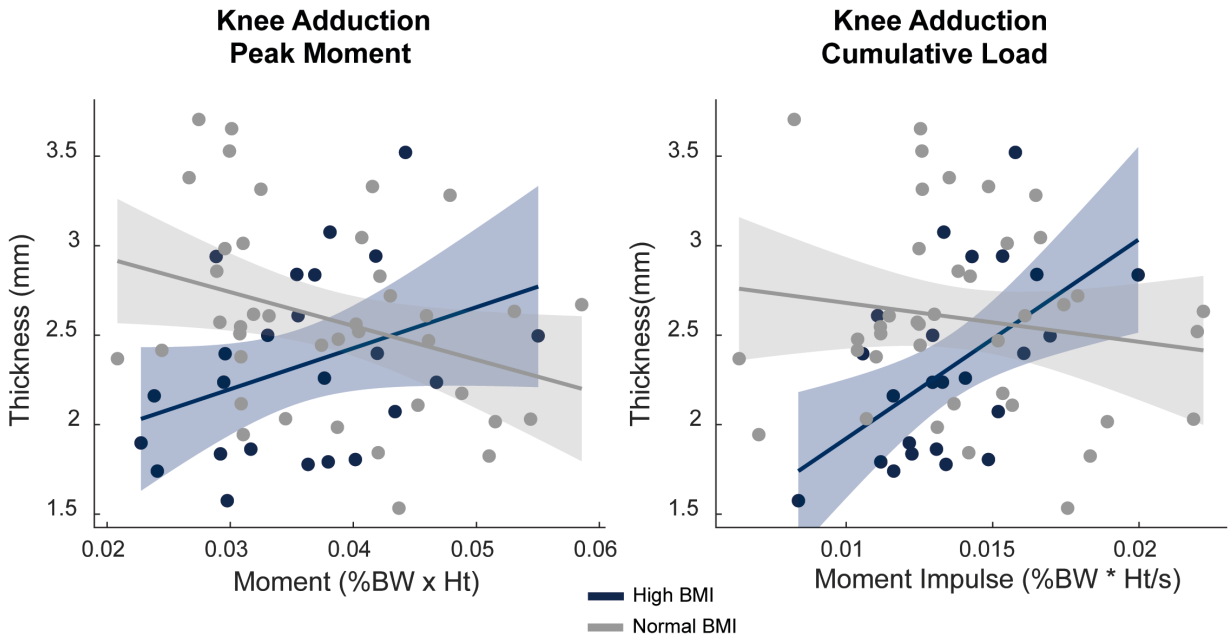

Figure 3.2. Scatter plots depicting BMI group x load predictor interactions for lateral thickness. Blue dots and fit represent high BMI data and gray dots and fit represent normal BMI data. KAM Slope for High BMI group: t = 2.58, p = 0.012. KAM cumulative load slope for High BMI group: t = 2.72, p < 0.01. Slopes for both outcomes in the normal BMI group were not significant.

For medial: lateral trochlear thickness ratios, covariates of age (t = 0.79, p = 0.43) and sex (t = 0.83 p = 0.41) were not significant and only accounted for a total of 2% of the total model variance (p = 0.54). We observed that BMI moderated the association between peak KAM ($F_{5,58}$ = 4.25, total R^2 = 0.268, p < 0.01), KAM cumulative load ($F_{5,58}$ = 5.73, total R^2 = 0.33, p < 0.01), and KFM cumulative load ($F_{5,58}$ = 4.011, total R^2 = 0.26, p < 0.01) and medial: lateral trochlear thickness ratios (Figure 3.3). The BMI group x KAM interaction (R^2 = 0.084, p = 0.013), indicated that greater peak KAM was associated with lesser medial: lateral trochlear thickness ratios in the ACLR limb but only in the high BMI

group (t = -3.15, p = 0.002, Figure 3.3). The BMI group x KAM cumulative load interaction (R^2 = 0.104, p < 0.01), indicated that greater KAM cumulative load was associated with lesser medial: lateral trochlear thickness ratios in the ACLR limb but only in the high BMI group (t = -3.92, p < 0.01, Figure 3.3). Lastly, the BMI group x KFM cumulative load interaction (R^2 = 0.055, p = 0.042) indicated that greater KFM cumulative load was associated with lesser medial: lateral trochlear thickness ratios in the ACLR limb but only in the high BMI group (t = -2.98, p < 0.01, Figure 3.3).

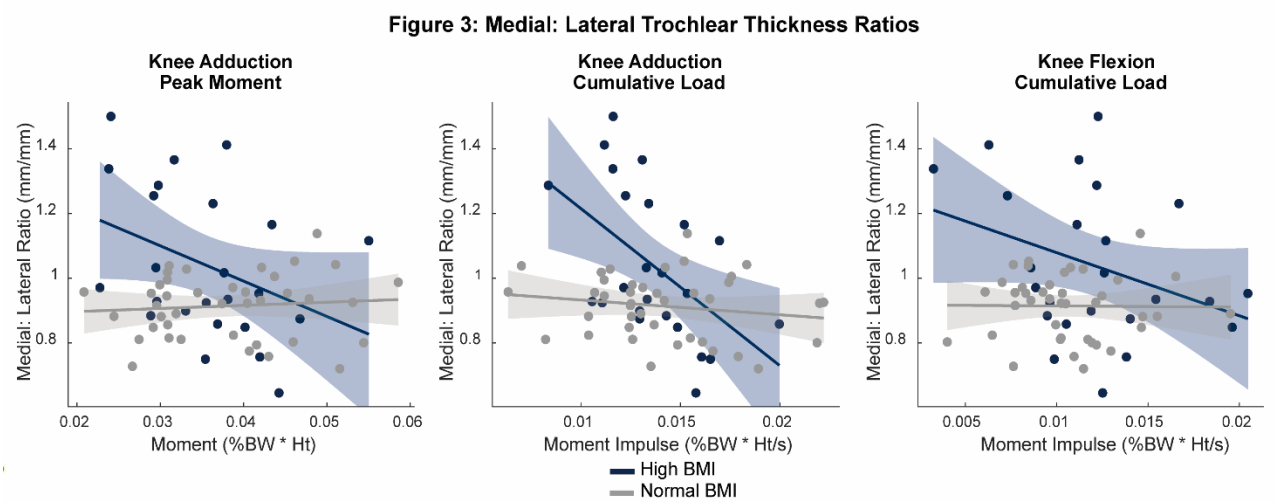

Figure 3.3 Scatter plots depicting BMI group x load predictor interactions from moderation analyses of Medial: Lateral Trochlear thickness ratios. Blue dots and regression fit represent high BMI ACLR data and gray dots and regression fit represent normal BMI ACLR data. KAM Slope for High BMI group: t = -3.15, p < 0.01. KAM cumulative load slope for High BMI group: t = -3.92, p < 0.01. KFM cumulative load slope for High BMI group: t = -2.98, p < 0.01. Slopes for both outcomes in the normal BMI group were not significant.

Reliability and Exploratory Analyses:

Interrater reliability ($ICC_{2,k}$: Two-way random effects, absolute agreement) for all cartilage outcomes were excellent (ICC range: 0.982-0.992) with SEMs ranging from 0.08 – 0.1 mm for cartilage thickness comparable to previously reported studies [246].

As supplementary analyses, we also aimed to evaluate how body composition (i.e., body fat) was associated with outcomes of interest presented above. Full results are

presented in the appendices below (section 3.7 of this chapter). Briefly, body fat (%) similarly moderated associations between gait (KAM and KAM cumulative load) and cartilage outcomes (lateral thickness and M:L ratios) in our cohort but associations were generally stronger when utilizing BMI as a predictor and the direction of associations were similar regardless of body composition surrogate (i.e., BMI or Body fat %).

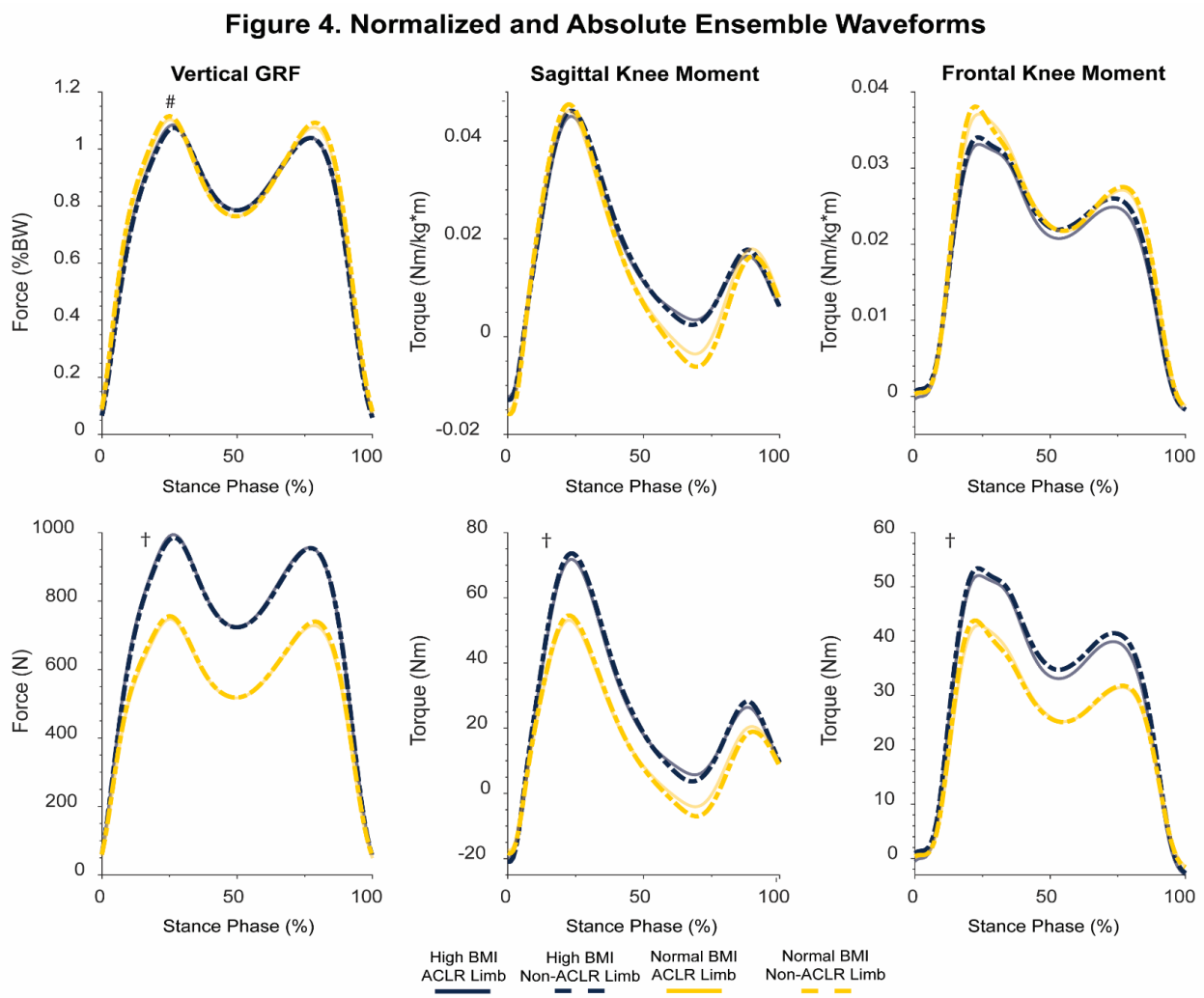

Figure 3.4. Ensemble waveforms for all gait outcomes stratified by group and limb for both normalized (top three panels) and absolute kinetics (bottom three panels). Solid lines indicate ACLR limb data while dashed lines indicate non-ACLR limb. Blue curves indicate High BMI groups, maize curves indicate normal BMI groups. # = Group Effect for Normalized Outcomes. † = Group Effect for Absolute outcomes.

3.5 Discussion:

The purpose of this investigation was to evaluate how BMI impacts gait biomechanics and ultrasound-based assessments of trochlear cartilage thickness after ACLR. We observed that gait biomechanics were largely similar between groups and limbs. In addition, high BMI uniquely impacted trochlear cartilage thickness and moderated associations between biomechanics and thickness outcomes. Overall, our data suggests that the combination of high BMI and ACLR may uniquely influence cartilage health and the association between walking kinetics and cartilage health in this population.

Here we observed that ACLR individuals with high BMI walked with lesser normalized peak vertical GRFs, and larger knee flexion moment cumulative loads bilaterally compared to normal BMI patients with ACLR. Findings of reduced peak vertical GRFs in those with high BMI partly agrees with prior research in healthy populations [322]. We reason the observations of reduced normalized vertical GRFs, and larger sagittal knee cumulative loads may likely be attributed to the slower self-selected walk speeds in the high BMI participants with ACLR in our study. Furthermore, those with high BMI also walk with altered temporal-spatial characteristics like reduced step-lengths and greater double-limb support times which are considered strategies to enhance gait stability [396]. Coupled with slower walking speeds, it can be speculated these compensatory gait strategies may also represent adaptations by which these individuals control support demands. It is also plausible that the sustained sagittal knee moment during mid-late stance (Figure 3.4) could be driving observations of higher knee flexion cumulative loads in those with high BMI after ACLR compared to normal BMI counterparts given that

moment magnitudes were relatively similar at other points in stance. Overall, the higher cumulative loads (and sustained moment in midstance) would reflect a more constant sagittal knee loading throughout stance in these individuals. Nonetheless, it is important to highlight our findings also showed those with ACLR regardless of BMI group exhibited relatively symmetrical walking GRFs (and in all other gait outcomes); data suggesting that gait appears to normalize at least in later phases post-op which contrasts with findings in earlier time-points [16, 388]. However, we are hesitant to conclude that load outcomes are fully normalized given the lack of comparison to normal and high BMI control groups. It is possible contralateral limb compensations are driving findings of "symmetrical" gait, and both limbs may be displaying aberrant loading characteristics.

Overall, knee-specific kinetics were largely similar between groups, with the exception of knee flexion moment cumulative loads which is contrary to our hypothesis. We note, however, that some inconsistencies exist when comparing gait between BMI groups as previous work has identified equivalent [319, 397], reduced [321, 322], and increased knee kinetics between groups [325]. Though, comparing gait between BMI group is difficult because of confounding factors such as sex, preferred walk speeds or how load outcomes are normalized. Our current analyses controlled for sex but not speed and we reason our normalized results may not necessarily represent biomechanically equivalent gait mechanics between normal and high BMI participants with ACLR. As mentioned above, those with high BMI walk slower which is considered a strategy to reduce support and metabolic cost demands given these individuals are heavier, and relatively weaker than normal BMI counterparts [325, 398]. Thus, the lack of differences in knee kinetics is interesting given that the approximately 8% slower walking speeds

observed in the high BMI group should generally contribute to a *reduction* in knee moments [368]. Nonetheless, these data may suggest individuals with high BMI after ACLR poorly adapt sagittal (or whole-body mechanics) in a manner that necessitates relatively higher knee functional demands. Further, load outcomes were also only comparable between groups when interpreting normalized outcomes as those with high BMI with ACLR walked with systematically greater absolute peak and cumulative loads (Table 3.3). Typical normalization procedures (i.e., BW x Height), however, assume equivalent mass distributions (i.e., lean vs. non-lean mass) across participant groups which may not be ideal when comparing BMI groups. For instance, those with high BMI in our cohort exhibited lesser relative lean mass (≈ 12% difference; Table 3.1) and thus, reduced proportions of active muscle may impair how these individuals' control/absorb higher absolute loads [322]. While speculative, it is plausible that abnormal internal joint loads (i.e., contact stresses) may be present in those with high BMI despite walking with relatively equivalent body weight normalized outcomes [399, 400].

We also observed that individuals with normal BMI tended to have *thicker* cartilage in the ACLR limb compared to contralateral limbs, whereas those with high BMI tended to have *thinner* lateral trochlear cartilage in the ACLR limb. Further, those with high BMI generally exhibited thinner lateral trochlear cartilage and greater medial: lateral thickness ratios bilaterally compared to normal BMI individuals with ACLR. In the patellofemoral joint, lateral cartilage is the primary area of joint contact [401] and thus, it is not surprising structural differences were observed in this ROI in our ACLR groups. We reason it is possible the differential structural features observed between our BMI subsets of individuals with ACLR (i.e., thinner vs. thicker ACLR limbs) may be representative of

cartilage at different stages of OA. For example, typically, an overall thickening or pathologic “swelling” of cartilage is considered an early structural signature of OA in response to early compositional derangement of the tissue’s matrix, whereas thinning behavior isn’t expected to occur until more “advanced” stages of the disease [115, 142]. Individuals with high BMI enter ACLR rehabilitation with biomechanical and metabolic risk factors (i.e., systemic inflammation) for OA that precede or are independent of the injury/surgery itself [8, 170, 316], which may accelerate the overall time-course of OA-related progression in these individuals. Further, patellofemoral cartilage undergoes both thinning and thickening in the first few years after ACLR [257, 402] and the structural differences observed between groups could be attributed to the largely heterogenous nature of post-traumatic OA. Longitudinal investigations combining more comprehensive measures of cartilage health (i.e., structure, composition, and mechanical function) are needed to better understand if these high BMI patients with ACLR display a differential time-course of disease features after ACLR.

Finally, we observed that BMI moderated the association between loading characteristics during walking and cartilage structure which partially agreed with our hypotheses as the direction of associations were different than initially expected. Greater normalized peak KAM, KAM and KFM cumulative loads were significantly and positively associated with both lateral trochlear thickness and medial: lateral cartilage thickness ratios but this was only significant in those with high BMI after ACLR (Figure 3.2). Previous studies in uninjured populations have demonstrated that BMI has a strong moderating effect on the association between knee loads and cartilage outcomes [252, 291], similar to that seen in OA populations [292]. While there is generally a positive relationship

between knee kinetics (KAM, KFM) and cartilage thickness/thickness distributions, these associations are often non-existent or negative (i.e., higher load may be detrimental to cartilage structure) in uninjured individuals with high BMI. However, our findings suggest the opposite as it appears that ACLR individuals with high BMI who walk with greater normalized KAM and KAM/KFM cumulative loads exhibited thicker lateral trochlear cartilage and lesser medial: lateral thickness ratios – associations not observed in our normal BMI cohort. As mentioned above, normal BMI individuals with ACLR displayed thicker lateral trochlear cartilage and lesser medial: lateral thickness ratios than our high BMI subset. Our findings could suggest that higher functioning high BMI individuals with ACLR (i.e., those who may potentially walk faster with increased normalized load outcomes) may exhibit more "normal" cartilage structure, similar to that seen in our normal BMI individuals with ACLR.

Further, the positive associations observed in our study suggest that higher joint loads may not necessarily be detrimental to those with high BMI. Cartilage homeostasis strongly relies on cyclic mechanical stimulation to maintain overall tissue health [6]. Inflammatory mediators, which are heightened in subjects with high BMI, impair chondrocytes function in response to mechanical signaling which has been considered one mechanism contributing to cartilage breakdown in these individuals [8]. Intuitively, many have often suggested gait modifications to reduce loading in those with high BMI as a means to mitigate cartilage breakdown and circumvent OA onset – yet these recommendations appear in contrast to our study findings. Nonetheless, we reason it is difficult to fully connect loading factors and cartilage health outcomes because many traditional gait metrics are limited to per-step assessments in a lab-based environment.

These measures, while providing substantial insight, may not fully capture how those with high BMI or pathological populations habitually load the joint throughout daily life. Physical activity disengagement is problematic after ACLR [403, 404], and it is possible individuals with high BMI are more likely to be sedentary compared to normal BMI individuals with ACLR. Thus, habitual disengagement in adequate activity after ACLR could represent a lifestyle factor in those with high BMI that contributes to an overall joint underloading profile, irrespective of the person's per-step gait patterns. Future research combining gait assessments with estimations of physical activity data (i.e., daily steps, PA engagement etc.) may help better connect gait biomechanics and cartilage adaptations after ACLR.

Our study is not without inherent limitations. Firstly, we prescreened individuals using a BMI cut-off of 27.0 kg/m^2 to group participants into normal BMI and high BMI groups and thus there was some similarities in participants who were near our cut-off values. Nonetheless, we performed sensitivity analyses where we excluded four participants from the normal BMI group that displayed body fat % values that would categorize them as high BMI and our results remained relatively unchanged (Appendix below: Tables 3.6-3.7). Further, all participants in our high BMI were correctly identified as "high BMI" using sex specific body fat % cut-offs (i.e., 25% for males, 35% for females) and thus we retained all participants in analyses. We also conducted exploratory analyses which indicated that body fat % similarly moderated associations between load and cartilage outcomes. However, only the peak KAM model was significant, and the associations were weaker (Figure 3.5). Thus, although BMI may not be the best determinant of body composition, our results suggest that there is still some value of using BMI to categorize participants which is beneficial clinically where body composition

assessments may be less feasible. Second, our gait and cartilage assessments were also limited due to the cross-sectional nature of our study design and whether high BMI individuals with ACLR exhibit unique post-operative recovery trajectories than their normal BMI counterparts remains unknown. While our data suggests that cartilage structural changes may differ between these two ACLR subsets, future research is needed to detail the potential unique differences in post-traumatic OA disease development in those with normal and high BMI after ACLR. Lastly, we note that we did not compare gait or cartilage outcomes to representative control participants with normal and high BMI. Nonetheless, we reason that comparison to contralateral limbs as done in our study still offers important insight, particularly given that we observed interactions between group and limb for cartilage thickness. Future studies with representative control groups may help us better understand if those with high BMI after ACLR experience worse biomechanical and joint health consequences.

3.6 Conclusion:

Individuals with high BMI after ACLR walk with similar normalized but greater absolute peak and cumulative joint loads compared to normal BMI individuals with ACLR. High BMI also influenced cartilage thickness alterations after ACLR which may suggest these subsets of individuals with ACLR experience different time-courses of OA-related events. Lastly, greater normalized knee loads positively influenced trochlear cartilage thickness in those with high BMI which may suggest cartilage may retain an adaptive response to joint loading even in the presence of high BMI in some individuals.

3.7 Supplementary Analysis

Associations between Body Fat Percent and Gait and Cartilage Outcomes:

Similar covariates of sex and age were utilized when evaluating associations between body fat percent and gait/cartilage outcomes and regression model results for covariates are included in Table 3.5 below. Overall, we found that body fat was significantly associated with greater knee flexion moment cumulative loads ($\Delta R^2 = 0.076$, $p = 0.028$) and lesser peak vertical GRFs in ACLR limbs ($\Delta R^2 = 0.076$, $p = 0.029$). However, no other significant associations were found between body fat percent, gait, or cartilage thickness outcomes. Beta's, t-statistics, and significance values for these data are listed in Tables 3.6 and 3.7.

Table 3.5 Full regression results from significant knee flexion moment cumulative load and peak vertical ground reaction force models.

		Standardized β	***t-statistic***	***p-value***	**Total R^2**
Knee Flexion Cumulative Load	*Covariates*				0.059
	Age	-0.069	-0.511	0.597	
	Sex	0.076	0.532	0.598	
	Predictor				**ΔR^2**
	Body Fat (%)	0.332	2.241	0.029	0.076*
Vertical Ground Reaction Force	*Covariates*				0.075
	Age	-0.169	-1.444	0.154	
	Sex	0.136	0.878	0.383	
	Predictor				**ΔR^2**
	Body Fat (%)	-0.335	-2.253	0.028	0.076*

Table 3.6. Regression results for between Body fat and cartilage outcomes in the ACLR limb. Model data presented accounts for sex and age as covariates.

Cartilage Outcomes	*Body Fat Percent*		
	Standardized β	***t-statistic***	***p-value***
Peak KFM	-0.038	-0.245	0.807
KFM Cumulative Load	**0.332**	**2.241**	**0.029**
Peak KAM	-0.255	-1.819	0.074
KAM Cumulative Load	-0.081	-0.531	0.598
Peak Vertical GRF	**-0.331**	**-2.253**	**0.028**
Vertical GRF Cumulative Load	0.146	1.030	0.308

Table 3.7. Regression results for between Body fat and cartilage outcomes in the ACLR limb. Model data presented accounts for sex and age as covariates.

Cartilage Outcomes	*Body Fat Percent*		
	Standardized β	***t-statistic***	***p-value***
Medial Thickness	-0.068	-0.444	0.659
Lateral Thickness	-0.192	-1.521	0.134
Medial: Lateral Ratio	0.227	1.479	0.145

Moderating Effects of Body Fat on the Association Between Walking Biomechanics and Cartilage Outcomes:

Exploratory analyses were performed to evaluate if body fat percent also moderated associations between gait and cartilage outcomes in our cohort. We observed that body fat moderated the association between peak KAM and lateral trochlear cartilage thickness ($F_{5,58}$ = 3.62, total R^2 = 0.25, p< 0.01) and medial: lateral cartilage thickness ratios ($F_{5,58}$ = 3.65, total R^2 = 0.25, p < 0.01). We also observed that body fat moderated

the association between KAM cumulative load and medial: lateral thickness ratios ($F_{5,58}$ = 3.69, total R^2 = 0.25, p < 0.01) but no other gait outcomes were associated with cartilage thickness outcomes. For lateral cartilage thickness, the BMI group x KAM interaction (R^2 = 0.064, p = 0.035), indicated that the association between KAM and thickness became stronger (and positive) as body fat percent increased (Figure 3.5): Below). For medial: lateral cartilage ratios, the BMI group x KAM interaction (R^2 = 0.093, p = 0.012) and KAM cumulative load interaction (R^2 = 0.06, p = 0.04), indicated that the association between peak KAM, KAM cumulative loads, and medial: lateral thickness ratios became stronger (and more negative) as body fat increased (Figure 3.5: Below). More specifically, higher KAM and KAM cumulative loads were associated with lesser medial: lateral thickness ratios and this association was stronger in individuals with higher body fat percent.

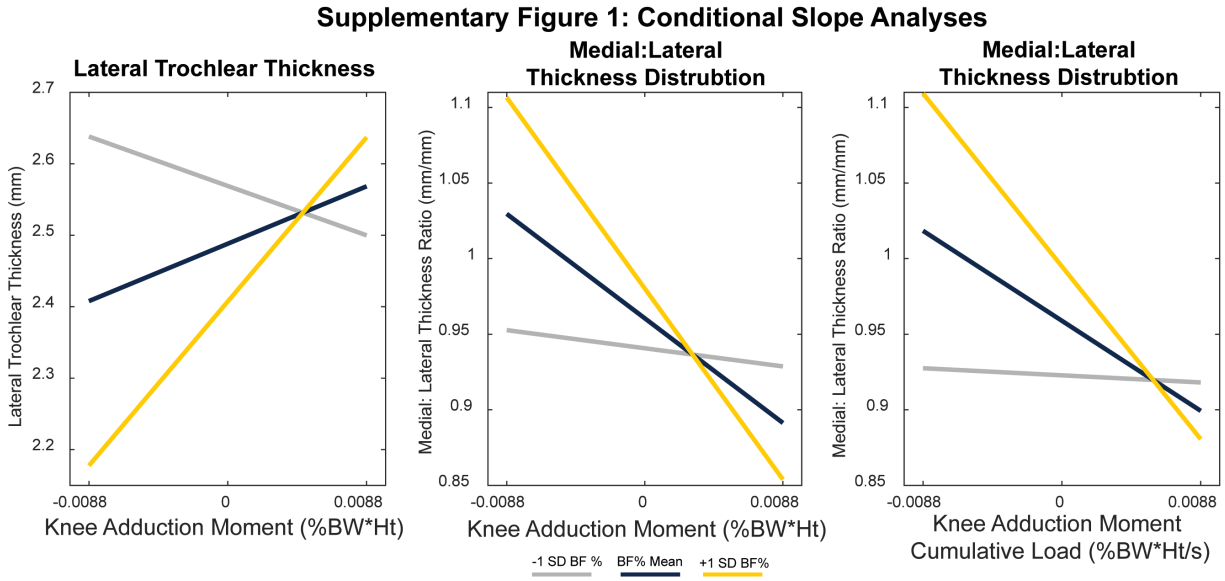

Figure 3.5. Figure 3.5 depicts the interaction between Body Fat x KAM on cartilage outcomes. Data displayed are post-hoc conditional slope analyzes the aid in visualizing interactions at different levels of the moderator variable (Body Fat Percent). **Note:** Data on the X axis represents mean centered KAM data.

Sensitivity Analysis (Removal of 4 Normal BMI) – Trochlear Cartilage Comparisons:

For medial trochlear thickness, we similarly observed that no interaction or main effects were observed (All p >0.05) indicating medial cartilage was similar between limbs and groups. For lateral trochlear thickness, the limb x group interaction remained significant when dropping the normal BMI participants (t = 2.00, p = 0.05). Post hoc comparisons indicated a significant difference in slopes between normal BMI and high BMI groups. In normal BMI ACLR patients, lateral cartilage tended to be *thicker* in the ACLR limb compared to contralateral limb (mean [95% CI]: 2.66 mm [2.50, 2.82] vs 2.59 mm [2.43, 2.76]) whereas high BMI individuals tended to exhibit *thinner* lateral cartilage in the ACLR compared to contralateral limb (mean [95% CI]: 2.35 mm [2.14, 2.56] vs 2.46 mm [2.25, 2.67]). Collapsed across limbs, normal BMI individuals with ACLR exhibited *thicker* lateral trochlear cartilage compared to high BMI individuals with ACLR (mean [95% CI]: 2.63 mm [2.47, 2.78] vs 2.41 mm [2.21, 2.61]).

Lastly, we observed a significant limb by group interaction for the medial: lateral trochlear cartilage thickness ratio (t = 2.29, p = 0.026), similar to our original analyses (t = 2.566, p = 0.013). Post-hoc pairwise comparisons indicated that the ACLR limb exhibited greater medial: lateral cartilage thickness ratios (i.e., thicker medial cartilage relative to lateral) compared to the contralateral limb but only in the high BMI group (mean [95% CI]: 1.031 mm/mm [0.97, 1.09] vs 0.951 mm/mm [0.89, 1.01], p = 0.017). We also observed that high BMI patients with ACLR exhibited greater medial: lateral cartilage thickness ratios compared to normal BMI patients, but this was only significant in ACLR limbs (1.031 mm/mm [0.97, 1.09] vs 0.918 mm/mm [0.86, 0.97], p = 0.033).

Table 3.8. Trochlear Cartilage Outcomes via US. Data are represented as Mean [95% CI]. BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction.

Cartilage Outcomes	Normal BMI (<27.0 kg/m^2)		High BMI (≥ 27.0 kg/m^2)	
	ACLR Limb	Non-ACLR Limb	ACLR Limb	Non-ACLR Limb
Medial Thickness (mm)	2.36 [2.20, 2.51]	2.35 [2.19, 2.51]	2.34 [2.19, 2.48]	2.28 [2.16, 2.40]
Lateral Thickness (mm)	2.59 *† [2.41, 2.77]	2.51† [2.35, 2.66]	2.33* [2.11, 2.54]	2.43 [2.23, 2.62]
Medial: Lateral Thickness Ratio	0.92*† [0.88, 0.95]	0.94† [0.90, 0.98]	1.04 * [0.94, 1.14]	0.96 [0.89, 1.03]

* = Indicates interaction effect of limb x group. † = Indicates main effect of group.

Sensitivity Analysis (Removal of 4 Normal BMI) – Gait Comparisons:

After removal of the 4 participants, similarly did not observe any limb x group interactions for other limb/joint load outcomes of interest (i.e., KFM, KAM, GRF etc.). For normalized knee moments and cumulative load outcomes there were no significant main effects for group (F range: 0.15 – 1.20, $p > 0.05$) or limb (F range: 0.15 – 2.84, $p > 0.05$) with exception of peak vertical GRFs and knee flexion moment cumulative loads which is identical to original analyses. We observed a main effect of group on peak vertical GRFs ($F_{1,57} = 6.71$, $p = 0.012$) indicating that those with high BMI after ACLR walked with lesser first peak vertical GRFs (i.e., 1.131 BW [1.109, 1.153] vs. 1.086 BW [1.059,1.113]). We also similarly observed a significant knee flexion moment cumulative load group effect ($F_{1,57} = 4.51$, $p = 0.038$) indicating that those with high BMI after ACLR walked with greater knee flexion moment cumulative loads (i.e., normal BMI: 0.0105 %BW *Ht/s [0.009, 0.0011] vs. high BMI: 0.0122 %BW *Ht/s [0.011 0.013]). For absolute load comparisons, we similarly observed characteristics group effects which indicated all peak moments and

cumulative load indices were larger in high BMI individuals with ACLR (F range: 14.42-65.46, $p < 0.05$).

Sensitivity Analysis (Removal of 4 Normal BMI) –Associations with Cartilage Outcomes:

Similar results for medial trochlear thickness when participants were removed from analyses and neither peak load or cumulative load outcomes predicted medial trochlear thickness and BMI did not moderate any relationships between predictors and cartilage outcomes (All $p > 0.05$).

For lateral cartilage thickness, nearly identical were observed as our original analyses. BMI moderated associations between peak KAM ($F_{5,54} = 3.78$, total $R^2 = 0.26$, $p < 0.01$), KAM cumulative load ($F_{5,54} = 3.63$, total $R^2 = 0.25$, $p < 0.01$) and lateral trochlear thickness (Figure S2). Post-hoc probing of the BMI group x KAM interaction ($R^2 = 0.103$, $p < 0.01$; Figure S2) indicated greater peak KAM was associated with thicker lateral trochlear cartilage in the high BMI group with ACLR ($t = 2.48$, $p = 0.017$) but not in the normal BMI group with ACLR ($t = -1.02$, $p = 0.31$). Similarly, for the BMI group x KAM cumulative load interaction ($R^2 = 0.07$ $p = 0.03$; Figure 3.6), *greater* peak KAM cumulative load was associated with thicker lateral trochlear cartilage in the high BMI group ($t = 2.67$, $p = 0.01$) but not in the normal BMI group with ACLR ($t = -0.07$, $p = 0.95$).

Similar to above, we observed similar results as our original analyses in that BMI moderated the association between peak KAM ($F_{5,54} = 3.88$, total $R^2 = 0.26$, $p < 0.01$), KAM cumulative load ($F_{5,54} = 5.23$, total $R^2 = 0.33$, $p < 0.01$), KFM cumulative load ($F_{5,54} = 3.64$, total $R^2 = 0.252$, $p < 0.01$) and medial: lateral trochlear thickness ratios (Figure 3). The BMI group x KAM interaction ($R^2 = 0.096$, $p = 0.01$), indicated that greater peak KAM was associated with lesser medial: lateral trochlear thickness ratios in the ACLR limb but

only in the high BMI group (t = -3.057, p < 0.01, Figure 3.6). The BMI group x KAM cumulative load interaction (R^2 = 0.102, p < 0.01), indicated that greater KAM cumulative load was associated with lesser medial: lateral trochlear thickness ratios in the ACLR limb but only in the high BMI group (t = -3.85, p < 0.01, Figure 3.6). The BMI group x KFM cumulative load interaction (R^2 = 0.06, p = 0.044) indicated that greater KFM cumulative load was associated with lesser medial: lateral trochlear thickness ratios in the ACLR limb but only in the high BMI group (t = -2.901, p < 0.01, Figure 3.6).

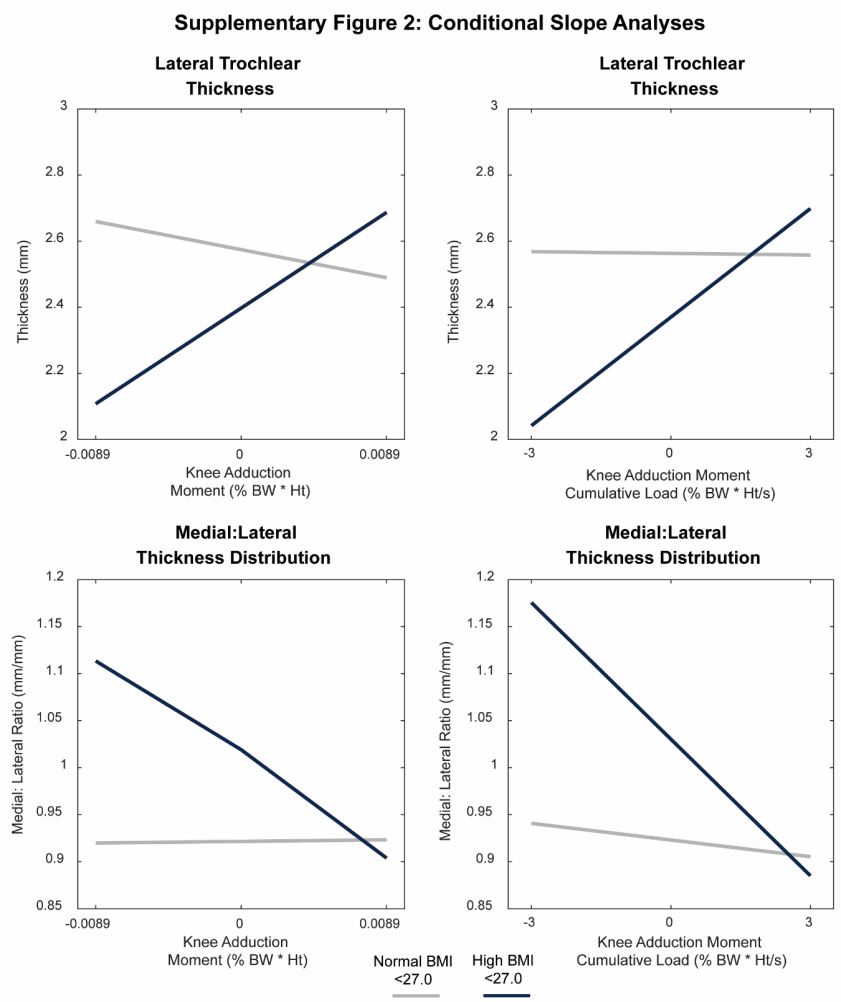

Figure 3.6 Figure 3.6 depicts the interaction between BMI x Gait Predictors on cartilage outcomes. Data displayed are post-hoc conditional slope analyzes the aid in visualizing interactions at different levels of the moderator variable (BMI) **Note:** Data on the X axis represents mean centered KAM and KAM cumulative load data. Top Panels indicate Lateral Trochlear Thickness data and bottom panels indicate Medial:Lateral Thickness ratios

Chapter 4 Body Mass Index and Knee Mechanics Predict Exercise-induced changes in Trochlear Cartilage Thickness and Echo Intensity following Walking in those with ACLR

4.1 Abstract

Background: Both high body mass index (BMI) and anterior cruciate ligament reconstruction (ACLR) independently influence knee osteoarthritis risk. Preliminary evidence shows the combination of these risk factors leads to poorer recovery and altered biomechanical outcomes after ACLR, but few studies have directly evaluated early changes in cartilage health between BMI groups in this population.

Purpose: Here, we evaluated ultrasound-based measures of cartilage strain and compositional changes (via echo-intensity [EI]) after an incline walking stress-test between normal and high BMI individuals with ACLR. Secondarily, we evaluated the associations between habitual walking biomechanics (i.e., ground reaction forces and sagittal knee kinetics and kinematics) and cartilage strain and EI outcomes.

Study Design: Controlled Laboratory Study

Methods: Gait biomechanical analyses and ultrasonography of the femoral trochlea were evaluated in sixty-four participants with ACLR who were considered normal BMI (BMI<27.0 kg/m^2, n=40) and high BMI (BMI>27.0 kg/m^2, n=24). Ultrasound images were collected bilaterally before and after an incline treadmill walk and medial and lateral trochlear strain and EI changes post-exercise were used to compare BMI groups. Gait

outcomes included ground reaction forces, peak sagittal plane knee moments, angles, and excursions to determine associations with cartilage outcomes.

Results: High BMI individuals with ACLR exhibited greater medial trochlear cartilage strain in the ACLR limb compared to normal BMI individuals (approx. 6%, $p<0.01$). In those with high BMI, the ACLR limb exhibited greater medial trochlear strain relative the non-ACLR limb (approx. 4%, $p<0.05$), but this was not observed in normal BMI groups. Medial trochlear EI changes were also greater bilaterally in those with high BMI compared to their normal BMI ACLR counterparts (approx. 10%, $p<0.01$). Lastly, individuals who walked with greater peak knee flexion angles exhibited lesser medial cartilage strain ($p=0.015$).

Conclusion: Our data suggests that high BMI disproportionately impacts cartilage functional properties after ACLR, while gait biomechanics consistent with quadriceps avoidance gait were associated with larger medial cartilage strain.

Clinical Relevance: High BMI individuals with ACLR may represent a subset of patients more susceptible to accelerated onset of post-traumatic OA and thus, may necessitate additional or more targeted interventions to mitigate or slow disease development.

4.2 Introduction

Post-traumatic osteoarthritis (OA) is a common long-term consequence in those with an anterior cruciate ligament injury, as half of all patients develop the disease within two decades, irrespective of surgical reconstruction (i.e., ACLR) [2]. Multiple mechanisms are thought to contribute to post-traumatic OA risk after ACLR, but strong evidence suggests that the odds of developing the disease are over three times greater in individuals with ACLR who were overweight (body mass index [BMI]: 25.0 kg/m^2) or obese (BMI>30.0 kg/m^2) [12-14]. Recent data from animal models has also suggested that the combination of obesity and joint injury may lead to a faster rate of post-traumatic OA progression that is more severe than either factor alone (i.e., obesity, joint injury) [332]. These findings are concerning as nearly one-third of patients undergoing ACLR are classified as obese [314, 315], a number that likely will grow given that obesity rates are expected to increase over 30% by 2030 [405]. As such, it may be beneficial to understand the influence of high BMI on post-traumatic OA in those with ACLR as this subgroup of patients may require unique treatment approaches.

Pathological changes to articular cartilage are characteristic of OA development, but the disease also involves significant derangement in nearly all tissues and structures within the joint (i.e., bone, ligaments, menisci etc.) [102]. In the earliest stages of OA, composition of the cartilage matrix is considerably altered (i.e., proteoglycan depletion, increased water content) which can adversely affect the mechanical function of cartilage and lead to a reduced ability to resist mechanical loading (i.e., decreased stiffness) [107, 171, 173, 179, 180]. Therefore, it has been suggested that assessing mechanical function of articular cartilage *in vivo* may be useful to evaluate the health status of cartilage and

offer insight into early pathological joint changes occurring in those at risk for OA (i.e., post-ACLR) [227-231, 236, 237].

Numerous studies have assessed cartilage mechanical function *in vivo* by measuring the pre-to-post change in cartilage thickness (i.e., cartilage strain) and composition (i.e., via T1ρ and T2 relaxation times) following acute exercise like walking or running, using magnetic resonance imaging (MRI) [226-231, 234-236, 261]. However, MRIs are costly, image segmentation is extremely time-consuming and clinical access is limited, all of which substantially reduce the scalability of this imaging modality for widespread and routine use [191, 199, 200, 204, 226, 234, 381, 406, 407]. Ultrasound is a cost-effective imaging alternative to MRI that can easily and reliably evaluate patellofemoral joint cartilage in the femoral trochlear regions [156, 193, 219, 243-245, 394] and has been used to evaluate trochlear cartilage strain behavior in healthy populations [219, 240]. Ultrasound can also indirectly evaluate cartilage composition via echo-intensity (EI) and recent work has shown higher EI is associated with longer femoral cartilage T2 relaxation times [408] – an MRI biomarker indicative of cartilage water content and type II collagen organization.. Importantly, the femoral trochlea seems to be the most susceptible region of cartilage in the patellofemoral joint to display early post-traumatic OA-related features after ACLR [93, 221, 224]. As such, ultrasound may be a convenient, low-cost, and clinically feasible OA imaging tool to characterize pathological alterations in the patellofemoral joint in ACL populations.

While data is scarce, evidence has shown that ACL-deficient knees undergo greater strain compared to the ACL-intact knee following walking and hopping, findings that may reflect early post-traumatic OA-related degeneration [235, 409]. These findings

are not surprising given that compositional changes assessed via MRI-based markers (i.e., T1ρ, T2 relaxation times) occur rapidly in ACL populations, presenting as early as 6 months post-operatively [258-260, 410]. High BMI and body composition metrics (i.e., body fat percent) have also been associated with greater magnitudes of cartilage strain and poorer cartilage composition (↑ T1ρ, and T2 times) in both healthy and OA populations [226, 411]. As previous evidence has connected high BMI with post-traumatic OA progression after ACLR [221, 332], it can be hypothesized that high BMI (and poor body composition) may also contribute to alterations in cartilage strain and composition changes after ACLR. However, no studies to date have evaluated the link between BMI, body fat percentage and surrogate measures of cartilage mechanical properties (i.e., strain) or EI changes post exercise after ACLR; data needed to understand the role of BMI in pre-clinical disease states.

Overall, assessment of cartilage strain is a non-invasive metric that may be sensitive to changes in cartilage composition and intrinsic mechanical properties (i.e., solid-matrix integrity) [106, 107]. Load-specific factors may also affect cartilage strain behavior, given the well-defined viscoelastic behaviors of the tissue [105, 135, 136]. For example, Paranjape et al., observed *in vivo* that progressively faster walking speeds lead to increased magnitudes of tibial cartilage strain in healthy adults [236]. Walking speed is known to influence ground reaction forces, loading rates and knee-specific loads [25, 412-414] and thus, the authors posited the increasing magnitude of joint loads and number of load cycles may likely explain the increased strains observed in response to faster walk speeds. Nonetheless, little research has connected habitual walking mechanics (i.e., knee angles, moments, ground reaction forces (GRFs) with cartilage strain

characteristics, and thus it remains unclear how poor walking biomechanics (i.e., under/overloading) influence how cartilage may respond to acute exercise. Such knowledge is critical given that alterations in walking mechanics are thought to contribute to cartilage breakdown with disease in both ACLR and high BMI populations and thus, these data may prove insightful into comprehensively understanding the unique influence of biomechanical factors on OA pathogenesis.

Here, we evaluated differences in femoral trochlear cartilage strain and echogenicity (EI) following a 30-minute incline walk in individuals with normal and high BMI after ACLR. Secondarily, we aimed to evaluate the associations between BMI, habitual gait biomechanics and cartilage strain and EI changes post-exercise. We also performed exploratory analyses using body composition metrics derived from Dual X-Ray Absorptiometry (DEXA) scans to evaluate associations between body fat percent and our cartilage outcomes while controlling for sex and time post-operative. For our main aims, we hypothesized those with high BMI after ACLR would exhibit greater cartilage strains and changes in cartilage EI following exercise compared to normal BMI individuals with ACLR after controlling for sex and time post-ACLR. We also hypothesized that greater GRFs, loading rates, knee moments and angles would be associated with greater and medial and lateral femoral trochlear cartilage strain and changes in cartilage echogenicity following exercise. For exploratory aims, we hypothesized that greater body fat percent would be associated with greater cartilage strains and changes in trochlear cartilage EI following exercise.

4.3 Methods

Sample Size Calculations and Participant Recruitment:

Sample size was determined using an *a priori* power analysis based on previously published research evaluating the effects of BMI, body fat % and OA-status on cartilage strain [226, 227]. Overall, medium-to-large effect sizes were identified for cartilage strain between OA-knees and healthy controls (Cohens *d* range: 0.62-0.68) and moderate-to-strong associations were identified between BMI, body fat % and cartilage strain in healthy knees (R^2 range: 0.38-0.58). Assuming a moderate effect (f^2=0.25) [415], an a level of 0.05, and an expected power of 0.80 (1-b), we estimated that 24 participants per BMI group were required to achieve 80% power to detect differences in cartilage strain between limbs and groups while accounting for covariates of sex and time post-operative. In total, we oversampled and recruited sixty-four individuals with an ACLR who were categorized into normal BMI (N=40; BMI < 27.0 kg/m^2) and high BMI groups (N=24; BMI ≥ 27.0 kg/m^2). Participants were eligible for this study if they: 1) were between 14-45 years of age, 2) free from lower extremity injury in the past 12 months, 3) between 1.5-3.5 years post-ACLR, and 4) had no prior/current diagnosis of arthritis. Participants were excluded if they 1) had a history of a previous meniscal or ACL tear to either knee and 2) had a reconstruction with an allograft. All participants provided written informed consent. Any minors recruited provided informed assent and consent from a parent or guardian was also acquired. This study was reviewed and approved by the University of Michigan Medical School Institutional Review Board (IRBMED: HUM00169174).

Table 4.1. Participant demographics. Data are represented as Mean (SD) unless otherwise stated. BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction. MCL = Medial collateral ligament. LCL = Lateral collateral ligament. †Indicates significant difference between normal and high BMI groups.

Demographics	**Normal BMI (N = 40: <27.0 kg/m^2)**	**High BMI (N = 24: ≥ 27.0 kg/m^2)**
	Mean (SD)	**Mean (SD)**
Age (yrs.)	23.33 (5.74) †	27.28 (8.07)
Height (m)	1.70 (0.08) †	1.72 (0.10)
Weight (kg)	69.16 (7.82) †	91.4 (13.28)
BMI (kg/m^2)	23.85 (1.95) †	30.88 (3.66)
Body Fat (%)	27.79 (7.05) †	37.45 (8.16)
Lean Mass (%)	72.80 (7.20) †	60.85 (14.66)
Body Fat (kg)	17.84 (5.22) †	32.79 (9.90)
Lean Mass (kg)	46.63 (8.46) †	54.83 (10.38)
Sex (N)	24 F, 16 M	15 F, 9 M
Time-Post ACLR (mo.)	27.23 (7.09)	28.33 (8.40)
Preferred Walking Speed (m/s)	1.27 (0.15) †	1.20 (0.18)
Graft Type (N)	Bone-Patellar-Tendon-Bone = 30 Hamstring Tendon = 10	Bone-Patellar-Tendon-Bone = 20 Hamstring Tendon = 4
Meniscal Surgeries (N)	None = 29 Meniscectomy = 4 Repair = 8	None = 10 Meniscectomy = 3 Repair = 10
Collateral Ligament Injuries (N)	MCL Injury = 1 LCL Injury = 1 Neither = 38	MCL Injury = 0 LCL Injury = 1 Neither = 23

Study Design:

Data included in the current study were a part of a larger investigation and were split across two study sessions. On the initial visit, gait biomechanics and US evaluation of the femoral trochlear cartilage before and after an incline walking exercise (Figure 4.1)

were gathered. Body composition scans via Dual X-Ray Absorptiometry (DEXA) were acquired at a separate testing session involving additional ultrasound assessments (not reported here). Briefly, participants were consented, self-selected speed was assessed overground, and gait biomechanics were then collected during three separate walking conditions: 1) self-selected speed, 2) a pre-determined speed (1.3 m/s) and 3) an incline walk at a fixed speed (5° slope, 1.3 m/s) – depicted in Figure 4.1 (non-randomized order). Further breakdown of gait testing protocols are listed below in subsequent sections. Data reported here are from the self-selected condition to capture how "typical" walking patterns are contributing to cartilage outcomes post-exercise. Following gait assessments participants completed a standardized 45-minute unloading period to minimize the effects of preceding walking as has been done previously (Figure 4.1). US assessments of femoral cartilage were conducted immediately following the rest period and immediately following a 30-minute incline walk (5° slope, 1.3 m/s). Detailed methods for gait and ultrasound assessments are described in the following sections.

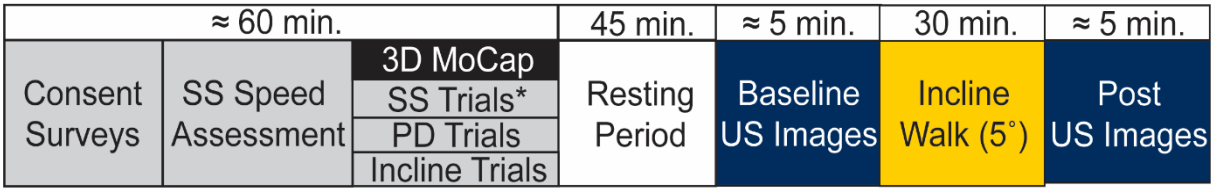

Figure 4.1. Study Design depicting gait biomechanics and ultrasound procedures. On a separate day, DEXA scans were collected with additional study procedures not included in the current manuscript. For 3D motion capture assessments, only data from the self-selected condition (* in figure) were included in current analyses. Baseline and Post images were acquired bilaterally at 140 degrees of knee flexion and the incline walk speed was standardized at 1.3 m/s to minimize potential effects of preferred speed differences between BMI groups. SS = Self-selected; PD = Pre-determined; US = Ultrasound; MoCap = Motion Capture.

Gait assessments were completed using a 10-camera Qualisys motion capture system (Gothenburg, Sweden). Marker data were sampled at 200 Hz and synchronized with an instrumented split-belt Bertec treadmill (Bertec, Columbus, Ohio) in which analog force signals were captured at 2000 Hz. To minimize variation in loading parameters attributed to shoe types (i.e., minimalist vs. maximalist), each participant was outfitted with laboratory standard neutral cushion footwear (Nike Flex Run 9, Beaverton OR). Our walking marker set consisted of a combination of 48 retroreflective markers placed bilaterally on the lower extremities. Markers consisted of a combination of single markers directly affixed to anatomical landmarks and rigid clusters of non-collinear markers secured to the lower-limb segments. Static markers consisted of markers on iliac crests, anterior superior iliac spine, greater trochanter, medial and lateral femoral epicondyles, malleoli, and the first and fifth metatarsal heads bilaterally. Joint centers were determined from static marker positions as noted previously [246, 322]. After marker set-up, a standing calibration trial was captured, and static markers were removed leaving only the calcaneus markers and rigid clusters affixed to the sacrum and the thigh, shank and foot segments for dynamic trials. Rigid clusters were chosen over single-based markers placed directly on the thigh to minimize the random variation attributed to soft-tissue artifact of single markers placed directly on skin [416].

After collection of the standing calibration trial, participants underwent approximately 15-20 minutes of treadmill walking across 3 blocks of separate walking condition: 1) self-selected speed, 2) a pre-determined speed (1.3 m/s) and 3) an incline walk at a fixed speed (5° slope, 1.3 m/s). Of note, only data from the self-selected speed condition are reported herein (Figure 4.1). Prior to trial recordings for each walking

condition, a 2-minute acclimation period was provided to allow participants to familiarize themselves with the treadmill speed/incline. After which three 1-minute motion capture trials were recorded for each walking condition. While walking, participants were affixed with a chest harness fastened over the shoulders and mid-chest (Petzl Chest'Air, West Valley City, UT). Participants were also provided standardized instructions for walking and were informed to maintain body position in the center of the treadmill, to avoid cross-stepping, and to keep their eyes looking forward as much as possible as if they were walking outside. Instructions were consistently verbalized throughout trials as reinforcement and participants were instructed to occasionally look down at their feet to gauge their position on the treadmill and to aide in not crossing over the treadmill belts.

Biomechanical Outcome Measures:

Biomechanical models were computed by combining raw marker and force plate data using Visual 3D (C-Motion Inc., Germantown MD). Marker positions and force data were filtered using a fourth order zero-phase lag digital Butterworth Filter with cut-off frequencies set at 6 Hz and 10 Hz, respectively. Hip joint centers were estimated from pelvis static markers using the Davis method [389] while knee centers were defined as the midpoint between medial and lateral epicondyle markers. Knee joint motions were defined as the motion of the shank relative to the thigh using a Cardan XYZ rotation sequence (x= flexion/extension, y=ab/adduction, z=internal/external rotation) [390]. Filtered kinematic and kinetic data were combined via inverse dynamics procedures and stance phases for all treadmill trials were identified using a 50N threshold to define heel-strike and toe-off [388]. Outcomes of interest included the first peak vertical GRF and instantaneous vertical GRF loading rates (defined as the peak value when differentiating

the GRF waveform with respect to time) extracted from the first 50% of stance phase. Knee-specific outcomes included the peak knee flexion moment (KFM), peak knee flexion angle (KFA), and knee flexion and extension excursions (KFE, KEE, respectively). Joint moments calculated from inverse dynamics were expressed as external and normalized to a product of body weight (N) and height (m) (referred to hereafter as % BW*Ht). For all biomechanical outcomes complete stance phases (i.e., heel strike to toe off) from each 1-minute trial were analyzed and the average of these were used in statistical analyses.

Ultrasound Evaluation of Femoral Trochlear Cartilage:

Following gait biomechanical assessments, femoral trochlear articular cartilage thickness was quantified using US [219, 220, 246, 394]. Prior to US image acquisitions, participants completed a 45-minute non-weightbearing period in which they were required to lay supine on a treatment table with their legs in full extension to allow adequate recovery of articular cartilage thickness from the effects of preceding weightbearing activity [232, 417, 418]. To ensure measurement consistency, a single investigator acquired all US images using a GE LOGIQe device (General Electric, Fairfield, CT, USA) with standardized imaging parameters (B-Mode, Frequency=12MHz, Depth=3.5cm, Gain=50, Dynamic Range=75). Bilateral US imaging of the femoral cartilage was conducted with participants knees placed in 140 degrees of flexion. The US transducer was placed transversely in line with the medial and lateral femoral condyles above the superior edge of the patella and tilted until the probe was perpendicular to the femoral cartilage surface and maximized the reflection of the cartilage surfaces. The intercondylar notch was centered on the screen and marked on a transparent grid for consistency and

repeatability of US images and a total of three images were acquired from each knee and right knees were always imaged first.

Following baseline cartilage images, participants immediately began a 30-minute standardized incline walking exercise on the treadmill speed (1.3 m/s, 5°). We chose a fixed walking speed as constraining speed and walking duration allowed for a standardization of walking distance, which could vary drastically if individuals walked at self-selected paces and either walk durations or step counts were constrained. We chose 1.3 m/s by averaging the gait speeds reported in existing literature investigating walking biomechanics in healthy, ACLR and high/normal BMI populations [25, 319, 321, 322, 330, 366, 419]. Incline walking was chosen over level-ground walking as walking at an incline increases compressive forces about the patellofemoral joint [420] which might assist in preferentially loading the femoral trochlea and improve the sensitivity of our US measures. Following the 30-minute walk, imaging protocols were repeated bilaterally. The pre-post change in femoral trochlear cartilage thickness was used to quantify cartilage strain (%); expressed as percent change scores between resting and post-walking acquisitions (Equation 1: $\left(\frac{\text{Post-Pre Cartilage Thickness}}{\text{Pre Cartilage Thickness}}\right)*100$).

Femoral trochlear cartilage thickness and echo-intensity were extracted from each image using an open-source app created in MATLAB [395] and readers are referred to the published paper for imaging-specific processing schemes. Briefly, images were optimized via SCOUT to improve contrast and clarity of the femoral cartilage borders. Investigators then manually traced two separate cartilage contours, the superior cartilage-synovium border and deep cartilage-bone interface, to define cartilage regions of interest. After delineating the cartilage contours, the SCOUT app defined three equal segments

across the image representing the medial, lateral, and intercondylar regions of the tissue. The intercondylar notch was defined as the deepest point within the central trochlear region (identified via SCOUT) and spanned the middle 25% of the manually drawn cartilage ROI. In cases where SCOUT incorrectly placed the intercondylar notch marker, investigators manually adjusted notch location to ensure anatomical correctness. We evaluated cartilage thickness in medial and lateral cartilage regions to align with previous work using US [219, 238, 246] by identifying the minimum Euclidean distance between cartilage-bone and cartilage-synovial space interfaces at every pixel in each ROI (Fig. 4.2). Within each region of interest, cartilage echo-intensity was also calculated as the average pixel intensity (0-255 arbitrary units [AU]). A higher EI is visually represented as a brighter border or tissue on images (i.e., 0 = black, 255 = white). Measurement reliability and precision for cartilage thickness and EI were assessed between 2 raters and across 2 days via intraclass correlation coefficients ($ICC_{2,k}$) and standard error of the measurement in a subset of participants (N=15). ICC's for inter-rater reliability for cartilage outcomes ranged from 0.971-0.991, and ICC's for test-retest reliability ranged from 0.874-0.976. Full results of this analysis can be found in Table 4.4 at the end of this chapter.

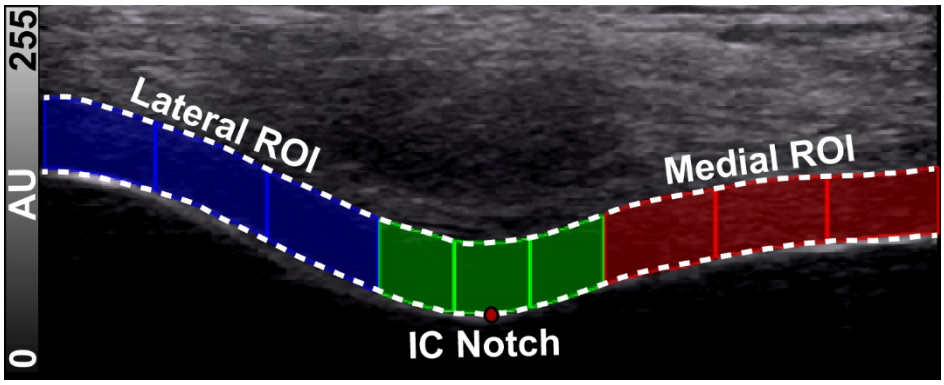

Figure 4.2 Medial and lateral trochlear regions of interest. Red dot is the identified center of the intercondylar notch (IC) which was used to segment regions. Dashed white lines depict the superficial and deep contours. AU (Arbitrary Units) refers to units for echo-intensity which are the gray scale pixel intensity in the image with 0 indicating black and 255 indicating white.

Body Composition Assessments:

On a separate day, body composition was assessed via dual-emission X-ray absorptiometry (DEXA: GE Lunar 2, GE Healthcare, Chicago IL). Clinically, BMI is a useful and routinely evaluated metric used to classify individuals into normal, overweight, or obese categories, but has inherent limitations to misclassify individuals as overweight or obese, particularly in young, active populations [421, 422]. Given that higher proportions of lean mass is generally considered protective of OA risk [423], more direct evaluations of body composition may provide a better assessment of the role of excess adiposity on post-traumatic OA outcomes after ACLR. Therefore, DEXA outcomes (i.e., total amount of lean and fat mass and body fat %) were also collected and used in exploratory statistical analyses (Table 4.5).

Statistical Approach:

To compare changes in trochlear cartilage thickness and EI between groups (high and normal BMI) and limbs (ACLR and non-ACLR), linear mixed models with a random factor of subject were completed via R Studio (lme4 package; R Core Team, Vienna, Austria). Significant interactions ($\alpha < 0.05$) were followed up with pairwise comparisons and significance values were corrected for multiplicity via Tukey's HSD. To evaluate the associations between BMI, gait biomechanics and cartilage thickness/EI changes in those with ACLR, stepwise regression analyses were conducted. Covariates included sex and time post-operative, and predictors of trochlear cartilage outcomes (i.e., thickness/EI) included BMI and gait outcomes (i.e., GRF, Loading KFA, KEE, KFM). Alpha level for significance was similarly set as $\alpha < 0.05$. Lastly, body composition outcomes (% fat mass, % lean mass, fat mass (kg), lean mass (kg)) and participant demographics were

compared descriptively using independent t-tests (Table 4.1) and exploratory analyses were performed to evaluate the associations between % body fat and cartilage outcomes via linear regression (reported in Appendix at end of this chapter; Table 4.5).

4.4 Results

Exercise-Induced Changes in Cartilage Strain and EI

For medial trochlear cartilage strain, we observed a significant limb x group interaction ($F_{1,64}$ = 6.20, p = 0.015). Post-hoc pairwise comparisons indicated that the ACLR limb exhibited greater cartilage strain compared to the non-ACLR limb (mean difference: 4.96%, t = 3.06, p < 0.01 – Table 4.2) in those with high BMI, but not the normal BMI group (t = 0.047, p = 0.963). Medial trochlear cartilage strain in the ACLR limb was greater in high BMI individuals with ACLR compared to normal BMI individuals with ACLR (mean difference: 7.54%, t = 4.64, p < 0.01 – Table 4.2), but the non-ACLR limbs were not different between groups (t = 1.551, p = 0.12). For lateral trochlear cartilage strain, no significant interaction ($F_{1,64}$ = 0.29, p = 0.591) or main effects (F range: 0.18-1.24, p > 0.268) were observed.

For medial trochlear EI changes, we observed a main effect of BMI group ($F_{1,64}$ = 9.019, p < 0.01), but limb and limb x group interactions were not significant (F range: 0.01 – 0.18, p >0.672). Group main effects indicated that high BMI individuals with ACLR exhibited greater medial trochlear EI changes post-exercise (i.e., greater increase in echogenicity post-exercise) compared to normal BMI individuals with ACLR (mean difference: 9.16%, t = 2.932, p <0.01 – Table 4.2). For lateral trochlear EI changes, no significant interaction ($F_{1,63}$ = 0.01, p = 0.95) or main effects (F range: 0.17-1.35, p > 0.25) were observed.

Table 4.2. Trochlear Outcomes Post-Walking. Data are represented as Mean [95% CI]. BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction. EI = Echo Intensity.

Gait Outcomes	Normal BMI (<27.0 kg/m^2)		High BMI (≥ 27.0 kg/m^2)	
	ACLR Limb	Non-ACLR Limb	ACLR Limb	Non-ACLR Limb
Medial Trochlear Strain (% Δ)	0.651 [-1.226, 2.528]	0.593 [-1.101, 2.198]	-6.60 † # [-3.18, -10.03]	-1.900 [-4.62, 0.82]
Medial Trochlear EI Δ (% Δ)	-1.038 [-4.372, 2.296]	-2.186 [-6.859, 2.448]	7.709 ‡ [1.411, 14.01]	7.638‡ [-0.34, 15.62]
Lateral Trochlear Strain (% Δ)	1.601 [-1.169, 4.370]	1.457 [-0.019, 2.932]	-0.508 [-3.84, 2.85]	0.773 [-2.55, 4.08]
Lateral Trochlear EI Δ (% Δ)	-3.469 [-12.09, 5.148]	-2.145 [-10.418, 6.127]	2.634 [-6.16, 11.43]	10.122 [-6.16, 28.46]

†: Interaction effect indicating significant difference compared to Normal BMI ACLR limb. **#:** Interaction effect indicating significant difference compared to High BMI non-ACLR limb. **‡:** Group main effect indicating significant difference compared to normal BMI group.

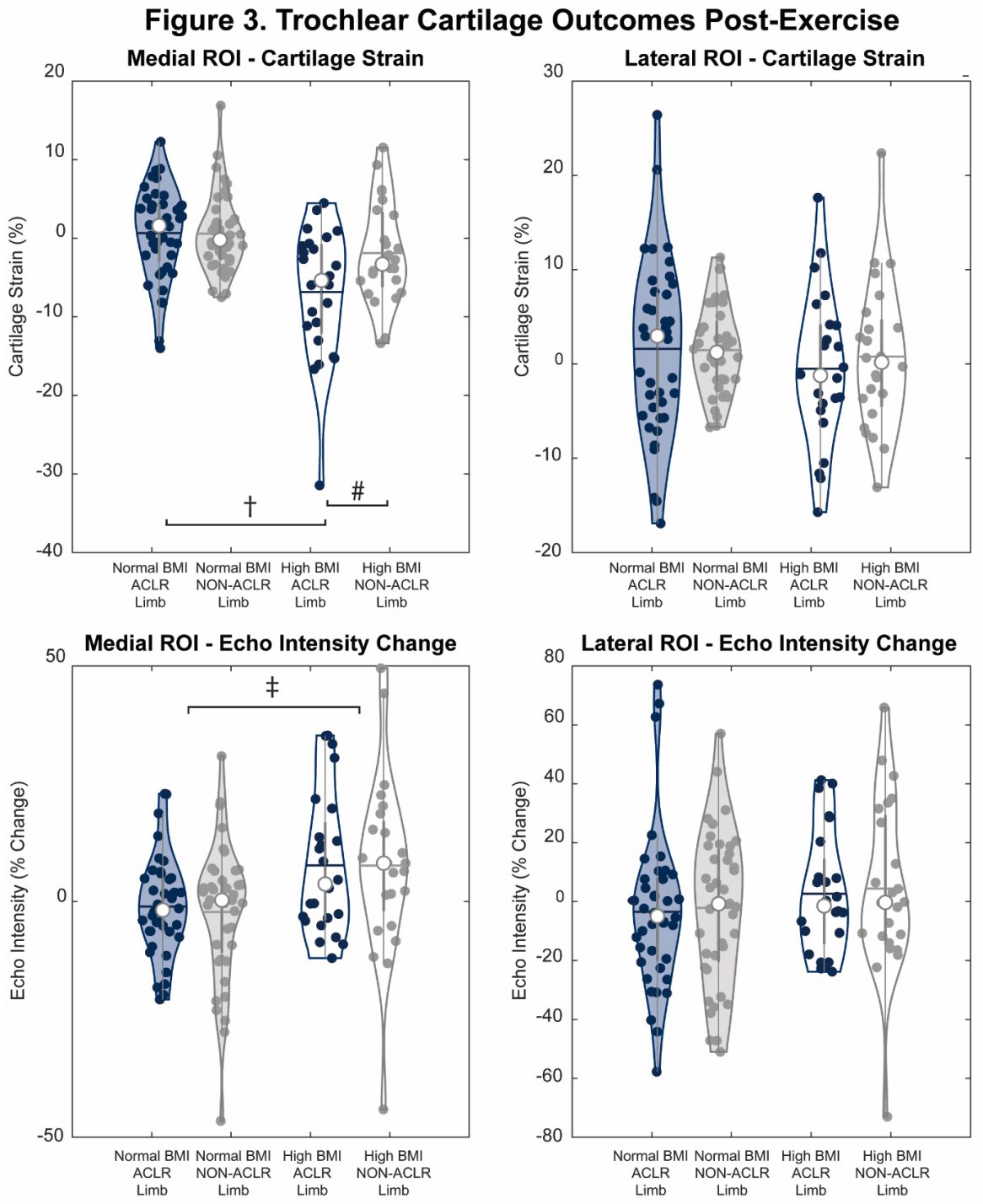

Figure 4.3. Violin plots depicting cartilage outcomes post-exercise. Filled violins indicate normal BMI groups with ACLR, unfilled violins indicate high BMI groups with ACLR. Blue violins represent ACLR limbs and gray violins indicate non-ACLR Limbs. Horizontal lines in violin indicate mean, white dots indicate median. **†:** Interaction effect indicating sig. difference compared to Normal BMI ACLR limb. **#:** Interaction effect indicating sig. difference compared to High BMI non-ACLR limb. **‡:** Group main effect indicating sig. difference compared to normal BMI group.

Associations between BMI, Gait and Cartilage Outcomes Post-Exercise:

Gait predictor means and standard deviations are reported below in Table 3. BMI was entered into the model after covariates and was positively associated medial trochlear cartilage strain (t = -2.92, p < 0.01). After accounting for BMI and covariates in the model, peak KFM (β = 0.243, t = 1.99, p = 0.05) and KFA (β = 0.275, t = 2.20, p = 0.03) were significantly associated with medial cartilage strain in the ACLR limb. However, only peak KFA was retained in the overall model, predicting an additional 6.5% of the variance (Δ t = 2.515, Δ p = 0.015) in medial trochlear cartilage strain (total R^2 = 0.213, $F_{4,59}$ = 4.00, p < 0.01). Knee excursions, peak vertical GRF and loading rates were not predictive of cartilage strain (t range: -0.943 – 1.80, p >0.07). For medial trochlear EI changes post-exercise, covariates of sex, and time since ACLR were not associated with EI changes ($F_{2,61}$ = 1.37, p = 0.26). After accounting for covariates, BMI was positively associated with greater increases in medial trochlear EI (t = 2.583, ΔR^2 = 0.096, Δ p = 0.012). No gait predictors were significantly associated with EI changes post-exercise (all p > 0.05).

For lateral trochlear cartilage strain, covariates of sex, and time since ACLR were not associated with EI changes ($F_{2,61}$ = 2.35, p = 0.10) and BMI was also not associated with lateral cartilage strain (t = -1.15, ΔR^2 = 0.02, Δ p = 0.26). No gait predictors were significantly associated with lateral trochlear cartilage strain (all p > 0.05). For lateral trochlear EI changes post-exercise, covariates of sex, and time since ACLR were not associated with EI changes ($F_{2,61}$ = 0.10, p = 0.907) and BMI was also not associated with lateral cartilage strain (t = -1.40, ΔR^2 = 0.03, Δ p = 0.17). No gait predictors were significantly associated with EI changes post-exercise (all p > 0.05).

Table 4.3. Normalized Biomechanical Outcomes. Data are represented as Mean (SD). BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction. ILR = Instantaneous loading rate. Data were not compared statistically and are compared in a separate investigation.

Gait Outcomes	Normal BMI (<27.0 kg/m^2)		High BMI (≥ 27.0 kg/m^2)	
	ACLR Limb	Non-ACLR Limb	ACLR Limb	Non-ACLR Limb
Vertical GRF (%BW)	1.124 (0.061)	1.133 (0.064)	1.089 (0.076)	1.083 (0.075)
Vertical GRF ILR (%BW/s)	14.05 (2.57)	14.02 (2.67)	12.25 (3.01)	12.13 (2.93)
Peak KFM (% BW*Ht)	0.0464 (0.012)	0.0486 (0.122)	0.0466 (0.015)	0.047 (0.014)
Peak KFA (deg.)	15.66 (4.85)	17.17 (4.12)	14.72 (6.24)	16.25 (5.81)
Peak KFE (deg.)	14.58 (2.94)	15.43 (2.66)	13.77 (3.66)	14.77 (3.50)
Peak KEE (deg.)	10.93 (4.36)	12.44 (4.07)	9.67 (4.68)	10.22 (4.47)

4.5 Discussion

Overall, the purpose of this investigation was to compare the acute changes in trochlear cartilage thickness (i.e., strain) and echogenicity (EI) after a 30-minute incline treadmill walk between high and normal BMI individuals with ACLR. Secondarily, we aimed to evaluate associations between gait mechanics and cartilage changes post-exercise. In agreement with our hypotheses, we observed that medial trochlear cartilage underwent greater strain bilaterally and larger increases in EI after exercise in those with high BMI compared to normal BMI. Further, the ACLR limb exhibited greater medial trochlear cartilage strain compared to the non-ACLR limb, but this was only observed in high BMI individuals with ACLR. Exploratory analyses using body composition metrics showed that greater body fat % was associated with greater medial trochlear cartilage

strain and medial EI changes post-exercise – suggesting adiposity may negatively impact cartilage mechanical integrity. Lastly, we observed that individuals with a lower BMI who walked with greater sagittal knee angles and moments experienced smaller decreases in medial cartilage thickness (i.e., reduced strain), but gait mechanics were largely not predictive of lateral outcomes post-exercise. Overall, these data provide novel insight on the effects of BMI (and body composition) on cartilage health outcomes after ACLR and suggest that post-traumatic OA related changes may be occurring earlier and present more drastically in those with high BMI after ACLR.

Our data revealed that medial trochlear cartilage strains were larger bilaterally in those with high BMI compared to normal BMI individuals with ACLR (Figure 4.3). We reason our findings likely reflect overall poorer cartilage matrix properties in high BMI ACLR individuals in our cohort. Cartilage strain behavior is tightly linked with the health of the cartilage matrix and the intrinsic mechanical properties of the tissue [134, 261]. In early OA states, cartilage composition is commonly altered and characterized by disorganization of the type II collagen network and reductions in proteoglycan content [191] – findings consistent with observations both in ACLR [260, 308] and high BMI cohorts [226]. Together, these compositional alterations have been shown to result in a mechanical weakening of the tissue [105, 134, 261] that may undergo increased cartilage strains when subjected to weight-bearing – as was partly observed in our study. For instance, previous work in uninjured populations has seen similar findings as Collins et al., observed higher BMI (and body fat %) was associated with greater cartilage strain and poorer cartilage compositional metrics (i.e., elevated T1ρ and T2 relaxation times) [226, 234]. Thus, it is possible some of these altered cartilage properties were partly

present prior to ACL injury, particularly given the increased strains observed in the non-ACLR limbs of our high BMI group.

Interestingly, we also observed that medial trochlear cartilage strains were greater in ACLR knees compared to the contralateral limb, but this was only present in high BMI individuals with ACLR (Figure 4.3. While speculative, these observations may be suggestive of a potential additive effect of high BMI and ACLR wherein early post-traumatic OA related cartilage alterations may present more rapidly and are more pronounced in ACLR patients with high BMI. Irrespective of joint injury, high BMI is thought to predispose the knee to OA partly due to the combination of excess weight and adiposity [8]. Those with high BMI walk with greater absolute joint loads [322, 325] and increased cartilage contact stress compared to normal BMI individuals [400]. Concurrently, high BMI also promotes excessive systemic inflammatory factors. Adipose tissue is metabolically active and facilitates production of adipokines (i.e., leptin, adiponectin) and pro-inflammatory cytokines (IL-6, TNF-α) that contributes to dysregulated cartilage metabolism and facilitates matrix breakdown [170, 335]. As such, it is reasonable that ACL injury further catalyzes the deleterious impact of high BMI on OA-related degeneration given the tissue is already experiencing an altered metabolic and mechanical environment. Our result and that of others provide some support for this tenet as BMI has been previously connected with greater odds of worsening patellofemoral features (i.e., cartilage defects, bone bruises etc.) in the first several years post-op [221]. Taken together, we reason that disease-modifying therapeutic approaches may be necessitated earlier and/or more aggressively in high BMI subsets of individuals after ACLR. Though, we note our data is cross-sectional in nature and further research

directly evaluating the effects of BMI on quantitative cartilage features after ACLR are needed to characterize the time-course of cartilage compositional and functional changes between normal and high BMI patients after ACLR.

We also observed that medial trochlear EI changes post-exercise were significantly greater bilaterally in high BMI individuals with ACLR compared to normal BMI individuals with ACLR. Here, we utilized cartilage EI as a compositional marker of trochlear cartilage and early-stage OA features like surface fibrillation increase cartilage echogenicity [192, 424]. Cartilage EI is also influenced by water content within the tissue as water on US images generally is anechoic and displays as darker pixel intensities [193, 425]. While speculative, we reason that observations of elevated medial trochlear EI changes post-exercise in high BMI individuals with ACLR may reflect greater magnitudes of fluid exudation following exercise. There is some support for this as recent preliminary data suggests trochlear cartilage EI is associated with T2 relaxation times at rest [408], an imaging biomarker associated with the density of the type II collagen matrix and intra-tissue water content. Further, data from OA populations show that individuals with OA similarly exhibit greater water loss following acute exercise, reflected as reduced T2 relaxation times via MRI [426]. Increases in the permeability of the cartilage matrix is a common feature of early OA-related degradation that also influences the tissues load-bearing capacity [134, 173]. Consequently, more degenerated tissue is considerably less capable of providing adequate compressive resistance to weightbearing and therefore, may be more susceptible to undergoing excessive strain and fluid loss [134]. Nonetheless, it is important to recognize that limited data has directly connected cartilage EI from ultrasound with more sensitive compositional MRI metrics and whether changes

in EI post-exercise follow similar patterns of cartilage compositional change post-exercise remains unclear. Given the accessibility and low costs of using US imaging, we propose further research should aim to validate cartilage EI measures to assess *in vivo* cartilage properties as this may be beneficial to permit more widespread and clinically viable imaging options to monitor OA in populations like those with ACLR.

Lastly, we observed that sagittal plane gait mechanics (i.e., peak KFM, KFA) were associated with medial trochlear strain in the ACLR limb, but peak KFA appeared to be the strongest predictor. However, gait outcomes were not predictive of changes in lateral cartilage outcomes post-exercise. After controlling for sex, time post-operative and BMI, we found that those who walked with greater peak KFA (and KFM) exhibited lesser medial cartilage strains in the ACLR limb. Reduced knee angles can impact contact locations and load distributions within the patellofemoral joint during walking wherein smaller angles contributes to reduced medial patellofemoral loads [427-429]. It is also possible that habitual restrictions in sagittal knee motion and lesser load magnitudes, consistent with a quad-avoidance gait strategy, may negatively impact cartilage in these regions due to an “underloading” load pattern. Nonetheless, our findings are in contrast to recent work by Bjornsen et al., who utilized similar US-protocols but did not observe any associations between gait outcomes and cartilage strain [430]. However, the sample in the aforementioned study was much earlier post-op (approx. 9 months) and was primarily in normal BMI individuals after ACLR. It is also noteworthy that we did not track 3D gait mechanics across our entire incline walking exercise. Thus, it is not clear if the associations observed in our sample are reflective of how individuals are actually loading their joints throughout our walking stimulus or if those who normally walk with larger KFA

exhibit healthier cartilage. Future work should consider pairing gait analysis during and throughout exercise stress-tests to better understand what mechanical factors may be predictive of cartilage strain in those with ACLR.

It is important to highlight that cartilage strain post-exercise was evaluated using an incline walking protocol in the current investigation. Previous studies using US-based assessments of cartilage strain post-walking have mainly used level walking protocols [219, 239, 418] and have reported heterogenous results on cartilage thickness changes. Incline walking is known to increase knee angles and patellofemoral joint loads that may preferentially load the trochlear regions that are accessible to US imaging which may improve sensitivity to detecting differences in cartilage properties. Indeed, we compared cartilage strains and EI changes between incline and level conditions in a subset of participants (N=15) and observed greater strain ($F = 5.75$, $p = 0.031$, mean difference: 3.9% [0.41, 7.37]) and greater EI increases ($F = 7.27$, $p = 0.017$, mean difference: 8.44% [1.73, 15.15]) during the incline condition. Further, we selected pre-determined durations (30-minutes) and speed (1.3 m/s) of the treadmill during our protocol to control for several factors. Those with high BMI often select slower self-selected walking speeds and we opted to minimize potential speed effects on cartilage strain outcomes. Controlling walk duration also ensured that all individuals walked identical distances and cartilage strain has been shown to increase with longer exercise durations [236]. While previous work has used standardized step protocols (i.e., 3000 steps) and/or self-selected speeds, those with high BMI often adopt slower speeds and smaller step lengths compared to normal BMI individuals which may confound group comparisons. Thus, our protocol constraints were selected such that both duration and speed effects were controlled

across participants – evidenced by the nearly identical steps during exercise between our groups (normal BMI steps: 3313.03 ± 141.07 vs. high BMI steps: 3325.75 ± 214.31). Nonetheless, we note it would be beneficial for future work to evaluate how manipulating exercise test constraints such as walk duration, walk distance or the number of loading cycles ultimately influences *in vivo* cartilage strain behaviors given the potential benefit of assessing cartilage mechanical properties via exercise.

Our study results should be considered in light of their limitations. We evaluated cartilage strain post-exercise between normal and high BMI individuals with ACLR, but no control groups without ACL injury were included. Thus, it is not fully clear whether BMI and joint injury interact and lead to disproportionately poorer cartilage outcomes. We note however, that our data provides important insight into this question given that we observed limb effects for cartilage strain that were only observed in the high BMI cohort. Such findings are important as it may suggest that deleterious post-traumatic OA related changes to cartilage are presenting earlier on in high BMI individuals with ACLR given that normal BMI individuals in our study exhibited lesser trochlear strains that were similar between limbs. Nonetheless, future study designs should include uninjured and ACL groups with high and normal BMI to fully understand if gait and/or cartilage outcomes are exacerbated by the combination of these two OA risk factors. We also had heterogenous graft types in our sample to increase generalizability of our findings and graft type distributions were slightly different between groups (25% HT in the normal BMI and 16% HT in high BMI group). However, we note that patellofemoral OA rates after ACLR are generally comparable between individuals who received BPTB or HT grafts [224, 431] and we did not observe associations between graft type and cartilage outcomes.

Nonetheless, it still may be beneficial to consider graft type comparisons more directly when evaluating early changes in cartilage health to better understand the influence of graft selection on cartilage health after ACLR.

4.6 Conclusions

We observed that ACLR limbs exhibited poorer tissue function compared to the contralateral limb, evidenced by greater medial trochlear strains post-walking, but this was only evidenced in those with high BMI. Overall, high BMI and body fat percent influenced the magnitude of medial trochlear strain and EI changes after an acute walking protocol, which may indicate that BMI and high body fat may contribute to deleterious changes to cartilage composition and mechanical function after ACLR. Lastly, those who walk with greater knee flexion angles, excursions, and moments tended to exhibit lesser cartilage strain, which may suggest that individuals who walk with less quadriceps-avoidance gait strategies may exhibit better cartilage health outcomes.

4.7 Supplementary Analysis

Reliability and Precision of US Assessments

We utilized two-way random effect models ($ICC_{2,\ k}$: Absolute Agreement) to evaluate both inter-rater and test-retest reliability for all outcomes. ICCs were classified as weak (< 0.05), moderate (0.5-0.69), or strong (>0.7). Standard error of the measurement (SEM: $SD\sqrt{1 - ICC}$) and minimal detectable change were also calculated (MDC: 1.645 x SEM x $\sqrt{2}$). For test-retest data, reliability and precision analyses were calculated between incline and level conditions that were completed on separate days (not reported herein). Overall, strong inter-rater and test-retest reliability were found for all outcomes (Table 4.4 below).

Table 4.4. Reliability Analyses.

	Inter-rater Reliability			**Test – Retest Reliability**		
	ICC	**SEM**	**MDC**	**ICC**	**SEM**	**MDC**
Medial Thickness (mm)	0.982	0.056	0.131	0.962	0.079	0.183
Medial EI (AU)	0.991	0.830	1.937	0.838	3.943	9.201
Lateral Thickness (mm)	0.971	0.096	0.224	0.976	0.067	0.159
Lateral EI (AU)	0.984	1.092	2.548	0.874	3.847	8.977

EI = Echo-intensity. AU = Arbitrary Units. ICC = Intraclass correlation. SEM = Standard error of the Mean. MDC = Minimal Detectable Change

Associations between Body Fat % and Cartilage Strain:

For original analyses we compared cartilage strain outcomes and EI changes between high BMI and normal BMI groups dichotomized at 27.0 kg/m^2. Given that BMI does not directly and adequately account for fat and lean mass distributions, we opted to assess body composition metrics via DEXA scans. We thus, performed linear regressions to evaluate how body fat % was also associated with cartilage strain and EI outcomes in our sample using similar covariates of sex and time post-operative.

For medial trochlear cartilage strain in the ACLR limb, covariates accounted for 2.0% of the variance (F = 0.595, p = 0.555). The addition of body fat % into the model accounted for an additional 18.7% of the variance (ΔR^2 = 0.186, p < 0.01, t = -3.65) wherein greater body fat % was associated with greater medial cartilage strain (i.e., decrease in thickness post-exercise). However, covariates (R^2 = 0.088, p = 0.070) and body fat % (ΔR^2 = 0.009, p = 0.465) did not predict any significant variance in medial trochlear cartilage strain in the non-ACLR limb. For medial EI changes post-exercise in the ACLR limb, covariates accounted for 5.4% of the variance (F = 1.646, p = 0.202). The addition of body fat % into the model accounted for an additional 12.0% of the variance (ΔR^2 = 0.120 p < 0.01, t = 2.877) wherein greater body fat % was associated with greater increases in medial cartilage EI post-exercise. However, covariates (R^2 = 0.009, p = 0.758) and body fat % (ΔR^2 = 0.047, p = 0.098) did not predict any significant variance in medial cartilage EI changes post-exercise in the non-ACLR limb.

For lateral trochlear cartilage strain, covariates and body fat % did not predict any significant variance in lateral strain in either the ACLR limb (total F = 2.069, p = 0.114) nor the non-ACLR limb (total F = 0.892, p = 0.451). Similarly, for lateral trochlear EI

changes post-exercise, covariates and body fat % did not predict any significant variance in lateral strain in either the ACLR (total F = 0.411, p = 0.746) nor the non-ACLR limb (total F = 0.458, p = 0.713).

Table 4.5. Full regression results from medial trochlear cartilage strain and medial trochlear EI changes post-exercise in the ACLR limb.

		Standardized β	***t-statistic***	***p-value***	**Total R^2**
Medial	*Covariates*				0.020
	Sex	0.146	1.078	0.286	
	Time Post-Op	-0.208	-1.713	0.092	
	Predictor				Δ R^2
	Body Fat (%)	-0.502	-3.65	< 0.001	0.187*
Medial EI Δ	*Covariates*				0.054
	Sex	-0.042	-0.302	0.764	
	Time Post-Op	0.271	2.184	0.033	
	Predictor				Δ R^2
	Body Fat (%)	0.404	2.877	0.007	0.120*

Chapter 5 Biomechanical Effects of Manipulating Preferred Cadence During Treadmill Walking in Patients with Anterior Cruciate Ligament Reconstruction

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5.1 Abstract:

Background: Abnormal gait is common after anterior cruciate ligament reconstruction (ACLR) which may influence osteoarthritis risk in this population. Yet few gait retraining options currently exist in ACLR rehabilitation. Cueing cadence changes is a simple, low-cost method that can alter walking mechanics in healthy adults, but few studies have tested its effectiveness in an ACLR population. Here, we evaluated the acute effects of altering cadence on knee mechanics in patients 9-12 months post-ACLR.

Hypothesis: Cueing larger steps will facilitate larger knee angles and moments, while cueing smaller steps would induce smaller knee angles and moments.

Study Design: Randomized Cross-sectional Design

Level of Evidence: Level 3

Methods: 28 patients with unilateral ACLR underwent gait assessments on a treadmill at preferred pace. Preferred walking gait was assessed first to obtain preferred cadence. Participants then completed trials while matching an audible beat set to 90% and 110%

of preferred cadence in a randomized order. 3D sagittal and frontal plane biomechanics were evaluated bilaterally.

Results: Compared to preferred cadence, cueing larger steps induced larger peak knee flexion moments and knee extension excursions bilaterally (p<0.01), whereas cueing smaller steps only reduced knee flexion excursions (p<0.01). Knee adduction moments remain unchanged across conditions and were similar between-limbs (p>0.05). Peak knee flexion moments and excursions were smaller in the injured compared to uninjured limb (p<0.01).

Conclusion: Frontal plane gait outcomes were unchanged across conditions suggesting acute cadence manipulations result in mainly sagittal plane adaptations. Follow-up studies using a longitudinal cadence biofeedback paradigm may be warranted to elucidate the utility of this gait retraining strategy after ACLR.

Clinical Relevance: Cueing changes in walking cadence can target sagittal plane knee loading and joint range of motion in ACLR participants. This strategy may offer high clinical translatability given it requires relatively minimal equipment (i.e., free metronome app) outside of a treadmill.

5.2 Introduction

Anterior cruciate ligament reconstruction (ACLR) is a common orthopedic procedure with an incidence rate in the United States of 74.6 per 100,000 person years [432]. In those with ACLR, functional impairments like reduced muscle strength and impaired movement during a variety of tasks are ubiquitous [10, 16, 433]. For example, persons with ACLR commonly adopt a walking strategy characterized by smaller peak knee flexion moments and flexion angles in the involved limb relative to the uninjured limb both early after surgery and for upwards of 10 years post-surgery [10, 15-20]. These altered movement patterns during walking are troubling as they have been linked to the early development of post-traumatic knee osteoarthritis [59, 258, 311, 434, 435]. Given the chronicity of gait deviations after ACLR and their negative consequences on long-term joint health, traditional post-operative rehabilitation appears insufficient to restore normal movement patterns post-ACLR. Thus, complimentary strategies that can enhance standard-of-care rehabilitation may be needed to enhance recovery of patient function after ACLR.

Gait retraining (i.e., a gait-specific intervention approach) involves targeting specific movement patterns through different modes of feedback to facilitate the restoration of pre-pathological gait patterns. Previous work suggests gait retraining programs are effective in reducing injury risks in runners [342, 343], improving gait asymmetries and walking speed post-stroke [344-349] and at improving pain symptoms and knee loading characteristics that influence disease progression in populations with knee osteoarthritis (OA) [350-352]. Nonetheless, despite a wealth of findings suggesting persons with ACLR walk with marked gait asymmetries linked with hazardous long-term

health outcomes like post-traumatic OA [10, 11, 20], gait retraining programs are scarcely included in standard-of-care post-operative ACLR rehabilitation programs [21, 22]. We contend that the absence of gait-specific training efforts in typical standard-of-care rehabilitation represents a missing and critical component of rehabilitation that if filled, may optimize current standard-of-care, help augment gait recovery, and potentially impede the development of post-traumatic OA in this population.

Appropriately, several recent works have focused on identifying gait retraining strategies capable of remediating walking gait deficits in those with ACLR [360, 361]. Although these investigations show promising acute improvements in walking biomechanics (e.g., increase knee moments and vertical ground reaction forces) [360, 361], a major drawback of current approaches are the use of expensive biomechanical devices to provide bio-feedback cues (i.e., real-time feedback of force data, split-belt treadmill paradigms), limiting overall clinical utility. Conversely, modifying simple spatial gait metrics such as step lengths may serve as an attractive feedback target that can be easily modified using auditory cues (i.e., a metronome). For example, manipulating step/stride lengths can directly modify knee kinetics and kinematics in healthy populations [342, 366, 369, 436]. At constrained speeds, cueing larger step lengths from preferred can increase both peak external knee flexion and adduction moments and knee flexion excursions [342, 366]. While temporal-spatial alterations have been seldom evaluated after ACLR, recent data by Hunnicutt et al., showed those with ACLR walked with smaller step lengths in the ACLR limb compared to the contralateral limb [278]. Coupled with findings that peak knee moments (i.e., external flexion and adduction) and knee excursions (both flexion and extension) are also commonly reduced in the ACLR limb

during walking [15, 282, 283, 437], modifying step-lengths may be a useful strategy to combat these gait deviations and facilitate larger ACLR limb loading. Nonetheless, it is not clear how responsive persons with ACLR may be to cadence manipulations and thus, studies aimed at characterizing the biomechanical effects of manipulations in cadence, in an ACL population are warranted to evaluate the merit of this potential strategy as a gait retraining option.

As such, the purpose of this investigation was to evaluate the acute effects of modifying step length via cadence manipulations during treadmill walking (i.e., 90%, 100%, and 110% preferred cadence) on knee joint biomechanics bilaterally in individuals 9-12 months after ACLR. Our primary biomechanical outcomes of interest included the peak external knee flexion moment (KFM) and knee flexion excursions (KFE). Our secondary outcomes of interest included the peak knee adduction moment (KAM) and knee extension excursion (KEE). We hypothesized that 1) knee biomechanics would increase linearly with step-lengths (i.e., peak moments, angles and excursions would be smallest at shorter step-conditions and greatest at longer step-conditions) and 2) that the magnitude of changes in biomechanical outcomes would be similar between both ACLR and the contralateral limb.

5.3 Methods

Participants

Sample size was determined *a priori* using effect sizes derived from previously published research evaluating the effects of cadence manipulations on walking biomechanics in healthy and ACLR individuals. Based on this previous data from Heiderscheit et al., [342], we estimated that using a moderate effect size for the KFM

(Cohen's f = 0.283), an α level of 0.025 to account for multiple outcome variables (KFM, KFE), and expected power of 0.80 (1-β), a minimum of 26 participants would be required to adequately power our study for our three cadence conditions (90%, 100%, 110% of self-selected cadence). Therefore, we enrolled thirty subjects with primary, unilateral ACLR who were between 9-12 months post-surgery to participate in this randomized crossover study. Participants were considered eligible for this study if they: 1) were between 14-45 years of age, 2) had no prior knee injury or surgery (other than current ACL in either leg 3) were between 9-12 months post-ACLR and 5) had no previous diagnosis of OA. Participants were excluded if they 1) have multiple ACLR's unilaterally or bilaterally, and/or 2) had an allograft reconstruction. Surgical information was verified via medical records review. All participants meeting criteria underwent a single two-hour session in which they completed gait analyses across a series of randomized conditions. The University of Michigan Medical School Institutional Review Board approved all methods used in the current investigation (IRB approval number: HUM00169174). Data from two subjects was excluded post-hoc before analyses due to incomplete or corrupt data and data from twenty-eight subjects are presented herein (Table 1).

Table 5.1. Participant demographics. Data are represented as Mean (SD) unless otherwise stated. BMI = Body Mass Index. ACLR = Anterior cruciate ligament reconstruction.

Demographics	**Mean (SD)**
Age (yrs.)	23.5 (5.9)
Height (cm)	172.0 (9.3)
Weight (kg)	74.7 (14.7)
BMI (kg/m^2)	25.1 (3.6)
Sex (N)	16 F, 12 M
Time-Post ACLR (wks.)	43.5 (4.7)
Pre-Injury Tegner Score (Median (25-75% IQR))	7.5 (7-9)
Current Tegner Score (Median (25-75% IQR))	5.5 (5-7)
Preferred Walking Speed (m/s)	1.25 (0.10)
Preferred Cadence (steps/min)	112.03 (6.43)
Graft Type	Bone-Patellar-Tendon-Bone = 24
	Hamstring Tendon = 4
Meniscal Surgeries	None = 16
	Meniscectomy = 4
	Repair = 9
Collateral Ligament Injuries	MCL Injury = 1
	LCL Injury = 1
	Neither = 26

Determining Self-Selected Walking Speed

To set the treadmill speed for each participant, self-selected over-ground walking speed was captured as participants walked across a 20-m walkway 5 times at a "comfortable pace as if they were walking to class or in the park". Infrared timing gates

(Tractronix, Lenexa, KS) were placed in the middle of the walkway 2 meters apart and the average of all trials was used to calculate their self-selected walking speed (m/s), which was used to set the treadmill belt speed for all conditions.

Gait Biomechanics and Walking Assessments

Lower extremity biomechanics were collected using a 12-camera motion capture system (Qualisys, Gothenburg, Sweden) sampling at 200 Hz as participants walked on a fully instrumented split-belt treadmill (Bertec, Columbus, Ohio) sampling at 2000 Hz. Participants were outfitted with a total of 48 retroreflective markers. Static markers were placed bilaterally on the iliac crests, anterior superior iliac spines, greater trochanters, medial and lateral femoral epicondyles, malleoli, and the first and fifth metatarsal heads to determine joint centers as we have done previously [246, 322]. Dynamic markers included bilateral calcaneus markers and 7 rigid clusters of 4 non-collinear markers affixed on the sacrum and bilaterally on the thigh, shank and foot segments to minimize soft-tissue artifact of single markers placed directly on skin. A standing calibration trial was first captured, and then the static markers described above were removed leaving only the rigid clusters and calcaneus markers.

After collection of the standing calibration trial, participants completed a total of 3 walking conditions (preferred cadence, 110% and 90% of preferred cadence). Participants always started testing with the preferred cadence condition, while the other 2 conditions were completed in a randomized order. Subjects performed three, 30-second walking trials at each cadence condition. All 3 walking conditions were performed at each subject's self-selected walking speed. For each condition, participants were allotted 2-minutes to familiarize to the treadmill speed and cadence target prior to initiating

motion capture trials. During the first minute of this familiarization period, no audible feedback was provided to allow participants to reach steady-state cadence. After the first minute, participants were provided with an audible beat set to their target cadence and were given an additional 60-seconds to match the target cadence (acceptable limits within ± 2 steps/min). Cadence was tracked in real-time using a Garmin Running Dynamics Pod attached to the sacrum that was linked via Bluetooth to a Garmin Forerunner 245 (Garmin USA, Olathe, KS). Instructions for treadmill walking were standardized for all conditions and participants were instructed to maintain the body position in the center of the treadmill, to avoid cross-stepping as much as possible and to keep their eyes looking forward. For 90 and 110% cadence conditions, participants were given standardized instructions to match each heel strike with each successive metronome beat. These instructions were verbalized throughout the walking trials as reinforcement.

Biomechanical Outcome Measures

Raw marker and force plate data from the walking trials were exported to Visual 3D (C-Motion Inc., Germantown MD) for model construction. Marker position and force data were low-pass filtered using a fourth order zero-phase lag digital Butterworth Filter with cut-off frequencies set at 6 Hz and 10 Hz, respectively. The hip joint center was estimated using the Davis method [389]. A joint coordinate system was used to derive knee joint motions defined as motion of the shank relative to the thigh [390]. Filtered kinematic and kinetic data were combined for standard inverse dynamics procedures using inertial parameters estimated from Dempster [391]. Joint moments calculated from inverse dynamics were expressed as external and reported as absolute (Nm). Stance phase was identified using a threshold of 50N to define heel-strike and toe-off (to mitigate

potential influence of excess treadmill baseline noise). Peak external knee flexion and adduction moments (KFM and KAM, respectively), and knee flexion excursion (KFE) were extracted from the first 50% of stance phase. Knee flexion excursion was defined as the difference between peak KFA and the KFA at initial contact. Knee extension excursion (KEE) was defined as the difference between peak KFA and the minimum knee flexion angle during mid/terminal stance. For all biomechanical outcomes, the average value across all stance phases identified was used in statistical analyses to compare across conditions.

Statistical Analyses

Descriptive statistics were used to explore distributions of each outcome measure and box plots were used to identify outliers. All data was normally distributed and treated as such. For descriptive purposes, average changes in step-length for each condition were calculated and reported below (i.e., distance between proximal foot segments at heel-strike). Separate two (limb: ACLR, contralateral limb) x three (cadence: 90%, 100% and 110% preferred cadence) repeated measures ANOVAs were used for each dependent variable (i.e., KFM, KAM, KFE, KEE). If an interaction was significant, we followed models with post-hoc pairwise comparisons Bonferroni adjusted for multiplicity (reported α levels are adjusted and significance for interactions will be maintained as $\alpha = 0.05$). For significant main effects, two planned contrasts were employed to compare gait outcomes of interested between: 1) 90% and 100% preferred cadence, and 2) 100% and 110% preferred cadence. Alpha levels for planned contrasts were Bonferroni-corrected to account for multiplicity and were evaluated at $\alpha = 0.025$ (0.05/2 contrasts).

5.4 Results

Twenty-eight participants post-ACLR completed this study (Table 1), and all were able to achieve cadence targets for each condition (Table 2). The average change in walking cadence resulted in a nearly 1:1 change in step-length. For example, we observed that cueing a 10% faster cadence (i.e., 110% cadence condition) resulted in an average 9.2% ± 2.6% and 9.1% ± 2.8% decrease in step length in the ACLR and contralateral limb, respectively. Likewise, cueing a 10% slower cadence (i.e., 90% cadence condition) resulted in an average 10.2% ± 2.0% and 10.1% ± 2.7% increase in step length in the ACLR and contralateral limb, respectively.

We observed a significant main effect for cadence ($F_{2,54} = 16.53$, $p < 0.01$) and limb ($F_{1,27} = 14.97$, $p < 0.01$) for the KFM (Table 2), but the interaction was not significant ($p > 0.05$). Cueing a slower cadence (i.e., larger steps) induced a larger KFM for both the ACL and non-ACL limb (mean difference: 8.95 Nm [95% CI: 4.73,13.16]). However, cueing a faster cadence (i.e., smaller steps) did not induce a change in the KFM ($p > 0.05$). Collapsed across cadence conditions, we observed smaller KFM in the ACLR relative to the contralateral limb (mean difference: 7.97 Nm [95% CI: 3.75,12.20]).

We observed a significant limb x cadence interaction ($F_{2,54} = 9.79$, $p < 0.01$) for the KFE. In general, both limbs responded similarly to cadence manipulations for all gait outcomes (i.e., direction of change). However, post-hoc pairwise comparisons indicated that the ACLR limb exhibited a smaller magnitude of change in KFE between the preferred and 110% cadence condition relative to the uninjured limb (mean difference: 1.76° [95%CI: 1.05, 2.48] vs. 2.91° [95%CI: 2.05, 3.77], respectively). Collapsed across cadence conditions, we observed smaller KFE in the ACLR relative to the contralateral

limb (mean difference: 2.36º [95% CI: 1.40, 3.32] $p<0.01$). Lastly, our main effect of condition showed that collapsed across limbs, cueing a *faster* cadence (i.e., smaller steps) resulted in reduced KFE (mean difference: 2.60º [95% CI: 1.66, 3.01], $p<0.01$). However, cueing a *slower* cadence (i.e., larger steps) did not induce a change in KFE ($p > 0.05$).

For KEE, we observed a significant main effect for cadence ($F_{2,54} = 22.30$, $p<0.01$) and limb ($F_{1,27} = 47.38$, $p <0.01$) (Table 2). Bilaterally, cueing a *faster* cadence (i.e., smaller steps) did not reduce KEE ($p>0.05$). However, cueing a *slower* cadence (i.e., larger steps) resulted in a greater KEE (mean difference: 2.45º [95% CI: 1.53, 3.78], $p<0.01$). Collapsed across cadence conditions, we observed smaller KEE in the ACLR relative to the contralateral limb (mean difference: 4.02º [95% CI: 2.82, 5.22] $p<0.01$).

Lastly, no significant main effects or interactions were found for the KAM (all $p >0.05$) indicating persons with ACLR walked with similar KAM between limbs and KAM was relatively unchanged across all cadence manipulations.

Table 5.2 Gait biomechanical outcomes across all walking conditions. Data are represented as Mean (SD).

	90% Preferred Cadence		100% Preferred Cadence		110% Preferred Cadence	
Outcomes	**ACLR Limb**	**Uninjured Limb**	**ACLR Limb**	**Uninjured Limb**	**ACLR Limb**	**Uninjured Limb**
KFM (Nm)	55.91 (20.18)*†	65.88 (24.69)	48.27 (15.05)†	55.62 (19.12)	51.25 (17.14)†	57.85 (22.32)
KFE (Δ°)	13.66 (3.56)†	16.64 (3.41)	13.39 (2.97)†	16.01 (3.13)	11.63 (3.02)*†	13.10 (3.60)
KEE (Δ°)	9.36 (4.69)*†	14.38 (5.30)	7.42 (3.80)†	11.42 (3.97)	7.30 (3.75)†	10.36 (3.86)
KFA (°)	15.71 (6.70)*†	19.83 (6.18)	13.66 (6.25)†	17.18 (5.10)	13.91 (6.19)†	17.05 (4.78)
KAM (Nm)	45.82 (11.03)	45.75 (12.44)	44.50 (11.19)	44.92 (12.99)	44.89 (10.59)	44.27 (11.92)
Step Length (cm)	67.38 (4.30)*	66.88 (4.45)	61.23 (3.97)	60.68 (3.91)	55.48 (3.70)*	55.16 (4.29)
Actual Cadence (steps/min)	100.79 (5.95)		111.36 (6.71)		123.16 (7.56)	
Target Cadence (steps/min)	100.67 (5.90)		111.86 (6.56)		123.05 (722)	

*: Sig. difference between cadence conditions. †: Sig. difference between limbs. ACLR – Anterior cruciate ligament reconstruction; KFM – Knee flexion moment; KFE – Knee flexion excursion; KFA – Knee flexion angle; KAM – Knee adduction moment.

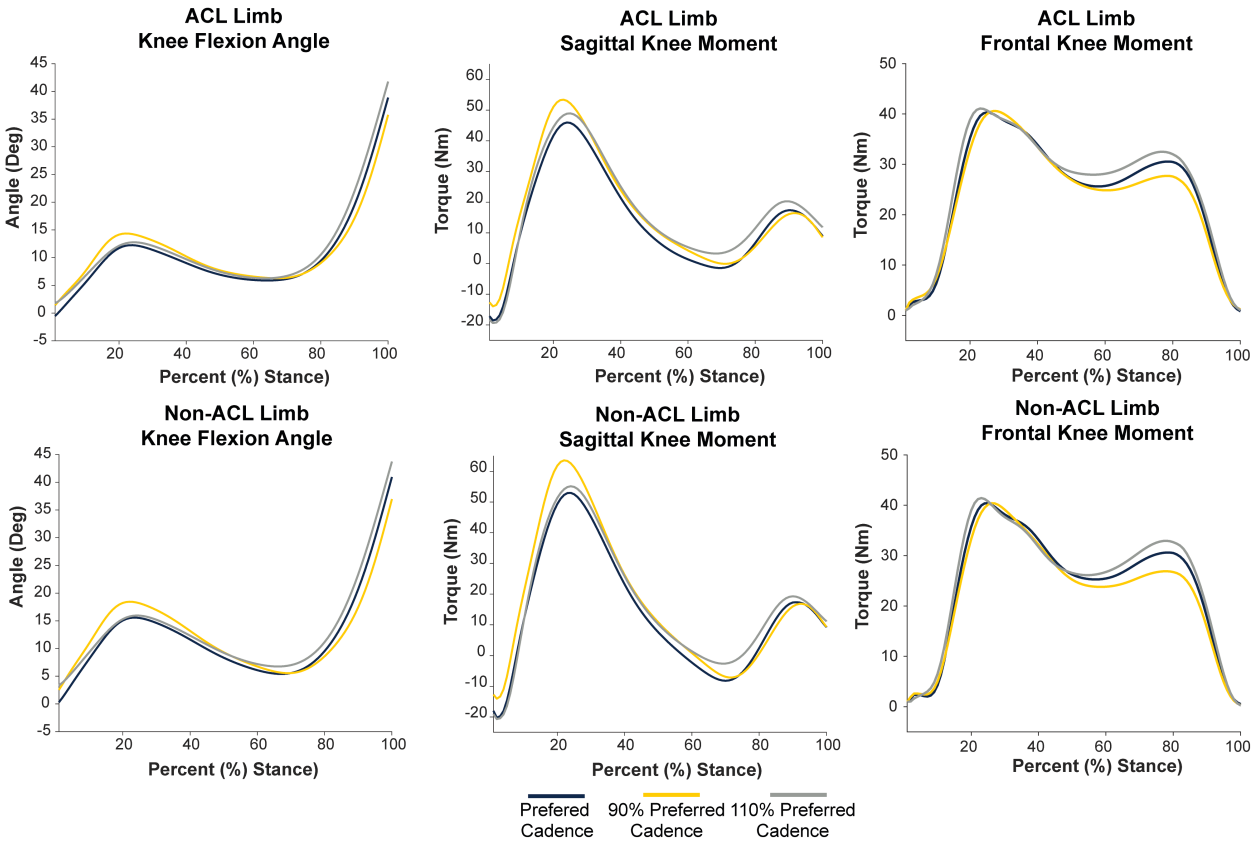

Figure 5.1. Ensemble waveforms for the sagittal plane knee flexion angle and moment across all cadence conditions. Blue solid lines denote the preferred cadence condition, while maize and gray denote the 90% and 110% cadence conditions, respectively.

5.5 Discussion

The purpose of this investigation was to evaluate the acute biomechanical effects of cadence manipulations on sagittal and frontal plane biomechanics in individuals between 9-12 months post-ACLR. In general, our findings show that manipulating cadence was able to acutely modulate knee kinetics and kinematics bilaterally in our cohort, but the biomechanical changes observed were limited to the sagittal plane. Overall, these data provide preliminary evidence that simple targets for gait retraining (i.e., cadence) can be effective to acutely target knee mechanics that are known to be affected in those with ACLR.

Data from our study suggest sagittal plane kinetics and kinematics are amenable to acute gait modifications in both the ACLR and contralateral limb. Our feedback condition inducing larger steps (i.e., 90% of preferred cadence) facilitated a larger KFM in the ACLR and uninjured contralateral limb compared to preferred walking conditions (Figure 1: approx. 15.71% and 18.91%↑, respectively). We reason greater peak KFM when cueing larger steps is likely attributed to the larger peak knee flexion angle observed during this condition (Table 2) as this may require a commensurate increase in the net knee extensor moment to provide bodyweight support. However, we did not find cueing smaller steps (i.e., 110% of preferred cadence) reduced the KFM, which is contrary to our hypothesis and previous research [369, 436, 438]. It is reasonable the small change in cadence we used (10% from preferred) was insufficient to impact the KFM given we did not observe any knee kinematic changes that would necessitate a smaller KFM (i.e., a smaller peak knee angle). Typically, reductions in knee angles are a common modification when adopting smaller steps during more dynamic tasks like running using similar 10% deviation from preferred cadence [23, 342]. However, walking is characterized by much smaller joint kinematics and thus, larger manipulations may be needed (i.e., 15-20% changes) as done in other investigations during walking ([369, 436, 438]. Nonetheless, our observations that modifications cueing larger steps can increase knee extensor demands may be one strategy to facilitate individuals to increase the load on their ACLR limb, at least in the sagittal plane. This could be potentially beneficial as a training stimulus during early rehabilitation when sagittal plane kinetics are substantially reduced in the ACLR limb.

We also observed that sagittal plane knee excursions were affected in response to cadence manipulations. When cueing smaller steps (i.e., faster cadence), we saw a reduction in KFE. Interestingly, our data suggests that the ACLR limb underwent a smaller change in the KFE compared to the uninjured contralateral limb in response to cadence manipulations (13.14% vs. 18.91% Δ°). We reason that this finding could be partly attributed to KFE already being smaller in the ACLR limb. Thus, it is not surprising the reductions in joint excursion in response to cadence manipulations were greater in the contralateral uninjured limb as alterations in joint excursions are generally less marked. However, we note cueing larger steps did not impact KFE which was unexpected and not consistent with our hypotheses. At fixed speeds, healthy individuals generally exhibit a positive linear relationship between peak knee angles and excursions and step-lengths during both walking and running [342, 369, 439]. Thus, we reasoned using a slower cadence feedback could be useful to increase joint excursions in the ACLR limb as a means to facilitate a less-stiff knee pattern. However, as we only provided feedback for a brief period (i.e., several minutes), a greater amount of feedback/training time, and/or the use of larger cadence manipulations (i.e., 15-20% above/below) may be required to alter knee kinematic outcomes like KFE.

In addition to changes in gait outcomes during early stance (i.e., KFM, KFE), we found that cadence manipulations also impacted midstance kinematics such as KEE. When cueing larger steps, we observed that KEE was increased approximately 2.5° bilaterally but KEE remained unchanged at smaller step conditions. In general, midstance gait outcomes like changes in KEE are less often reported after ACLR and thus, not well characterized in this population [437]. In those with knee OA, stiffened knee mechanics

in midstance such as reduced KEE and knee greater extension moments are linked to increased pain and worse disease severity. These gait deviations are often accompanied by reduced knee extensor strength and aberrant muscular activation patterns (i.e., co-contraction) during mid/terminal stance suggesting that impaired neuromuscular function, may drive these stiffened-knee patterns in midstance [327, 440, 441]. Stiff-knee mechanics in midstance are also seen in ACL populations as recent data from our lab suggests those with ACLR walk greater dynamic joint stiffness in the ACLR relative to the uninvolved limb, which was partly attributed to a smaller KEE (In Review). Thus, the ability of larger steps to increase knee excursions in midstance may represent a positive adaptation to our biofeedback cue and could be a means to help remediate some common walking impairments like stiffened knee behavior in those with ACLR. Nonetheless, it may be beneficial for future work to evaluate how biofeedback strategies, such as cadence cues, also impact dynamic knee stiffness metrics in both early and mid-stance after ACLR to more comprehensively understand the biomechanical effects of these gait manipulations.

We were also interested in evaluating secondary effects of our gait modification strategy (cadence manipulations) on kinetic outcomes such as the KAM. Here, we found that the peak KAM (i.e., first 50% of stance) remained relatively unchanged across all cadence conditions (Figure 1). Two previous investigations in healthy populations have shown cueing smaller steps induced a reduction in the first peak KAM, though, the KAM did not appear to increase in larger step conditions, which is partly in agreeance with our data [366, 442]. However, these studies evaluated gait over-ground and thus, some differences in our protocols may partly explain the lack of observed changes in KAM

across our conditions. In our study, we utilized a split-belt instrumented treadmill for gait assessments and asked participants to attempt to limit cross-over steps between belts which may have induced a slightly larger base-of-support than typical over-ground walking. Consequently, this may have impacted how our gait manipulations affected the KAM as compared to studies providing feedback over-ground. It is also plausible that individuals may adopt slightly different biomechanical strategies between over-ground and treadmill training given that feedback durations are likely longer and more continuous (i.e., cyclic) during treadmill walking. It is important to note we assessed peak KAM in the first 50% of stance given this metrics strong association with medial tibiofemoral contact forces in both healthy and ACLR populations. It is possible that cadence changes evoked additional changes in the KAM waveform in later phases of stance which may be missed when discrete analyses are employed. Future follow-up investigations may consider using functional data statistics (i.e., SPM, Functional ANOVA's) when evaluating the effects of gait retraining strategies given that these methods are capable of comparing the entire gait waveform across stance phase. Nonetheless, our results suggest at least acutely, the biomechanical effects of manipulating step-length during treadmill walking mostly impact the sagittal plane during the first half of stance.

Overall, our data adds promising results to the growing efforts to identify potential options for gait retraining in populations with ACLR. Although scarce, previous gait retraining efforts to remediate poor gait mechanics in those with ACLR have been generally accomplished via real-time feedback paradigms such as providing feedback targets to manipulate the vertical GRF (i.e., to increase or decrease). For example, Luc-Harkey et al., showed that cueing a small 5% increase in the vertical GRF was able to

elicit greater KFM in the ACLR and contralateral limb of approximately 13.22% and 14.89%, respectively [360, 361]. Although effective, real-time feedback of force data provides some issue for clinical use due to the need for an instrumented treadmill. Though, it is possible to estimate vertical GRF via low-cost load soles' and/or inertial sensors [443, 444], however, it is unclear if similar effects on knee outcomes like the KFM would be seen using these technologies. Alternatively, we observed relatively similar magnitudes of changes in the KFM using simple cadence manipulations in both the ACLR and contralateral limb of approximately, 15.71% and 18.91%, respectively. As such, our findings may provide some support for the use of simple gait metrics as retraining targets given the need for relatively minimal equipment, and comparable magnitudes of biomechanical changes relative to more sophisticated feedback paradigms.

It is important to highlight that between-limb differences in all our gait outcomes were maintained across all our cadence manipulations meaning both limbs exhibited relatively similar increases or decreases to gait changes. While regaining symmetry is considered an important outcome of ACLR rehabilitation, it is not entirely clear if this is a sufficient goal for gait outcomes to mitigate risk for post-traumatic OA development or if generally improving ACLR limb loading magnitude is more important to minimize early joint degeneration associated with limb unloading in this population [258, 312]. Nonetheless, targeting gait symmetry may also have some benefit as a gait retraining outcome. It is possible that manipulating cadence in combination with other task-constraints (i.e., walking speed) may allow researchers/clinicians to target both loading magnitude and gait asymmetries. For example, we have shown previously between-limb differences in GRFs reduce (i.e., participants walked more symmetrically) when

individuals with ACLR walk at slower than preferred speeds [25]. As such, it may be beneficial for future work to evaluate the combined effects of manipulating both preferred speed and cadence on gait mechanics and between-limb symmetries after ACLR, knowledge that will help better understand potential strategies to restore gait in this population.

This study has inherent limitations. Overall, our gait modification strategy was performed in a single session and aligned with the study purpose focused on evaluating the acute biomechanical effects of manipulating cadence/step-lengths in those with ACLR. As such, we did not evaluate the ability of our participants to recall the feedback conditions or test any after-effects of these gait manipulations. Given we only provided feedback for a total of approximately 5-7 minutes (total time including acclimation phases and testing trials), it is not likely these acute gait changes persisted. We also utilized relatively small cadence manipulations of 10% above and below preferred walking cadence which may have explained our lack of differences in outcomes like the KFE. During our pilot testing, we evaluated cadence manipulations upwards of 30% (in 5% increments) but participants expressed difficulty in meeting cadence targets greater than 20% from preferred. As our pilot data, and that from others [369, 436, 438] suggested that 10% was a sufficient stimulus to induce sagittal plane gait changes, we chose this cadence change so as to ensure all participants in our study we are able to meet gait targets. Indeed, everyone was capable of meeting the 10% cadence targets and we observed a near 1:1 change in step-length as a result of the cadence manipulations (Table 1). Nonetheless, it may be beneficial to evaluate how manipulating cadence 15% or 20% above preferred to evaluate if the magnitude of biomechanical changes scale with

cadence/step-length changes. Lastly, we believe it is important to highlight that we evaluated only external knee moments in our cohort, which are not fully reflective of the internal joint contact forces and neglect compensatory changes that may be occurring about adjacent joints. While both the KFM and KAM assessed in our cohort both uniquely contribute to tibiofemoral contact force estimates [445], it may be beneficial that future work includes estimates of knee contact forces via musculoskeletal modeling (i.e., EMG driven models). Further, follow-up studies may consider evaluating how the hip and ankle may be compensating/adapting to cadence biofeedback interventions.

5.6 Conclusion

Cueing individuals with ACLR to walk with a faster or slower cadence during treadmill walking is capable of acutely altering sagittal plane knee kinetics and kinematics. We contend that manipulating cadence has strong clinical utility as a gait retraining strategy as it requires relatively minimal equipment (outside of a treadmill) given freely available phone apps can provide audible cues. As gait deviations continue to persist in persons with ACLR, additional rehabilitative efforts, such as gait-specific interventions may be required to ensure the proper restoration of normal gait in this population.

Chapter 6 Walking Speed Differentially affects Lower Extremity Biomechanics in Individuals with Anterior Cruciate Ligament Reconstruction compared to Controls

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6.1 Abstract

Background: Walking biomechanics are commonly affected after anterior cruciate ligament reconstruction and differ compared to uninjured controls. Manipulating task difficulty has been shown to affect the magnitude of walking impairments in those early after knee surgery but it is unclear if patients in later phases post-op are similarly affected by differing task demands. Here, we evaluated the effects of manipulating walking speed on between-limb differences in ground reaction force and knee biomechanics in those with and without anterior cruciate ligament reconstruction.

Methods: We recruited 28 individuals with anterior cruciate ligament reconstruction and 20 uninjured control participants to undergo walking assessments at three speeds (self-selected, 120%, and 80% self-selected speed). Main outcomes included sagittal plane knee moments, angles, excursions, and ground reaction forces (vertical and anterior-posterior).

Findings: We observed walking speed differentially impacted force and knee-outcomes in those with anterior cruciate ligament reconstruction. Between-limb differences increased at fast and decreased at slow speeds in those with anterior cruciate ligament

reconstruction while uninjured participants maintained between-limb differences regardless of speed (partial η^2 = 0.13 – 0.33, p<0.05). Anterior cruciate ligament reconstruction patients underloaded the surgical limb relative to both the contralateral, and uninjured controls in GRFs and sagittal plane knee moments (partial η^2 range= 0.13 – 0.25, p < 0.05).

Interpretation*:* Overall, our findings highlight the persistence of walking impairments in those with anterior cruciate ligament reconstruction despite completing formal rehabilitation. Further research should consider determining if those displaying larger changes in gait asymmetries in response to fast walking also exhibit poorer strength and/or joint health outcomes.

6.2 Introduction

Anterior cruciate ligament reconstruction (ACLR) is a common lower extremity orthopedic procedure that imposes serious short- and long-term repercussions on a person's physical function and psychological well-being[12, 446-448]. Individuals with ACLR, for example, consistently display profound gait asymmetries and compensatory gait patterns when compared with uninjured individuals [10, 11, 16, 20, 69] The inability to restore walking patterns after ACLR is troubling from a post-traumatic osteoarthritis (post-traumatic OA) perspective given that articular cartilage is a highly conditioned and mechanosensitive tissue. As such, unresolved walking impairments after ACLR have been widely considered to precipitate post-traumatic OA development in this population [5, 75]. For example, evidence has linked unresolved walking abnormalities with early and chronic degenerative changes to knee articular cartilage (e.g., lesser proteoglycan density and cartilage thinning) after ACLR [59, 246, 258, 312, 378, 380, 449]. Thus, identifying and monitoring the recovery (or persistence) of gait asymmetries in individuals with ACLR is likely an essential objective when evaluating post-operative patients, as such knowledge may help inform clinical decision-making and monitor patient progress throughout rehabilitation.

Walking biomechanical assessments are often aimed at capturing movement patterns that closely resemble how individuals may ambulate in free-living conditions. Typically, this is estimated by testing individuals at what they perceive as their "preferred" or "comfortable" walking speed. Nonetheless, in-lab gait assessments do not fully mimic free-living conditions as walking is inherently variable and can require ambulating at a variety of speeds [450] and under a variety of conditions; thus, the overall difficulty of

walking also presumably changes as individuals modulate speed. Walking speed is well known to have strong systematic effects on joint-level demands as fast speeds often increase the muscular demand required to support and propel the body forward while slower speeds reduce active muscle contributions to support [368, 414, 451, 452]. Consequently, it is possible that when tasked with heightened demands for support (and propulsion), individuals with ACLR may differentially alter movement patterns compared to uninjured individuals without musculoskeletal impairments.

Indeed, a host of recent studies have investigated whether those with ACLR may differentially alter interlimb mechanics in response to varying task demands to better understand the extent of compensatory movement strategies post-surgery [25, 68, 69, 304]. For example, it has been shown that more difficult motor tasks like navigating stairs, hopping, or running often lead to more discernible differences in joint-level mechanics in ACLR patients, both between limbs and when compared to uninjured controls [37, 67-69]. In uninjured populations, however, between-limb differences in joint mechanics (i.e., right vs. left or dominant vs. non dominant) are generally small and seem to be relatively invariant across tasks [25, 69], suggesting tasks requiring increased muscular and/or attentional demands may provoke additional joint-level compensations in those with ACLR. Thus, it may be beneficial to adjust task demands to capture a patient's functional capacity, as this may help reveal more subtle movement deficiencies during the later phases of ACLR rehabilitation.

Recently, we have demonstrated that manipulating walking speed (i.e., fast or slow walking) is a feasible task-specific approach that may help better capture between-limb differences in patients early post-ACLR [25]. Specifically, we observed that vertical and

posterior ground reaction force (GRF) between-limb differences were exacerbated when walking at speeds faster than self-selected. Importantly, speed changes had negligible effects on between-limb differences in healthy groups (which were largely symmetrical) suggesting that uninjured individuals can maintain similar between-limb mechanics regardless of the demand or type of movement task. However, an important limitation in this previous study was that it only included individuals with acute ACLR (i.e., ~ 2 months post-surgery) who typically exhibit more pronounced gait impairments that are readily discernable even with a small increase in task difficulty [25]. Moreover, we only examined measures of limb loads (i.e., ground reaction forces) that may not be indicative of biomechanical changes at the knee. As a result, it is unclear if faster walking speeds are similarly sufficient at exacerbating gait impairments in individuals at later-phases of rehabilitation where asymmetries may be more subtle. Further, while ground reaction force components have been previously associated with sagittal plane knee kinematics and kinetics [15, 453], it may be useful to directly evaluate the effects of walking speed on knee-specific gait metrics (e.g., knee angles and moments) to gain a better understanding of this approach to detect compensatory gait strategies in this population.

Therefore, the primary purpose of this study was to comprehensively evaluate the effects of manipulating walking speed (i.e., 20% above and below self-selected speeds) on limb (vertical and anterior-posterior GRF) and joint (knee flexion moment, angle, and excursions) between-limb differences in gait biomechanics in individuals who were between 9-12 months post-ACLR and uninjured controls. A secondary purpose of the study was to evaluate gait biomechanical differences between ACLR participants and matched controls. Our primary hypothesis was that faster walking speeds would induce

larger between-limb differences in gait mechanics in those with ACLR but not healthy controls. Our secondary hypothesis was that those with ACLR would walk with lesser GRFs, and knee flexion moment and angles bilaterally compared to uninjured control participants.

6.3 Methods

Participant Recruitment:

We enrolled twenty-eight participants with primary, unilateral ACLR who were between 9-12 months post-surgery (Demographics listed in Table 1) and a convenience sample of twenty control participants who were matched on sex, age (± 3 yrs.), and body mass index (± 2 kg/m^2). Sample size was determined using an *a priori* power analysis (G Power, 3.1) [454] based on our previous published data examining the effects of walking speed on ground reaction force asymmetries in individuals acutely after ACLR [25]. Using an observed partial η^2 of 0.125, an α level of 0.017 to account for multiple main outcome variables (knee flexion moment [KFM], angle [KFA], and excursion [KFE]), and expected power of 0.80 (1-β), a minimum of 18 participants per group would be required to observe differences in speed effects between groups. Participants with ACLR were considered eligible if they: 1) were between 14-45 years of age, 2) had no prior knee injury or surgery (other current ACL), 3) were between 9-12 months post-ACLR and 4) had no previous diagnosis of OA. Participants were excluded if they 1) had multiple ACLR's, and/or 2) had an allograft reconstruction. For uninjured controls, participants were required to be free of any orthopedic lower-extremity surgeries over their lifetime and free from any lower-extremity injury within the previous six months. All participants meeting these criteria

underwent a single two-hour session in which they completed gait analyses across a series of randomized walking speed conditions.

Table 6.1. Participant demographics. Data represented as mean (SD) unless otherwise stated. BMI = Body Mass Index. ACLR = Anterior cruciate ligament re construction. IQR = Interquartile Range (25% and 75% IQR).

Demographics	ACLR Participants	Healthy Controls
	Mean (SD)	Mean (SD)
Age (yrs.)	23.5 (5.9)	24.2 (4.1)
Height (cm)	172.0 (9.3)	171.1 (7.8)
Weight (kg)	74.7 (14.7)	74.0 (12.4)
BMI (kg/m^2)	25.1 (3.6)	25.3 (3.7)
Sex (N)	16 F, 12 M	12 F, 9 M
Time-Post ACLR (wks.)	43.5 (4.7)	NA
Preferred Speed (m/s)	1.25 (0.10)	1.30 (0.19)
IKDC Score (Median (IQR))	86.8 (77.9-92.3)	100.0 (94.3-100.0)
KOOS Total (Median (IQR))	88.0 (80.8-91.0)	99.0 (97.0-100.0)
Graft Type	Bone-Patellar-Tendon-Bone = 24 Hamstring Tendon = 4	NA
Meniscal Surgeries	None = 16 Meniscectomy = 4 Repair = 9	NA
Collateral Ligament Injuries	MCL Injury = 1 LCL Injury = 1 Neither = 26	NA

Determining Self-Selected Walking Speeds:

Treadmill speeds were set for each participant by first assessing self-selected over-ground walking speed as participants walked across a 20-m walkway 5 times at a comfortable pace “as if they were walking to class or in the park”. Infrared timing gates (Tractronix, Lenexa, KS) were placed in the middle of the walkway 2 meters apart and the average of all trials was used to calculate their self-selected walking speed (m/s) which was used to set the treadmill speed for all subsequent conditions as has been done previously [25, 361]. We opted to assess self-selected speeds overground to approximate each individual’s habitual speed during normal activity.

Gait Biomechanics and Walking Assessments:

Walking biomechanics were assessed using a 10-camera motion capture system (Qualisys, Gothenburg, Sweden) sampling at 200 Hz as participants walked on a fully instrumented split-belt treadmill (Bertec, Columbus, Ohio) sampling at 2000 Hz. Participants were outfitted with a total of 48 retroreflective markers placed bilaterally consisting of both static and dynamic markers. Static markers were placed bilaterally on the iliac crests, anterior superior iliac spine, greater trochanter, medial and lateral femoral epicondyles, malleoli, and the first and fifth metatarsal heads to determine joint centers as we have done previously [246, 322]. Dynamic markers included bilateral calcanei markers and rigid clusters of four non-collinear markers affixed on the sacrum and bilaterally on the thigh, shank, and foot segments to minimize soft-tissue artifact of single markers placed directly on skin. A standing calibration trial was captured, and then static markers described above were subsequently removed leaving only the rigid clusters and calcaneus markers.

After the standing calibration trial, all participants completed a total of five walking conditions as part of a larger study [388]. These included a self-selected condition, two cadence conditions, and two speed conditions. For the purposes of this manuscript, only speed conditions are presented for each group (self-selected, 20% above and 20% below self-selected). Speed manipulations of 20% were chosen based on previous research both in ACLR [25] and uninjured populations [455, 456] to sufficiently increase (or decrease) muscular demand while not being too extreme (i.e., 30 or 40%) where individuals may near the walk-run transition (particularly in fast walkers). Uninjured control participants also completed an additional sixth walking condition where the treadmill was set to their matched-ACLR participants' self-selected pace which allowed us to make group comparisons at matched-speeds, minimizing any potential effect of speed differences in groups. Self-selected speeds were always completed first, and all subsequent cadence or speed manipulations were fully randomized. For each walking condition, participants were given two minutes to acclimate to the target treadmill speed prior to initiating motion capture trials and participants received standardized instructions to maintain the body position in the center of the treadmill, to avoid cross-stepping as much as possible and to keep their eyes looking forward. These instructions were verbalized throughout the walking trials as reinforcement. After the acclimation period, a total of three, successive 30-second motion capture trials were recorded for each condition. Uninjured control participants always completed matched speed conditions last.

Biomechanical Outcome Measures

Raw marker and force plate data from the walking trials were exported to Visual 3D (C-Motion Inc., Germantown MD) for model construction. Marker position and force data were low-pass filtered using a fourth order zero-phase lag digital Butterworth Filter with cut-off frequencies set at 6 Hz and 10 Hz, respectively. The hip joint center was estimated using the Davis method [389]. A joint coordinate system was used to derive knee joint motions, which was defined as motion of the shank relative to the thigh [390]. Filtered kinematic and kinetic data were combined for standard inverse dynamics procedures using inertial parameters estimated from Dempster [391]. Joint moments calculated from inverse dynamics were expressed as external moments and reported normalized to a product of body weight times height (%BW*Ht.). Stance phase was identified using a threshold of 50N to define heel-strike and toe-off (to mitigate potential influence of excess treadmill baseline noise).

For GRF outcomes, we evaluated the first peak vertical GRF, peak braking GRF and peak propulsive GRF. For knee joint outcomes, the peak external KFM, KFA, and KFE were extracted from the first 50% of stance phase. Knee flexion excursion was defined as the difference between peak KFA and the KFA at initial contact. On average, approximately 25 stance phases were typically identified across all walking conditions and the average value from all stance phases in all 3 trials was used in subsequent statistical analyses. Ensemble averages of all gait outcomes for each limb are depicted in Figure 3. For uninjured control participants, representative “ACL” and “Non-ACL” limbs were determined by random assignment based on the proportion of left and right ACL limbs from our injured cohort. Our individuals with ACLR had an even distribution of

affected limbs (i.e., 14 left and 14 right ACLRs) and thus, we randomly assigned 10 uninjured control participants as left “ACL” limbs and 10 uninjured control participants with right ACL” limbs.

Statistical Analyses:

Statistical models were conducted using SPSS version 28 (IBM, Armonk, NY USA). Ground reaction force (vertical GRF, braking GRF, propulsive GRF) and knee biomechanical outcomes (KFM, KFA, KFE) were compared across speeds in ACLR and control participants via separate two (group) x two (limb) x 3 (speed) repeated measures ANOVA models. Significant 3-way interactions were followed by separated 2 x 3 repeated measures ANOVA models for each group and accordingly, main effects or interactions were followed with post-hoc t-tests that were Bonferroni corrected to account for multiplicity of comparisons. Alpha levels for significance were retained at the level of $\alpha = 0.05$ as SPSS corrects the *p*-value of the observed test result, rather than correcting the alpha level. To compare GRF and knee biomechanics between groups, we used a 2 (limb) x 2 (group) ANOVA. Data from these between-group comparisons were from the self-selected speed condition in ACLR participants and from the matched speed condition in healthy control participants.

To aid in interpretations of statistical analyses, all changes in knee and force variables were also compared to previously reported minimally clinically important differences (MCID) or minimal detectable change (MDC) where available using between-limb differences (i.e., ACLR – Non-ACLR limbs) which are reported in Table 2. Previous data from Di Stasi et al., reports MCID for the sagittal knee moments and angles as 0.003 BW * Ht. and 3°, respectively [285]. For GRF variables, few data in the ACLR literature

have reported MCID or MDC values and thus, we are interpreting MDC data from treadmill walking post-stroke [457]: Vertical GRF MDC = 0.017 BW, AP GRF = 0.008 BW.

6.4 Results:

Ground Reaction Force Characteristics – Speed Comparisons:

We observed a significant group by limb by speed 3-way interaction for the braking GRF ($F_{2,92}$ = 5.021, partial η^2 = 0.10, p < 0.01). We followed the significant 3-way interaction with separated ANOVA models for each group and found that in the ACLR cohort, there was a significant limb by speed interaction ($F_{2,54}$ = 13.31, partial η^2 = 0.33, p < 0.01) wherein between-limb differences in the braking GRF increased as a function of walking speeds (i.e., 80% < SS < 120% -- Figure 1 and Table 2, all p < 0.047). Compared to previously reported values, the change in braking GRF between-limb differences between self-selected and fast speeds (≈0.009 BW) exceeded MDC values of 0.008 BW [457]. However, for uninjured control participants we did not observe any significant limb by speed interactions for the ($F_{2,38}$ = 0.60, partial η^2 = 0.031, p > 0.56) or limb main effects ($F_{1,19}$ = 0.23, partial η^2 = 0.01, p = 0.64) but the speed main effect was significant ($F_{2,38}$ = 128.85, p < 0.01) where braking GRF magnitudes increased linearly with speeds (i.e., smallest at slow speeds, greatest at fast speeds – Figure 1).

We did not observe a significant group by limb by speed 3-way interaction for the vertical or propulsive GRF ($F_{2,92}$ = 5.021, partial η^2 = 0.10, p < 0.01). For the ACLR cohort, we observed limb main effects for the vertical GRF ($F_{1,27}$ = 6.12, partial η^2 = 0.19, p = 0.02) but not for the propulsive GRF (p = 0.52) shown in Figure 1. Individuals with ACLR walked with smaller first peak vertical GRF in the ACLR limb relative to their uninjured limb (Table 2. mean difference: 0.018 BWs [-0.003, -0.034]) which exceed MDC values.

For uninjured control participants, no limb main effects were observed for either the vertical GRF ($F_{1,20}$ = 1.58, partial η^2 = 0.08, p = 0.22) or propulsive GRF ($F_{1,20}$ = 0.39, partial η^2 = 0.02, p = 0.54). In both ACLR and uninjured control groups, main effects for speed were observed ($F_{2,54}$ = 105.08 – 760.64, partial η^2 = 0.81 – 0.97, p < 0.01) wherein all GRF magnitudes increased linearly with speeds (i.e., smallest at slow speeds, greatest at fast speeds – Figure 1).

Knee Kinetics and Kinematics – Speed Comparisons:

We observed a significant group by limb by speed 3-way interaction for the peak KFM ($F_{2,92}$ = 10.13, partial η^2 = 0.18, p < 0.01) and peak KFA ($F_{2,92}$ = 6.60, partial η^2 = 0.13, p < 0.01) but not for KFE ($F_{2,92}$ = 5.794, partial η^2 = 0.06, p = 0.054). We followed the significant 3-way interaction with separated ANOVA models for each group and found that in the ACLR cohort, there was a significant limb by speed interaction for the peak KFM ($F_{2,54}$ = 12.40, partial η^2 = 0.32, p < 0.01) and KFA ($F_{2,54}$ = 3.81, partial η^2 = 0.12, p = 0.03) wherein between limb differences in the KFM and KFA increased as speeds increased (i.e., between-limb differences were greatest at fast speed – Figure 2 and Table 2). At 80% of self-selected speed, KFM was not different between the ACLR and contralateral limb (p = 0.10). In general, between-limb differences in the KFM and KFA exceeded established MCIDs (0.003 % BW*Ht) at self-selected and 120% self-selected speeds (Table 2) but only the change in KFM between-limb differences met this MCID value (Table 2). For the KFE, we observed limb main effects ($F_{1,27}$ = 25.01, partial η^2 = 0.48, p < 0.01) indicating that, collapsed across speeds, individuals with ACLR walked with lesser KFE in the ACLR limb compared to the contralateral (mean difference: 2.6°) which was comparable to reported MCIDs of 3°. For uninjured control participants, no

significant limb by speed interaction ($F_{2,38}$ = 0.76 – 2.87, partial η^2 = 0.04 – 0.13, p > 0.07) or limb main effects ($F_{1,19}$ = 0.02 – 1.12, partial η^2 = 0.01 – 0.06, p > 0.30) were observed for any knee-specific outcomes indicating that uninjured controls exhibited similar sagittal knee moments, angles, and excursions between limbs (Figure 2). For both the ACLR and uninjured control groups, we observed significant speed main effects on all knee joint outcomes ($F_{2,38}$ = 57.62 – 138.85, partial η^2 = 0.75 – 0.88, p < 0.01) wherein peak moments, angles and excursions increased linearly across speeds (p<0.01).

Ground Reaction Force Characteristics - Between Group Comparisons:

We observed significant group effects for the vertical GRF ($F_{1,46}$ = 7.88, partial η^2 = 0.146, p = 0.01) wherein collapsed across limbs, individuals with ACLR walked with reduced first peak vertical GRFs compared to uninjured control participants (mean difference [95% CI]: 0.048 BWs [0.013, 0.082], p = 0.01). We also observed significant group x limb interactions for the braking GRF ($F_{1,46}$ = 9.276, partial η^2 = 0.168, p < 0.01). Between groups, the ACLR limb exhibited smaller braking GRFs compared to healthy controls participants (mean difference [95% CI]: 0.033 BWs [0.017, 0.048], p < 0.01) but the non-ACLR limb did not (p = 0.14). Lastly, no significant group or interaction effects were observed for the propulsive GRF (p > 0.08).

Knee Kinetics and Kinematics – Between Group Comparisons

We observed significant group by limb interactions for all knee-specific outcomes ($F_{1,46}$ = 6.779 – 15.30, partial η^2 = 0.13 – 0.25, p < 0.012). Between groups, the ACLR limb exhibited smaller peak KFMs (mean difference [95% CI]: 0.011 %BW*Ht. [0.003, 0.018], p = 0.035), and peak KFE (mean difference [95% CI]: 2.34° [0.64, 4.04], p < 0.01) compared to uninjured control participants, however, the non-ACLR limb was not different

compared to uninjured controls (p = 0.38). No differences between groups were observed in the peak KFA in either the ACLR (p = 0.18) or the non-ACLR limb (p = 0.67). Ensemble waveforms depicting limb comparisons can be found in Figure 3.

Table 6.2. Between-limb differences for gait outcomes across speeds in individuals with ACLR. Data presented as mean between-limb difference and 95% CI's denoted in brackets []. Bolded data indicates significant interaction effects of speed x limb. Interlimb difference data from healthy controls were small and non-significant and are only depicted graphically in Figures 1 and 2.

Interlimb Differences	ACLR Cohort		
	80% SS Speed	**SS Speed**	**120% SS Speed**
Vertical GRF (%BW)	-0.009 [-0.002, -0.021]	-0.018 [-0.003, -0.034]	-0.026 [-0.006, -0.047]
Braking GRF (%BW)	**0.007 [0.0001, 0.013]**	**0.013 [0.005, 0.022]**	**0.022 [0.012, 0.032]**
Propulsive GRF (%BW)	-0.001 [-0.006, 0.005]	-0.041 [-0.034, -0.048]	-0.002 [-0.007, 0.003]
KFM (%BW*Ht)	**-0.003 [-0.007, 0.001]**	**-0.006 [-0.002, -0.010]**	**-0.009 [-0.005, -0.0014]**
KFA (°)	**-2.697 [-0.961, -4.432]**	**-3.518 [-2.196, -4.841]**	**-3.852 [-0.961, -4.432]**
KFE (Δ°)	-2.254 [-1.149, -3.360]	-2.615 [-1.535, -3.696]	-2.830 [-1.682, -3.978]

SS = Self-selected. ACLR = Anterior cruciate ligament reconstruction. GRF = Ground reaction force .BW = Bodyweight. KFM = Knee flexion moment. KFA = Knee flexion angle. KFE = Knee flexion excursion.

Figure 1. Ground Reaction Force Outcomes Across Walking Speeds

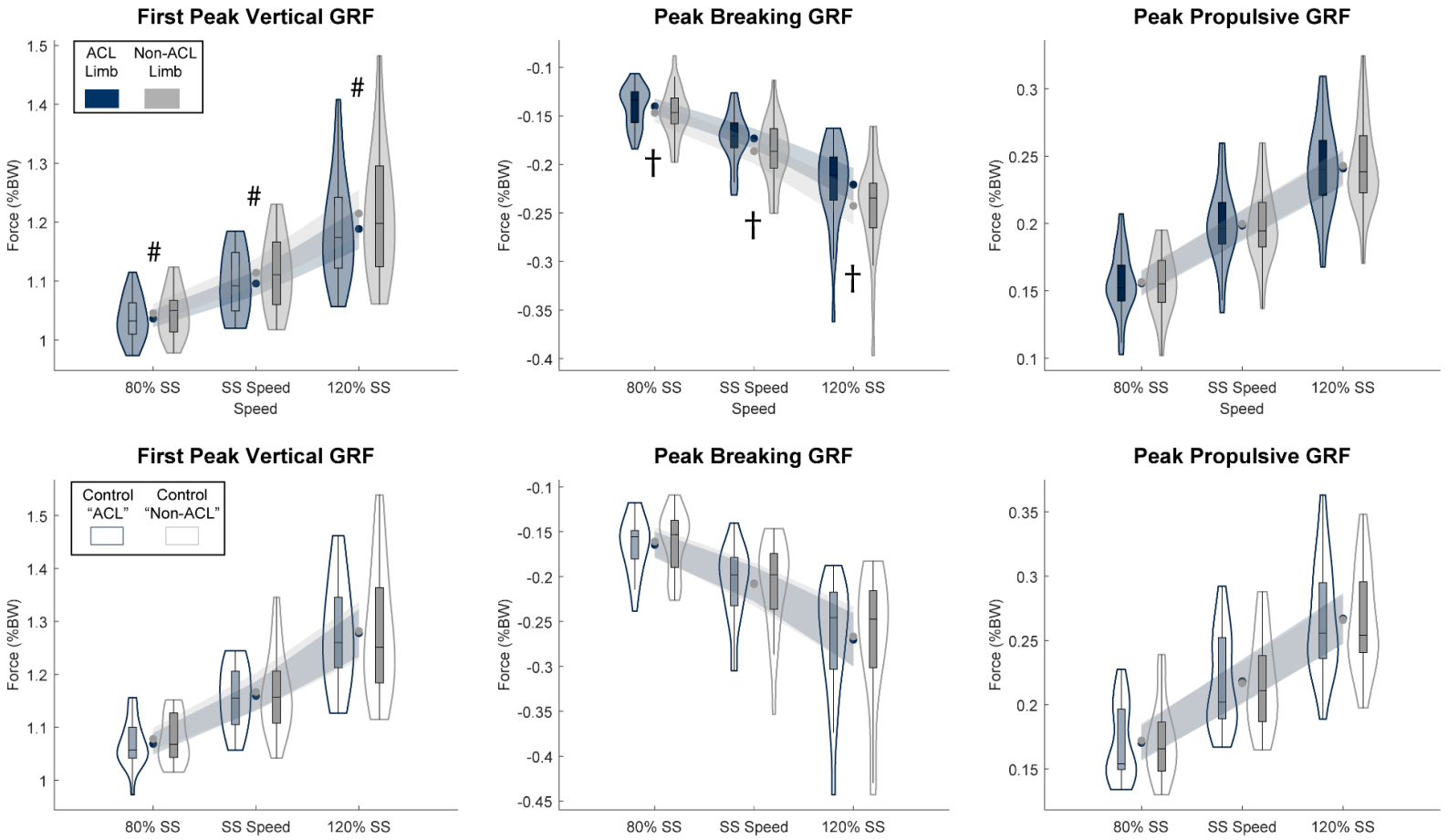

Figure 6.1 Violin plots depicting ground reaction force (GRF) outcomes between limbs and across speeds for ACLR and control groups. Shaded violins represent data from individuals with ACLR and unshaded violins represent data from uninjured controls. Box plots show median and IQR. Dots and shaded regions depict group means and 95% confidence intervals. † Indicates where interaction effects were present. # Indicates where limb main effects were present. Speed effects were not depicted symbolically as all GRF outcomes increased as a function of speed (i.e., smallest magnitudes at 80% speed, largest at 120% speed). SS = self-selected.

Figure 2. Knee Joint Outcomes Across Walking Speeds

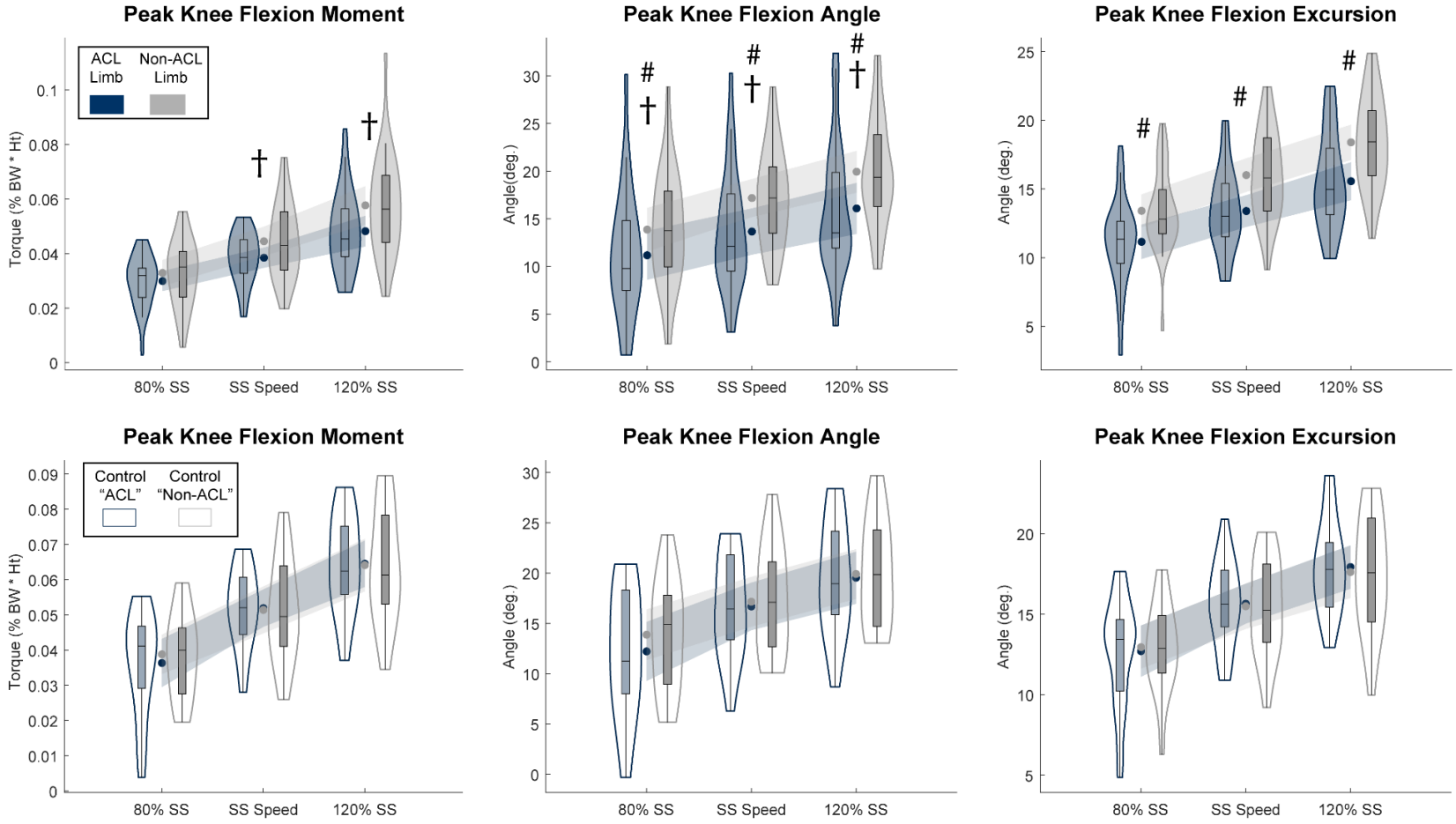

Figure 6.2 Violin plots depicting knee-specific outcomes between limbs and across speeds for ACLR and control groups. Shaded violins represent data from individuals with ACLR and unshaded violins represent data from uninjured controls. Box plots show median and IQR. Dots and shaded regions depict group means and 95% confidence intervals. † Indicates where interaction effects were present. # Indicates where limb main effects were present. Speed effects were not depicted symbolically as all knee outcomes increased as a function of speed (i.e., smallest magnitudes at 80% speed, largest at 120% speed). SS = self-selected.

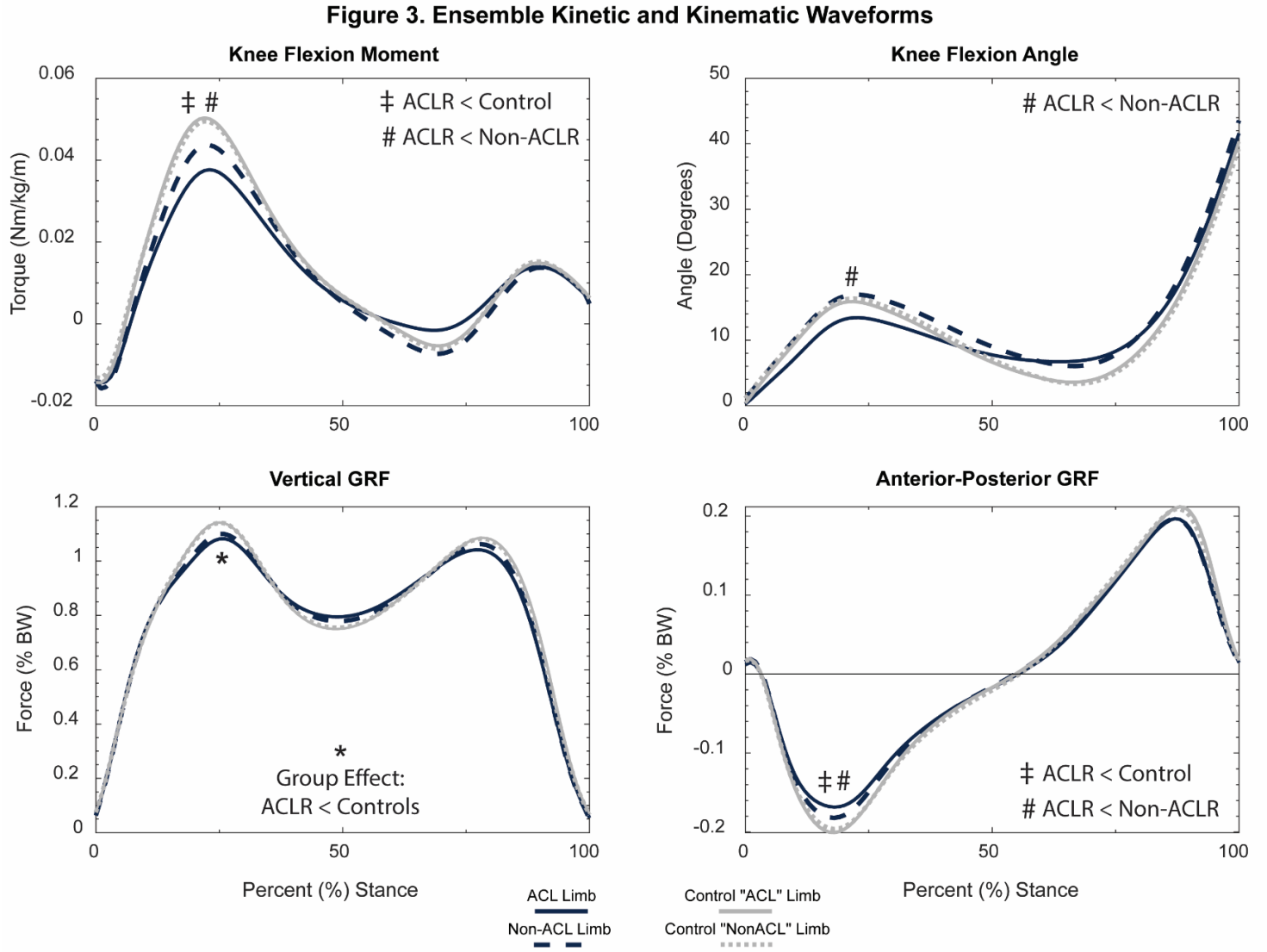

Figure 6.3 Ensemble waveforms from gait outcomes for both limbs in each group. Blue solid and dashed lines indicate the ACL and non-ACL limb data from the ACLR group, respectively. Gray solid and dashed lines indicate the “ACL” and “non-ACL” limb from representative controls, respectively. Note the readers are referred to the online version of the article for interpretation of color.

6.5 Discussion:

The purpose of this study was to evaluate the effects of modifying walking speed on limb and knee joint biomechanics in individuals with ACLR and uninjured controls. Our primary hypothesis was that faster walking speeds would induce larger between-limb differences in gait biomechanics in those with ACLR but not in uninjured controls. Our results support this hypothesis, as faster walking speeds induced larger between-limb differences in the braking GRF, peak KFM and KFA in those with ACLR. Further, uninjured controls displayed minimal between-limb differences regardless of speed demands. Lastly, our secondary hypotheses were that individuals with ACLR would exhibit smaller GRFs, peak knee moments and angles/excursions compared to uninjured controls. Our data largely confirm this hypothesis, as we observed differences in nearly all biomechanical variables in the ACLR limb compared to controls.

Overall, our data support recent works showing that individuals with ACLR differentially modulate lower-limb mechanics in response to varying task demands compared to uninjured controls [25, 68, 304, 458]. We observed that between-limb differences in the braking GRF were significantly impacted by walking speed demands but only in those with ACLR, confirming previous data from our lab in individuals with acute ACLR (i.e., 9-12 weeks post-ACLR) [25]. The braking or posterior GRF is the force component opposing ambulatory progression during the first half of stance, a period wherein there are large support demands placed on the hip and knee extensors [451, 452]. Those with ACLR commonly exhibit lasting deficits in knee extensor function [459] which may impair their ability to effectively resist large braking forces. The commensurate increase in between-limb differences in the braking GRF observed in our ACLR cohort at

the fast speed also exceeded previously established MDC values. It is plausible these increased asymmetries may be attributed to several compensatory strategies aimed at offloading or minimizing heightened knee demand while walking fast. For example, those with ACLR individuals often walk with reduced knee angles and/or walk with slower knee extension angular velocities compared to the uninjured limb [10, 15, 16, 64, 283, 297, 460]. Together, these gait deviations likely contributed to reduced braking GRFs at impact and presumably, decrease knee extensor contributions for support. Lin et al., observed that independent of these changes in knee kinematics, those with ACLR may also modulate anterior center of mass (COM) velocities when transitioning from double- to single-limb stance which could be subtle whole-body adjustments to further “protect” loading about the surgical limb and reduce braking GRFs [461]. As such, compensatory gait strategies are not fully normalized 9-12 months after ACLR and seem to be dependent on walking speed - faster walking exacerbates braking GRF between-limb differences while slower walking reduces them.

Interestingly, we observed that between-limb differences in the vertical GRF were not affected by walking speed demands in both individuals with ACLR and uninjured controls, contrary to our hypotheses and previous works [25, 455]. Individuals with ACLR in our cohort exhibited characteristic reductions in vertical GRFs (albeit relatively small) in the surgical limb relative to their contralateral while uninjured controls, as expected, walked with minimal between-limb differences. Nonetheless, our data show that despite the apparent asymmetry shown in our ACLR cohort, both groups were able to maintain relatively consistent between-limb differences in vertical GRF magnitudes regardless of walking speed demands. Generally, peak vertical GRFs differences between-limbs after

ACLR are less pronounced than other gait impairments (i.e., knee moment or angles differences) [15, 25] evidenced by an average limb symmetry index of 98.5% in our ACLR cohort. Early post-surgery when gait deficits are pronounced (i.e., approx. 2-3 months), faster walking magnified between-limb differences in the vertical GRF whereas slower speeds minimized these differences. However, our data suggest ACLR patients who either completed or are close to completing standard-of-care rehabilitation/return-to-activity training do not appear to display as noticeable compensations at the whole-body level when performing more difficult tasks (i.e., speed demands). As such, it appears that at least in the vertical GRF, between-limb differences somewhat improve throughout rehabilitation but may not be sufficiently restored given the observed differences compared to controls. Our data also suggests using vertical GRF as an outcome to differentiate poor movement patterns across task demands after ACLR may offer less "resolution" during later rehabilitation phases compared to other outcomes like the braking GRF.

We also observed that the sagittal plane KFM and KFA were differentially impacted by walking speed in those with ACLR compared to uninjured controls. For instance, those with ACLR exhibited large between-limb differences in the peak KFM, KFA and KFE at preferred walking speeds while uninjured controls walked symmetrically as expected from previous works [20, 462]. However, faster than preferred speeds exacerbated between-limb differences in the KFM and KFA in those with ACLR while uninjured controls were able to maintain symmetry regardless of speed demands. It is important to note however, that clinically meaningful differences existed at both self-selected and fast walking speeds for KFM and KFA and it appears that speed had stronger effects on the KFM (Table 2).

Nonetheless, these findings provide additional supporting evidence that walking biomechanics are not normal between 9-12 month post-operatively and appear to worsen when performing increasingly difficult task [16, 25, 304], suggesting that additional, targeted rehabilitation may be need to sufficiently restore walking gait. Further, we want to highlight that manipulating walking speed to better identify poor movement patterns is promising given that speed is easily modifiable and using relatively small speed changes (i.e., 20%) appears sufficient to evoke greater asymmetry. This could be advantageous for clinicians or researchers when monitoring walking biomechanics throughout post-operative recovery as gait assessments (or training stimuli) can be individually tailored based on patient progress (i.e., faster walking speeds at later-phases of recovery) or symptoms (i.e., using smaller speed manipulations). Together, this knowledge could be useful to inform timing or dosage of rehabilitation interventions. It is also reasonable that incorporating gait assessments at several walking speeds may help better capture a patient's functional capacity than allowing individuals to self-select "comfortable" speeds. However, we note there is still a lack of understanding on what biomechanical variables or movements are best at differentiating between those with poorer functional outcomes after ACLR. There may be of benefit for future studies to fully examine the utility of incorporating fast walking speed assessments or more difficult tasks throughout ACLR recovery to understand what offers researchers and clinicians the greatest resolution to detect abnormal biomechanical patterns.

Lastly, we observed that those with ACLR walked with bilaterally smaller vertical GRFs compared to uninjured controls, and reductions in nearly all gait outcomes in the ACLR limb relative to the contralateral limb and to limbs of uninjured controls. However,

contrary to our hypothesis and previous findings from similar cohorts with ACLR (i.e., 6-12 months post-surgery) [16], we did not find that the non-ACLR limb exhibited differences in knee-specific outcomes compared to controls. Davis-Wilson et al., observed that those with ACLR near the time to return-to-activity exhibited bilateral deficits in sagittal plane knee angles and moments compared to controls [16]. Although, authors noted the reductions in preferred walking speed over time partly explained the reductions in joint loads magnitudes. In our study, we had uninjured control participants walk at their matched ACLR participants preferred walk speed to ensure gait comparisons would not be impacted by speed which may explain conflicting findings. Though we note our lack of 1:1 matching, many of the "unmatched" ACLR participants exhibited similar self-selected speeds as controls and thus they were in group analyses. Nonetheless, lack of differences could also be attributed to differences in testing protocols (overground vs. treadmill walking, shod vs. barefoot walking) and/or the rehabilitation status of patients in our cohort.

There are some limitations to this study. We evaluated how walking speed impacted between-limb differences in gait biomechanics between groups to gain insight on how ACLR patients may differentially navigate varying task-demands. While at the group level, individuals with ACLR responded differently than uninjured individuals, our study did not include common clinical metrics such as knee extensor strength, hop tests, or other components of return-to-sport batteries [70, 463, 464]. It would be ideal to evaluate how those displaying greater walking compensations to faster walk speeds may present on typical clinical metrics to better understand avenues to identify patients with poorer functional outcomes. We also note that between-limb differences in several

outcomes (i.e., braking GRF, KFM, KFA) were already present at self-selected speeds and comparable to previously established MCIDs. Nonetheless, the impact of speed on magnifying these differences may still improve sensitivity of detecting poor biomechanical function. Further, the gait outcomes chosen in our study generally necessitate 3-D motion capture equipment, which is not widely available nor time-effective for routine clinical use. With the continual development of inertial measurement units and pressure-sensing load soles, gait assessments in typical clinical practice may become more readily accessible through assessing surrogate measures of load/knee-specific metrics (i.e., vertical GRF estimation, shake accelerations etc.). As such, it would be beneficial for future work to verify if the effects of speeds on between-limb differences could also be similarly detected when using wearable sensors or if manipulating speed improves the sensitivity of detecting gait impairments via these surrogate metrics — knowledge which could be of value for improving clinical translation.

6.6 Conclusion:

Between-limb differences in walking biomechanics (GRFs and knee kinetics/kinematics) tend to magnify as individuals walk faster above MCID values while walking slower reduces between-limb differences below MCID values. Conversely, uninjured controls can maintain relatively consistent, symmetrical walking patterns across speeds. Individuals with ACLR also exhibited reduced GRFs, knee moments, and angles compared to healthy controls indicating that normal gait patterns are not restored after rehabilitation which may warrant incorporation of gait-specific rehabilitation throughout post-operative recovery.

Chapter 7 Summary and Future Directions

7.1 Introduction:

The overarching goal of this dissertation was to globally evaluate how modifiable risk factors for post-traumatic OA such as altered gait mechanics and high BMI acutely influence knee cartilage health outcomes in those with ACLR and evaluate potential strategies to improve gait mechanics post-ACLR. Although post-traumatic OA development is largely multifactorial, aberrant walking mechanics and high BMI are well established (and modifiable) risk factors that are linked with higher OA risks in those suffering from ACL injury and ACLR. Given the considerable burdens attributed to post-traumatic OA development, it is imperative that research is conducted to understand the mechanisms by which these risk factors contribute to disease development and to illuminate potential rehabilitative or intervention approaches. Ultimately this knowledge is critical to improving patient care, optimizing rehabilitation outcomes, and mitigating or circumventing the development of post-traumatic OA after ACLR. Overall, the data generated from this dissertation provides novel insight on the impact of BMI and gait biomechanics on structural and functional measures of cartilage health post-ACLR. We reason our findings may help drive future research aimed at characterizing the potentially unique OA trajectories in those with high BMI after ACLR. Secondarily, our work also provided clinical insight for the development of potential gait retraining paradigms to target knee mechanics after ACLR while also providing clinicians and researchers interested in

gait assessment with insight on how modifying task constraints could be beneficial when evaluating patient functioning. Below is a brief summary of each study aim, the key outcomes and how the goals of this dissertation were achieved. Study limitations and insight on future follow-up research directions are also presented herein.

7.2 Summary:

In chapter 3, we evaluated the associations between BMI, gait biomechanics and ultrasound-based evaluations of femoral trochlear health in those after ACLR. This investigation provides novel data combining gait biomechanical assessments with direct assessments of cartilage structure (i.e., thickness) and function (i.e., strain and EI changes) to comprehensively evaluate the impact of high BMI on OA-related factors after ACLR. Overall, this study provides important insight on cartilage structural changes in those with ACLR and demonstrates that BMI may accelerate the post-traumatic OA time-course in this patient population. The key outcomes of this study were that cartilage thickness was differentially affected by BMI wherein normal BMI individuals with ACLR exhibited apparent cartilage thickening whereas high BMI individuals with ACLR exhibited cartilage thinning in ACLR limbs – findings that may be suggestive of more “advance” degenerative cartilage changes in those with high BMI. Further, BMI moderated the associations between walking biomechanics and cartilage structural outcomes such that individuals with high BMI who walked with larger joint loads exhibited thicker, and potentially healthier cartilage after ACLR – this relationship, however, was not observed in normal BMI participants. While important, our data only provides preliminary insight on the impact of BMI on post-traumatic OA after ACLR and future work assessing more sensitive metrics of early OA-related changes in cartilage health (i.e., MRI-based

compositional or functional assessments of cartilage) with longitudinal study designs are warranted.

In chapter 4, we evaluated additional metrics of femoral trochlear health by leveraging an exercise stress-test to assess cartilage functional properties and how BMI impacted these outcomes after ACLR. As highlighted throughout this dissertation, knowledge of the earliest pathological tissue alterations is essential for clinicians and researchers to appropriately intervene and implement disease-modifying interventions at times when cartilage has yet to undergo significant deterioration. While structural alterations are hallmark signs of OA, cartilage compositional and functional decline precedes overt changes in cartilage structure. Thus, functional assessments of cartilage properties (i.e., via imaging stress-tests) could be advantageous and serve as an imaging OA-biomarker after ACLR. Nonetheless, limited research has evaluated changes in cartilage functional properties after ACLR and thus, it remains relatively unclear how BMI further influences these deleterious cartilage changes. This investigation was completed to fill this important knowledge gap and extend findings from chapter 3. We observed that higher BMI and body fat % were associated with poorer cartilage functional properties in ACLR knees. Further, across our entire cohort, smaller knee angles, and lesser knee loads, gait characteristics consistent with a limb underloading or quad-avoidance gait pattern, were associated with poorer cartilage functional properties. These findings provide further support that BMI strongly influences cartilage health outcomes post ACLR and may lead to early presentation of post-traumatic OA features.

Together, we contend that findings from chapters 3 and 4 suggest a potential interaction between high BMI and ACLR on cartilage health outcomes and thus, it is

plausible this subset of patients may necessitate unique post-op treatment plans. Findings of cartilage thinning and excess cartilage strains both between limbs and across our BMI groups, for example, provide some support of this notion given that cartilage outcomes were relatively similar both structurally (i.e., thickness) and functionally (i.e., strain assessments) in our normal BMI group. As such, our data appears to suggest that changes in these cartilage properties appear to be influenced by BMI likely representing more pronounced and rapid cartilage changes in this subset of patients. Nonetheless, early PTOA-related cartilage alterations are largely considered reversible and amenable to intervention. Given this, it may be beneficial to integrate weight management and/or physical activity interventions into post-operative rehabilitation programs after ACLR for those who may be high BMI. These interventions have been shown to be beneficial in older OA populations to improve gait/mobility and may be useful tools to implement into ACLR rehabilitation.

In chapter 5, we focused on gait biomechanical alterations after ACLR by assessing the acute effects of manipulating preferred cadence on knee biomechanics as a potential option for gait retraining. Currently, relatively few strategies have been proposed to directly retrain gait mechanics after ACLR and thus, a key gap of current rehabilitation programs is the absence of dedicated gait interventions. Cadence manipulations are common strategies for running retraining programs and can easily be adapted for both clinical and free-living conditions given the need for relatively minimal equipment (i.e., metronome/smart watch etc.). In our investigation, we found manipulating cadence may be a feasible retraining strategy as it was capable of acutely targeting knee mechanics known to be altered after ACLR. Specifically, we found cueing individuals with

ACLR to walk with larger steps facilitated greater knee excursions and increased sagittal knee moments bilaterally. Conversely, smaller steps reduced knee excursions, but peak angle and moments remain unchanged. Given that those with ACLR commonly adopt walking patterns characterized by reductions in sagittal plane angles, excursions, and moments, cueing larger step lengths may be one strategy to promote more normal gait patterns and hopefully, mitigate or slow OA development in these individuals. Such findings are promising given that this retraining strategy could be easily scaled for widespread clinical implementation given that cadence manipulations can be cued using freely available mobile metronome apps and commercially-available motorized treadmills. Nonetheless, these findings are limited to a single-session and future research extending our findings to a more longitudinal design/program is needed to fully elucidate the benefits (or consequences) of this gait retraining strategy.

Lastly, in **chapter 6** we aimed to evaluate how altering walking speed influenced interlimb biomechanics in individuals with and without ACLR. Previously, we have shown that altering walking speed modifies ground reaction force interlimb differences after ACLR wherein faster walk speeds exacerbates interlimb differences in GRFs while slower speeds minimize these differences. Although, in this preliminary work we only assessed speed effects on interlimb differences using global limb loading outcomes (i.e., ground reaction forces) which do not reflect joint-level compensations. Furthermore, our ACLR cohort was only 2-3 months post-op – a time where gait impairments are pronounced. Thus, it is not clear if speed would similarly affect interlimb differences in gait in ACLR patients who were farther along in rehabilitation where gait impairment may be more subtle. Thus, this aim of the dissertation was focused on extending our work to understand

if manipulating walking speeds could represent a task-specific modification that can improve the detection of poor gait biomechanics in those with ACLR who were near the completion of rehabilitation and a cohort of uninjured controls. Alternatively, faster walking speeds could be a strategy to facilitate increased limb/joint loads in those with ACLR and reasonably, could be a potential option to explore as a retraining stimulus in the context of combating the deleterious effects of joint underloading on cartilage health. Overall, we observed that walking speed differentially impacts interlimb differences in knee mechanics in those with ACLR compared to controls. Namely, interlimb differences in sagittal knee angles and moments appeared to increase as a product of increasing walk speeds in those with ACLR. Conversely, uninjured controls largely maintained minimal interlimb differences in gait mechanics regardless of speed demands. Overall, we reason that leveraging fast walk speeds could be an avenue to test an individual's functional capacity during walking. It is well known that speed strongly influences musculoskeletal demands and thus, in pathological populations, it is possible additional impairments may arise during more demanding tasks – as shown here. This may be advantageous clinically where access to normative data from uninjured populations is not viable as faster walking speeds may help identify individuals who require additional treatment. Alternatively, faster walking speeds may also feasibly serve as an intervention approach given its ability to induce larger joint/limb loads and knee range of motions. It can be speculated that long-term exposure to walking at faster than preferred speeds (and thereby the heightened neuromuscular demands) may serve as a potential gait training stimulus, even if gait asymmetries are acutely elevated. Similar error-augmentation approaches that magnify gait asymmetries such as split-treadmill training have been shown to induce positive gait

adaptation in other clinical populations. Thus, it is possible a progressive walking speed training could also be useful in a gait retraining context after ACLR.

7.3 Limitations and Future Research Directions:

Overall, the studies comprising this dissertation represent advancements in our understanding of modifiable risk factors implication in post-traumatic OA development after ACLR and provide novel insight into potential options for gait retraining strategies. Nonetheless, the findings of these studies must be interpreted in light of their limitations and future research is warranted to extend and verify our findings.

A primary limitation of chapters 3 and 4 of this dissertation is a lack of uninjured normal and high BMI control participants. The main purpose of these aims were to test the hypothesis that high BMI and joint injury interact to disproportionately affect cartilage outcomes after ACLR. However, we only recruited high and normal BMI groups with ACLR and to test a true interaction between factors, all four groups would be needed to adequately test this hypothesis. We note, however, that this would likely require 100-120 participants, which was not feasible in our time frame. Furthermore, we utilized the non-ACLR limb as our comparison limb between groups and observed interesting findings that we speculate may support the presence of a potential interaction between factors. For instance, in **chapter 3** we observed that cartilage structural changes differed between BMI groups – high BMI individuals exhibited *thinner* ACLR limb cartilage relative to the non-ACLR limb whereas normal BMI individuals exhibited *thicker* ACLR limb cartilage. Subsequently, **chapter 4** showed that those with high BMI exhibited greater cartilage strains after an incline walk compared to normal BMI individuals with ACLR and further, cartilage strains were greater in ACLR limbs compared to non-ACLR limbs but only in the

high BMI group. The time-course of post-traumatic OA involves progressive changes to both the composition of the cartilage matrix and to the tissues' structure. Thus, we reason findings of thinner and functionally weaker cartilage (i.e., higher strains) in those with high BMI may be suggestive that this subset of patients is at a more advanced stage of OA. Nonetheless, we note that our data was cross-sectional in nature, and it is ultimately unclear how these cartilage structural and functional differences between BMI groups after ACLR evolve during and after post-operative rehabilitation. Future research including uninjured control groups and using more longitudinal analyses would be beneficial to extending our findings and aid in improving our understanding of the factors contributing to OA development after ACLR.

Further, it is important to recognize that **chapters 3** and **4** utilized ultrasound imaging to evaluate cartilage structural and function alterations after ACLR. While ultrasound has substantial utility as an OA imaging tool, imaging windows are limited with this modality as generally, only a the anterior femoral trochlear within the patellofemoral joint is accessible via ultrasound. As such, we were unable to image patellar cartilage, or even more distal femoral and tibiofemoral articulations. Patellofemoral OA-related changes after ACLR are common and present rapidly, but nonetheless, MRI-based imaging options allow for more comprehensive assessment of cartilage changes throughout patellofemoral and tibiofemoral joints. Future research should consider evaluating the deleterious impact of high BMI after ACLR using quantitative MRI metrics. We also had heterogenous graft types in our sample to increase generalizability of our findings and graft type distributions were generally similar between groups (25% HT in the normal BMI and 21% HT in high BMI group). However, we note that patellofemoral

OA rates after ACLR are generally comparable between individuals who received BPTB or HT grafts [224, 431] and we did not observe associations between graft type and cartilage outcomes. Nonetheless, it still may be beneficial to consider graft type comparisons more directly when evaluating early changes in cartilage health to better understand the influence of graft selection on cartilage health after ACLR.

We also evaluated the potential utility of cadence manipulations as a gait retraining strategy but did so in a cross-sectional single session design. The testing blocks in this study involved approximately 5-7 minutes of dedicated walking while matching cadence beats which is likely insufficient to cause any plastic changes in gait. Future longitudinal research leveraging motor learning principles to vary the dosage, feedback type and frequency are needed to identify ways to optimize gait improvements from this retraining strategy. Further, we note that our retraining approach was conducted during treadmill walking in which walking speeds are constant. Providing metronome cues when speeds are not constant and controlled may lead to differential gait adaptations and thus, it may be beneficial to understand how cueing cadence changes during overground walking in constrained and unconstrained manners may influence knee mechanics. Such knowledge is crucial to fully vetting this retraining strategy given that the ability to implement gait retraining programs outside of a lab or clinical environment may improve patient involvement and potentially maximize gait improvements over the long-term.

Lastly, we note that the use of speed manipulations to alter biomechanical asymmetries, although convenient, has not been fully explored in a clinical context. For example, we did not examine any subjective or objective clinical assessments that are typically assessed at return-to-sport testing after ACLR. Thus, it is not clear if those

showing the worst “decay” in gait mechanics at faster walk speeds also exhibit poorer clinical and/or functional outcomes. Although clinical gait assessments are not typical in standard of care, they may become more feasible given the increased availability of wearable technology that permits gait monitoring. Thus, providing clinicians with options to improve their ability to detect patients who require additional treatment is important to optimizing functional recovery. Future research should consider extending our study design to include patient-reported outcomes, strength and hop metrics to evaluate the benefits of manipulating walking speed demands to detect poor biomechanical function in patients. Alternatively, and as noted above, faster walking speeds could also be useful from a rehabilitation perspective. Long term manipulation of walking speeds (i.e., increasing above preferred) throughout rehabilitation could feasibly serve as a training stimulus that may translate to improvements in loading patterns and knee motions after ACLR. Walking speed is a simple task-constraint that could be easily modified/adapted by clinicians throughout rehabilitation to appropriately challenge patients as they show improvements (or decrements) in walking patterns. Nonetheless, the use of walking speed manipulations as a rehabilitation is largely speculative and requires further study.

Appendices

Appendix A: Survey Research

Subject ID: ☐☐☐☐☐☐☐ **Date:** ☐☐ / ☐☐ / ☐☐
Month Day Year

Demographic Form

Instructions: Below is a list of questions about your demographics and health history. Please answer the following questions as completely as possible.

1. What is your sex? (circle all that apply)
   a. male
   b. female
   c. intersex

2. How do you identify? (circle all that apply)
   a. man
   b. woman
   c. non-binary
   d. prefer to self-describe: _____
   e. prefer not to identify

3. Have you been previously diagnosed with radiographic or symptomatic knee osteoarthritis?
   a. yes
   b. no
   c. n/a

4. Date of ACL injury (if exact date is unknown, please provide an approximate date):
   a. date:_____
   b. n/a

5. Date of ACL surgery (if exact date is unknown, please provide an approximate date):
   a. date:_____
   b. n/a

6. Which is your ACL injured limb?
   a. right
   b. left
   c. n/a

7. What ACL graft type do you have?
   a. patellar tendon (aka bone-patellar tendon bone)
   b. hamstring (aka semitendinous gracilis)
   c. quadriceps tendon
   d. tibialis anterior
   e. other: _____
   f. n/a

8. Was your graft type an allograft (e.g. donated tissue) or an autograft (e.g. your own tissue)?
   a. allograft (e.g. donated tissue)
   b. autograft (e.g. your own tissue)
   c. unknown
   d. n/a

9. Did you have any additional knee injuries besides your ACL rupture? (circle all the apply)
   a. meniscal injury
   b. meniscal surgery
   c. bone bruise
   d. articular cartilage lesion
   e. collateral ligament damage
   f. none
   g. unknown
   h. n/a

10. If you have experienced an ACL injury, what was the type of injury?
    a. contact
    b. non-contact

    If your injury was non-contact, did it occur during:
    1) cutting/pivoting
    2) landing
    3) decelerating (slowing down)
    4) other

11. If you have experienced an ACL injury, what activity where you involved in when the injury occurred
    a. soccer
    b. football
    c. volleyball
    d. basketball
    e. skiing/snowboarding
    f. other

12. For magnetic resonance imaging (MRI) procedures, do you have any body piercings that you are unwilling and/or unable to remove?
    a. yes
    b. no

Subject Initials ☐☐☐ **Subject ID** ☐☐☐☐☐ **Testing day** ☐☐ / ☐☐ / ☐☐
Month Day Year

Pre-Screening Form

Subject U of M Record Number: ☐☐☐☐☐☐☐

First Name:
Middle Name (or initial):
Last Name:
Mailing Address:
Phone Number:
Email:

Birthday: ☐☐ / ☐☐ / ☐☐ **Height (m): _____ Weight (kg): _____**
Month Day Year

Instructions: Below if a list of questions about your health history. In order to meet pre-screening criteria, it is important for the research team to know if you have ever been diagnosed or experienced one of the following:

1) Do you have a previous history of ACL injury or knee surgery other than your current ACL reconstruction?

2) Does your current ACL reconstruction use an allograft (e.g., donated tissue) graft type?

3) Have you suffered a lower extremity injury within the past 6 months, other than your current ACL reconstruction?

4) Have you been previously diagnosed with radiographic or symptomatic knee osteoarthritis?

5) Do you have a history of untreated diabetes?

6) If you are female, are you currently pregnant?

Please check one of the following boxes:

☐ **Yes, I have been diagnosed or experienced one of the above-mentioned items**
☐ **No, I have not been diagnosed or experienced one of the above-mentioned items**

Signature of staff completing screening: _____ **Date:**_____

2000 IKDC SUBJECTIVE KNEE EVALUATION FORM

Your Full Name_____

Today's Date: _____/_____/_____ (Day / Month / Year) Date of Injury: _____/_____/_____ (Day / Month / Year)

SYMPTOMS*:

*Grade symptoms at the highest activity level at which you think you could function without significant symptoms, even if you are not actually performing activities at this level.

1. What is the highest level of activity that you can perform without significant knee pain?

   4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   3❑Strenuous activities like heavy physical work, skiing or tennis
   2❑Moderate activities like moderate physical work, running or jogging
   1❑Light activities like walking, housework or yard work
   0❑Unable to perform any of the above activities due to knee pain

2. During the past 4 weeks, or since your injury, how often have you had pain?

	0	1	2	3	4	5	6	7	8	9	10	
Never	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	Constant

3. If you have pain, how severe is it?

	0	1	2	3	4	5	6	7	8	9	10	
No pain	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	Worst pain imaginable

4. During the past 4 weeks, or since your injury, how stiff or swollen was your knee?

   4❑Not at all
   3❑Mildly
   2❑Moderately
   1❑Very
   0❑Extremely

5. What is the highest level of activity you can perform without significant swelling in your knee?

   4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   3❑Strenuous activities like heavy physical work, skiing or tennis
   2❑Moderate activities like moderate physical work, running or jogging
   1❑Light activities like walking, housework, or yard work
   0❑Unable to perform any of the above activities due to knee swelling

6. During the past 4 weeks, or since your injury, did your knee lock or catch?

   0❑Yes 1❑No

7. What is the highest level of activity you can perform without significant giving way in your knee?
   4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   3❑Strenuous activities like heavy physical work, skiing or tennis
   2❑Moderate activities like moderate physical work, running or jogging
   1❑Light activities like walking, housework or yard work
   0❑Unable to perform any of the above activities due to giving way of the knee

SPORTS ACTIVITIES:

8. What is the highest level of activity you can participate in on a regular basis?

4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
3❑Strenuous activities like heavy physical work, skiing or tennis
2❑Moderate activities like moderate physical work, running or jogging
1❑Light activities like walking, housework or yard work
0❑Unable to perform any of the above activities due to knee

9. How does your knee affect your ability to:

		Not difficult at all	Minimally difficult	Moderately Difficult	Extremely difficult	Unable to do
a.	Go up stairs	4❑	3❑	2❑	1❑	0❑
b.	Go down stairs	4❑	3❑	2❑	1❑	0❑
c.	Kneel on the front of your knee	4❑	3❑	2❑	1❑	0❑
d.	Squat	4❑	3❑	2❑	1❑	0❑
e.	Sit with your knee bent	4❑	3❑	2❑	1❑	0❑
f.	Rise from a chair	4❑	3❑	2❑	1❑	0❑
g.	Run straight ahead	4❑	3❑	2❑	1❑	0❑
h.	Jump and land on your involved leg	4❑	3❑	2❑	1❑	0❑
i.	Stop and start quickly	4❑	3❑	2❑	1❑	0❑

FUNCTION:

10. How would you rate the function of your knee on a scale of 0 to 10 with 10 being normal, excellent function and 0 being the inability to perform any of your usual daily activities which may include sports?

FUNCTION PRIOR TO YOUR KNEE INJURY:

	0	1	2	3	4	5	6	7	8	9	10	
Couldn't perform daily activities	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	No limitation in daily activities

CURRENT FUNCTION OF YOUR KNEE:

	0	1	2	3	4	5	6	7	8	9	10	
Can't perform daily activities	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	No limitation in daily activities

Scoring Instructions for the 2000 IKDC Subjective Knee Evaluation Form

Several methods of scoring the IKDC Subjective Knee Evaluation Form were investigated. The results indicated that summing the scores for each item performed as well as more sophisticated scoring methods.

The responses to each item are scored using an ordinal method such that a score of 0 is given to responses that represent the lowest level of function or highest level of symptoms. For example, item 1, which is related to the highest level of activity without significant pain is scored by assigning a score of 0 to the response "Unable to perform any of the above activities due to knee pain" and a score of 4 to the response "Very strenuous activities like jumping or pivoting as in basketball or soccer". **For item 2, which is related to the frequency of pain over the past 4 weeks, the responses are reverse-scored such that "Constant" is assigned a score of 0 and "Never" is assigned a score of 10. Similarly, for item 3, the responses are reversed-scored such that "Worst pain imaginable" is assigned a score of 0 and "No pain" is assigned a score of 10.** Note: previous versions of the form had a minimum item score of 1 (for example, ranging from 1 to 11). In the most recent version, all items now have a minimum score of 0 (for example, 0 to 10). To score these prior versions, you would need to transform each item to the scaling for the current version.

The IKDC Subjective Knee Evaluation Form is scored by summing the scores for the individual items and then transforming the score to a scale that ranges from 0 to 100. **Note:** The response to item 10a "Function Prior to Knee Injury" is not included in the overall score. To score the current form of the IKDC, simply add the score for each item (the small number by each item checked) and divide by the maximum possible score which is 87:

$$\text{IKDC Score} = \left[\frac{\text{Sum of Items}}{\text{Maximum Possible Score}}\right] \text{x } 100$$

Thus, for the current version, if the sum of scores for the 18 items is 45 and the patient responded to all the items, the IKDC Score would be calculated as follows:

$$\text{IKDC Score} = \left[\frac{45}{87}\right] \text{x } 100$$

$$\text{IKDC Score} = 51.7$$

The transformed score is interpreted as a measure of function such that higher scores represent higher levels of function and lower levels of symptoms. A score of 100 is interpreted to mean no limitation with activities of daily living or sports activities and the absence of symptoms.

The IKDC Subjective Knee Form score can be calculated when there are responses to at least 90% of the items (i.e. when responses have been provided for at least 16 items). In the original scoring instructions for the IKDC Subjective Knee Form, missing values are replaced by the average score of the items that have been answered. However, this method could slightly over- or under-estimate the score depending on the maximum value of the missing item(s) (2, 5 or 11 points). Therefore, in the revised scoring procedure for the current version of a form with up to two missing values, the IKDC Subjective Knee Form Score is calculated as (sum of the completed items) / (maximum possible sum of the completed items) * 100. This method of scoring the IKDC Subjective Knee Form is more accurate than the original scoring method.

A scoring spreadsheet is also available at: www.sportsmed.org/research/index.asp This spreadsheet uses the current form scores and the revised scoring method for calculating scores with missing values.

KOOS KNEE SURVEY

Today's date: _____/_____/_____ Date of birth: _____/_____/_____

Name: _____

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

Symptoms

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

S1. Do you have swelling in your knee?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

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

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

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

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S4. Can you straighten your knee fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

S5. Can you bend your knee fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

Stiffness

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

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

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

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

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Pain

P1. How often do you experience knee pain?

Never	Monthly	Weekly	Daily	Always
☐	☐	☐	☐	☐

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

P2. Twisting/pivoting on your knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P3. Straightening knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P4. Bending knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P5. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P6. Going up or down stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P7. At night while in bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P8. Sitting or lying

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P9. Standing upright

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, daily living

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

A1. Descending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A2. Ascending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A3. Rising from sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A4. Standing

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A5. Bending to floor/pick up an object

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A6. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A7. Getting in/out of car

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A8. Going shopping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A9. Putting on socks/stockings

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A10. Rising from bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A11. Taking off socks/stockings

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

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

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A13. Getting in/out of bath

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A14. Sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A15. Getting on/off toilet

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

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

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

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

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, sports and recreational activities

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

SP1. Squatting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP2. Running

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP3. Jumping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP4. Twisting/pivoting on your injured knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP5. Kneeling

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Quality of Life

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

Never	Monthly	Weekly	Daily	Constantly
☐	☐	☐	☐	☐

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

Not at all	Mildly	Moderately	Severely	Totally
☐	☐	☐	☐	☐

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

Not at all	Mildly	Moderately	Severely	Extremely
☐	☐	☐	☐	☐

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

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Thank you very much for completing all the questions in this questionnaire.

TEGNER ACTIVITY LEVEL SCALE

Please indicate in the spaces below the HIGHEST level of activity that you participated in BEFORE YOUR INJURY and the highest level you are able to participate in CURRENTLY.

BEFORE INJURY: Level_____ CURRENT: Level_____

Level 10	**Competitive sports- soccer, football, rugby (national elite)**
Level 9	**Competitive sports- soccer, football, rugby (lower divisions), ice hockey, wrestling, gymnastics, basketball**
Level 8	**Competitive sports- racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing**
Level 7	**Competitive sports- tennis, running, motorcars speedway, handball** **Recreational sports- soccer, football, rugby, bandy, ice hockey, basketball, squash, racquetball, running**
Level 6	**Recreational sports- tennis and badminton, handball, racquetball, down-hill skiing, jogging at least 5 times per week**
Level 5	**Work- heavy labor (construction, etc.)** **Competitive sports- cycling, cross-country skiing,** **Recreational sports- jogging on uneven ground at least twice weekly**
Level 4	**Work- moderately heavy labor (e.g. truck driving, etc.)**
Level 3	**Work- light labor (nursing, etc.)**
Level 2	**Work- light labor** **Walking on uneven ground possible, but impossible to back pack or hike**
Level 1	**Work- sedentary (secretarial, etc.)**
Level 0	**Sick leave or disability pension because of knee problems**

Y Tegner and J Lysolm. ***Rating Systems in the Evaluation of Knee Ligament Injuries.*** **Clinical Orthopedics and Related Research. Vol. 198: 43-49, 1985.**

Appendix B: Data Collection Forms – Aims 1-2

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Subject ID: ☐☐☐☐☐ **Testing Date:** ☐☐ / ☐☐ / ☐☐ (Month / Day / Year) **Time In:** ☐☐☐ **Time Out:** ☐☐☐

Data Collection Form – Gait Biomechanics

File Naming: F31_SubjectID (i.e. S01, 02, 03 etc.)

ACL Leg: R or L (Circle One) **Mass:** _____ kg (w/shoes) **Height:** _____ m

Leg Length: (GT → Ground) _____ cm

Survey Forms: IKDC KOOS Tegner IPAQ

Block Order: A (Level) B (Incline)

Selected Walking Speed: Collect Overground (Use GaitSpeed.xlsx to convert from time in sec. to m/s):

Walking Speed (m/s)	Practice	1	2	3	4	5	AVG

Marker Set Notes:

7 Clusters (2x Foot, 2x Shank, 2x Thigh, Sacrum)

20 Single Markers (IC, ASIS, GT, M/L Knee, M/L Ankle, 1st/5th, Calc)

48 Static Markers Total (Clusters + Single)

30 Dynamic Markers Total (Clusters + Calcanei)

Testing Trial Notes:

Allow for 2 minutes of familiarization at the set treadmill speed prior to initialization of testing trials.

5 Trials per Condition (i.e., Level or Incline).

Notes: _____

_____

_____

_____

_____

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Data Collection Form – Quadriceps Strength

Limb Randomization (Circle One): R **or** L

Test Randomization (Circle One): Isokinetic **or** Isometric

Instructions:

- Setup Cybex with **"BFB_Strength.Vi"** LabVIEW Program
- Examiner to secure chest, waist, and leg straps to participant
- Position chair and dynamometer arm so that dynamometer arm aligns with knee joint center
- Dynamometer arm pad should be position **two finger widths above the medial malleolus.**
- Cybex should be set to measure strength at 60 deg/sec in Con/Con mode, Isometrics @ 60 deg.
- Instruct subjects that they are to kick as hard as they can out and pull as hard as they can in; Examiners to provide vigorous encouragement
- **Practice Trials:** 1 repetition at 25%, 50%, 75%, and 100% of perceived maximal effort.

Chair/Dynamometer Positions:
Dynamometer Arm: _____
Dynamometer Rotation: _____
Chair Fore/Aft: _____
Chair Height: _____

Quadriceps and Hamstring Isokinetic Strength (60-degrees/s):

Trial #	Right Leg Extension	Right Leg Flexion	Left Leg Extension	Left Leg Flexion
1				
2				
3				
Average				

Quadriceps Isometric Strength (60-degree Knee Angle):

Rep #	Right Leg			Left Leg		
	Peak Torque	RTD 50-100ms	RTD 100-200ms	Peak Torque	RTD 100	RTD 200
1						
2						
3						
Average						

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Subject ID: ☐☐☐☐☐ **Testing Date:** ☐☐ / ☐☐ / ☐☐ (Month / Day / Year) **Time In:** ☐☐☐ **Time Out:** ☐☐☐

Data Collection Form – Ultrasound Incline

Folder Naming US: F31SubjectID_Day (i.e. D1, D2, D3 etc.)

Survey Forms: IKDC KOOS Tegner

Ultrasound Parameters: Frequency – 12Mhz Gain – 50 dB Depth – 3.5 cm

Daily Steps (Collect at arrival to lab): _____

Steps after Biomechanics: _____

Testing Speed: **1.3 m/s**

For baseline cartilage measures record knee angle (140 degrees or maximum flexion), distance from greater trochanter to heel (cm) and probe tilt below. For subsequent sessions, use these measures to ensure similar patient positioning.

Right Leg			Left Leg		
Knee Angle (°)	GT → Heel (cm)	Probe Tilt (°)	Knee Angle (°)	GT → Heel (cm)	Probe Tilt (°)

Collect Cadence via Metronome App/Garmin for Each Trial at Each Condition

	Minute 15	Minute 30
Cadence (BPM)		
Step Count		

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Subject ID: ☐☐☐☐☐ **Testing Date:** ☐☐ / ☐☐ / ☐☐ (Month / Day / Year) **Time In:** ☐☐☐ **Time Out:** ☐☐☐

Data Collection Form – Ultrasound Level (Optional)

Folder Naming US: F31SubjectID_Day (i.e. D1, D2, D3 etc.)

Survey Forms: IKDC KOOS Tegner

Ultrasound Parameters: Frequency – 12Mhz Gain – 50 dB Depth – 3.5 cm

Daily Steps (Collect at arrival to lab): _____

Steps after Biomechanics: _____

Testing Speed: **1.3 m/s**

For baseline cartilage measures record knee angle (140 degrees or maximum flexion), distance from greater trochanter to heel (cm) and probe tilt below. For subsequent sessions, use these measures to ensure similar patient positioning.

Right Leg			Left Leg		
Knee Angle (°)	GT → Heel (cm)	Probe Tilt (°)	Knee Angle (°)	GT → Heel (cm)	Probe Tilt (°)

Collect Cadence via Metronome App/Garmin for Each Trial at Each Condition

	Minute 15	Minute 30
Cadence (BPM)		
Step Count		

Appendix C: Data Collection Forms – Aims 3-4

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Subject ID: ☐☐☐☐☐ **Testing Date:** ☐☐ / ☐☐ / ☐☐ (Month / Day / Year) **Time In:** ☐☐☐ **Time Out:** ☐☐☐

Data Collection Form – Gait Biomechanics

File Naming: BFBSubjectID (i.e. 01, 02, 03 etc.)

ACL Leg: R or L (Circle One)

Dominant Leg R or L (Circle One) **Mass:** _____ kg (w/shoes) **Height:** _____ m

Leg Length: (GT → Lateral Malleolus): _____ cm (GT → Ground) _____ cm

Survey Forms: IKDC KOOS Tegner Tampa Marx

Block Order (Speed/Cadence): A (Cadence First) B (Speed First)

Speed Order: A (120% SS) B (80% SS)

Cadence Order: A (110% Cad.) B (90% Cad.)

Speed Conditions					Cadence Conditions		
Self-Selected	120% SS	80% SS	Match Speed		Cadence @ SS	110% Cadence	90% Cadence
				steps/min.			

Collect Cadence via Metronome App/Garmin for Each Trial at Each Condition

Trial #	Speed Conditions (Steps/min)			Trial #	Cadence Conditions (Steps/min)		
	Self-Selected	120% SS	80% SS		Cadence @ SS	110% Cadence	90% Cadence
1				1			
2				2			
3				3			

Notes: _____

_____

_____

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Data Collection Form – Quadriceps Strength

Instructions:

- Setup Cybex with **"BFB_QuadStrength.Vi"** LabVIEW Program
- Instruct subject to sit in dynamometer chair
- Examiner to secure chest, waist, and leg straps to participant
- Position chair and dynamometer arm so that dynamometer arm aligns with knee joint center
- Dynamometer arm pad should be position **two finger widths above the medial malleolus.**
- Cybex should be set to measure strength at 60 deg/sec in Con/Con mode, Isometrics @ 60 deg.
- Instruct subjects that they are to kick as hard as they can out and pull as hard as they can in; Examiners to provide vigorous encouragement
- **Practice Trials:** 2 repetition at 25%, 50%, 75%, and 1 repetition at 100% of perceived maximal effort.

Chair/Dynamometer Positions:
Dynamometer Arm: _____
Dynamometer Rotation: _____
Chair Fore/Aft: _____
Chair Height: _____

Quadriceps and Hamstring Isokinetic Strength (60-degrees/s):

Trial #	Right Leg Extension	Right Leg Flexion	Left Leg Extension	Left Leg Flexion
1				
2				
3				
Average				

Quadriceps Isometric Strength (60-degree Knee Angle):

Rep #	Right Leg			Left Leg		
	Peak Torque	RTD 50-100ms	RTD 100-200ms	Peak Torque	RTD 100	RTD 200
1						
2						
3						
Average						

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Subject ID: ☐☐☐☐☐ **Testing Date:** ☐☐ / ☐☐ / ☐☐ (Month / Day / Year) **Time In:** ☐☐☐ **Time Out:** ☐☐☐

Data Collection Form – Gait Biomechanics

File Naming: BFBSubjectID (i.e. 01, 02, 03 etc.)

ACL Leg: R or L (Circle One) **Mark as Non-Dominant Limb for Controls**

Dominant Leg: R or L (Circle One) **Mass:** _____ kg (w/shoes) **Height:** _____ m

Survey Forms: IKDC KOOS Tegner

Match order below with matched ACLR participant (from BFB_StudyInfo_Demographics.xlsx)

Block Order (Speed/Cadence): A (Cadence First) B (Speed First)

Speed Order: A (120% SS) B (80% SS)

Cadence Order: A (110% Cad.) B (90% Cad.)

Speed Conditions (m/s)					Cadence Conditions		
Self-Selected	120% SS	80% SS	Match Speed		Cadence @ SS	110% Cadence	90% Cadence
				steps/min.			

Collect Cadence via Metronome App/Garmin for Each Trial at Each Condition

Trial #	Speed Conditions (Steps/min)				Trial #	Cadence Conditions (Steps/min)	
	Self-Selected	120% SS	80% SS	Match Speed		110% Cadence	90% Cadence
1					1		
2					2		
3					3		

Notes: _____

_____

_____

PI: Garcia, IRB Number: HUM00169174, Protocol Title: A Systems Based Approach to Understand Factors Affecting Knee Articular Cartilage (Study 1)

Data Collection Form – Quadriceps Strength

Instructions:

- Setup Cybex with **"BFB_QuadStrength.Vi"** LabVIEW Program
- Instruct subject to sit in dynamometer chair
- Examiner to secure chest, waist, and leg straps to participant
- Position chair and dynamometer arm so that dynamometer arm aligns with knee joint center
- Dynamometer arm pad should be position **two finger widths above the medial malleolus.**
- Cybex should be set to measure strength at 60 deg/sec in Con/Con mode, Isometrics @ 60 deg.
- Instruct subjects that they are to kick as hard as they can out and pull as hard as they can in; Examiners to provide vigorous encouragement
- **Practice Trials:** 2 repetition at 25%, 50%, 75%, and 1 repetition at 100% of perceived maximal effort.

Chair/Dynamometer Positions:
Dynamometer Arm: _____
Dynamometer Rotation: _____
Chair Fore/Aft: _____
Chair Height: _____

Quadriceps and Hamstring Isokinetic Strength (60-degrees/s):

Trial #	Right Leg Extension	Right Leg Flexion	Left Leg Extension	Left Leg Flexion
1				
2				
3				
Average				

Quadriceps Isometric Strength (60-degree Knee Angle):

Rep #	Right Leg			Left Leg		
	Peak Torque	RTD 50-100ms	RTD 100-200ms	Peak Torque	RTD 100	RTD 200
1						
2						
3						
Average						

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