ASSOCIATIONS BETWEEN THE BIOLOGICAL, STRUCTURAL, AND MECHANICAL COMPONENTS OF CARTILAGE HEALTH FOLLOWING ACUTE LOADING IN HEALTHY PARTICIPANTS

Matthew S. Harkey

A dissertation submitted to the faculty of The University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Curriculum of Human Movement Science in the School of Medicine.

Chapel Hill
2017

Approved by:

Brian Pietrosimone

J. Troy Blackburn

Michael Lewek

Anthony C. Hackney

Randy Schmitz

© 2017
Matthew S. Harkey
ALL RIGHTS RESERVED

ABSTRACT

Matthew S. Harkey: Associations Between the Biological, Structural, and Mechanical Components of Cartilage Health following Acute Loading in Healthy Participants
(Under the direction of Brian Pietrosimone)

Context: A systems-based approach to assess cartilage health uses an acute bout of joint loading to signal acute changes in cartilage structure and metabolism. Evaluating how multiple systems, specifically cartilage structure and metabolic outcome measures, are influenced by loading may be a more sensitive measurement approach to understand the prognosis of cartilage related diseases. **Objective:** The objectives of this study are: 1) to compare the response of cartilage ultrasonography (US) outcome measures and serum cartilage oligomeric matrix protein (COMP) to a walking, drop-landing, and control condition; 2) to determine the association between lower extremity loading measures during walking and drop-landing and the change in cartilage US outcome measures and serum COMP; 3) to determine the association between cartilage US outcome measures and serum COMP. **Participants:** 43 healthy individuals. **Interventions:** A femoral cartilage US assessment and an ante-cubital blood draw were performed in healthy individuals before and after three independent sessions: walking, drop-landing, and control conditions. We assessed walking and drop-landing biomechanics at the beginning of the respective condition. **Main Outcome Measures:** Femoral articular cartilage was assessed with US to determine the thickness, area, and echo-intensity. Cartilage metabolism was quantified with serum

COMP. **Results:** US provides a reliable and precise modality for detecting the *in vivo* cartilage deformation and recovery response following walking and drop-landing, but the majority of US measures are not associated with lower extremity loading. COMP increases following walking and drop-landing in healthy individuals, but these changes are not associated with lower extremity loading measures. While the majority of cartilage structure and metabolism markers were not associated within the entire cohort, sex may influence the association between these measures. **Conclusions:** Cartilage structure and cartilage metabolism acutely respond to joint loading. The majority of lower extremity loading biomechanics selected in this study did not associate with acute changes in cartilage structure and metabolism.

ACKNOWLEDGEMENTS

To my advisor Brian Pietrosimone, it has been a long time coming. We are finishing up our 7th year together, and I could not have dreamed it up any better. I don't know how you did it, but you took an unmotivated kid straight out of undergrad and inspired and pushed me for seven years to get me to the point where I am excited to come in to work and learn something new every day. I will never be able to let you know how much it meant for you to put your faith in Brittney and I when you brought us with you from Toledo. Your passion for what you do and your ability to make people excited about your research is remarkable, and I would not be anywhere if it was not for your guidance over this last 7 years.

To my committee member Randy Schmitz, thank you for you your help and experience with the ultrasound technique. We would not have been able to design and complete this project.

To my committee member Mike Lewek, thank you for your help with the biomechanical instrumentation and the instruction you provided in your LabView class.

To my committee member Anthony Hackney, thank you for the countless hours you spent teaching us how to how to draw blood, not to mention you sacrificing your arms during the phlebotomy training. Also, thank you for the long days in the biochem lab helping run our assays.

To my committee member Troy Blackburn, you are one of the best teachers that I know. Throughout my entire 4 years here you have been a constant source of

knowledge for the biomechanics, ultrasound, LabVIEW, and basically anything else regarding how anything in the lab worked.

To my research assistants, you devoted hours upon hours of your time helping me collect and process the data for this project. This project definitively could not have happened without each and every one of yours help. (Daniel Farrell, Gabrielle Smith, Nicole Thomas, Arianna Douglas, Ryan Fockler, Leslie Sierra-Arevalo, Kyle Wolfe, Samantha Hammock).

To my funding sources from my last year, thank you for the funding you provided over the last year to allow me to successfully complete my dissertation. To the UNC HMSC program, thank you for selecting me as the recipient of the Duquette Scholarship. To the Royster Society of Fellows, thank you for selecting me as a recipient of a Dissertation Completion Fellowship. To the NATA Research & Education Foundation, thank you for selecting me as a recipient of a Doctoral Scholarship.

To all the other doctoral students, thank you for helping me successfully navigate through the program. Whether it was struggling in class, figuring out stuff in the lab, or just having a little fun. This process was a lot easier and a lot more fun having all of you around to lean on.

Last but not least, to Dr. Brittney Harkey, I would not have been able to make it through this without you there every step of the way. One of the most common questions I get, is “how does it works sharing an office with your wife, isn’t it really hard?”. I just tell people that I cant think of anything better than getting to spend all day everyday with my best friend. So thank you Dr. Harkey, I couldn’t have done this without you.

TABLE OF CONTENTS

LIST OF TABLES

Manuscript 1 Tables

Manuscript 2 Tables

Manuscript 3 Tables

LIST OF FIGURES

Manuscript 1 Figures

Manuscript 2 Figures

Manuscript 3 Figures

CHAPTER I

1.1 Introduction

Osteoarthritis (OA) is one of the most common joint diseases worldwide, affecting an estimated 10% of men and 18% of women.[1] OA is characterized by progressive degradation of cartilage, subchondral bone, and synovium that ultimately lead to synovial joint failure.[2] Specifically, knee OA leads to impaired mobility and decreased quality of life and is the sixth leading cause of years lived with disability worldwide.[3] With OA expenses estimated at approximately $81 billion per year in direct medical costs in the United States,[4] being able to effectively prevent OA is needed to limit its increasing financial burden. However, despite efforts to prevent OA progression,[5] the incidence of OA is on the rise. The lack of preventative strategies is in part due to the maintenance of cartilage health being controlled by a complex, multifactorial process that is dependent on three primary components: mechanical,[6] biological,[7] and structural.[8] Rather than the classical approach that seeks to independently treat either the mechanical, biological, or structural factors associated with OA,[9,10] a novel systems-based view of OA states that the development of disease is due to a continuously shifting interaction of each component that will determine the development of irreversible clinical OA.[11,12] Healthy cartilage homeostasis is maintained when each of the primary components of joint health is operating within “normal ranges”, but if one or more of these components moves out of their normal range, abnormal stresses are placed on the cartilage potentially leading to OA. Therefore,

understanding the relative state and interaction between each measure of joint health in healthy cartilage will be imperative for determining the “normal ranges” of a healthy joint. Once these normal ranges are established we can monitor individuals at risk for OA development with a goal of better identifying the early stages of cartilage decline.

Structural Component of Cartilage Health

The first component of cartilage health is structural, which relates to cartilage morphology, as measured by different imaging modalities.[8] Alterations in cartilage morphology are considered the hallmark sign of the disease,[2] and radiographic evidence of joint space narrowing is most commonly used to diagnosis of knee OA.[13] However, classic radiographs are not capable of providing insight to very early declines in joint health because they are unable to directly visualize cartilage and lack sensitivity to capture early changes in cartilage structure,[14] Therefore, more cartilage specific imaging modalities (i.e. magnetic resonance imaging [MRI] and ultrasound) are needed to closely monitor joint health to determine who may be at risk for OA development.[8] While MRIs are capable of visualizing articular cartilage, MRIs are costly, time consuming, and not easily accessible.[15] Within recent years, ultrasound has become an assessable, cost-effective, and easy to conduct alternative to MRI.[16] Additionally, previous studies have demonstrated high agreement with ultrasound measured cartilage thickness when compared to cross-sectional cadaver measurements[17] and MRI,[18,19] indicating that ultrasound is a valid tool for quantitatively assessing knee cartilage structure. Ultrasound has been used extensively in populations with established knee OA;[16] however, there is yet to be any evidence of whether ultrasound

is capable in detecting earlier subtle changes in cartilage health that may be indicative of future OA development. Additionally, while end-stage OA is characterized by decreased cartilage thickness, there is evidence that the earliest stages of OA result occur to the cartilage biology prior to overt changes in cartilage thickness.[20] Therefore, utilizing measures of cartilage structure, while also monitoring changes in cartilage biology will be very important in understanding the relationship between structure and biology in the early changes in cartilage health.

Biological Component of Cartilage Health

The biological component of cartilage health is defined by cartilage metabolism and composition factors that are involved in the maintenance of normal tissue.[21] The development of OA is often considered a slow-progressing continuum that ultimately leads to decreased cartilage thickness and eventually joint failure.[2] However, the earliest signs of OA are often metabolic and compositional alterations that occur without overt changes in cartilage structure.[20] Examples of these changes in cartilage biology are initial depletion of proteoglycans, disorganization of collagen fibers, and increased water content;[22] and since these alterations occur prior to changes in cartilage size, most conventional imaging modalities are unable to capture these early deleterious changes in cartilage health. Therefore, using techniques such as assessing fluid-based cartilage metabolism biomarker concentration[21] or compositional imaging techniques[20] are needed to assess the biological component of joint health. Whether assessing serum, synovial fluid, or urine, these fluids can be assessed to determine circulating biomarkers to provide insight into biological processes that are occurring within the

body.[21] Depending on the biomarker being tested, tissue metabolism ranging from type II collagen,[23] proteoglycan,[24] and bone[25] can be quantified to provide information of subtle changes in joint metabolism that may eventually lead to cartilage degradation. Cartilage oligomeric matrix protein (COMP) is a specific biomarker of cartilage degradation and is important in organization of the cartilage-collagen matrix.[25] COMP is one of the most studied biomarkers of cartilage metabolism and is one of the most promising biomarker candidates for assessing early OA risk.[26,27] Besides assessing biomarker concentrations, novel compositional imaging techniques are capable of providing insight into the biology of cartilage by allowing for characterization and quantification of the composition of cartilage.[20] While ultrasound has not been utilized as a cartilage compositional imaging technique, the echo-intensity of ultrasound may be able to assess cartilage water content.[28] Since early proteoglycan depletion and collagen disorganization results in an influx of water into the cartilage,[29] echo-intensity of the cartilage may be a surrogate measure of cartilage composition. While assessing biomarkers of cartilage health and compositional imaging techniques provide evidence of early biological declines in cartilage health, there is evidence that coupling these tools with a mechanical stimulus (i.e. loading) may enhance the diagnostic specificity to discover even earlier alterations in cartilage health.[30,31]

Mechanical Component of Cartilage Health

The mechanical component of cartilage health deals with the joints ability to withstand and cope with forces applied to the joint during physical activity. Referred to as a “disease of mechanics”,[9] OA is often thought of as a “wear and tear” disease that is

developed and progressed due to abnormal cyclic loading that occurs during walking gait.[6] Cartilage health is not only susceptible to an abnormal increase in the magnitude of joint loading, but is also highly vulnerable to an increased rate of loading during gait. Due to the viscoelastic properties of cartilage, rapidly applied loads (i.e. impulsive loading) decrease the ability of cartilage to dampen loads during gait.[32] These increases in magnitude and rate of loading (i.e. vertical ground reaction force [vGRF]) during gait have been observed in patients with clinically diagnosed OA,[33] as well as a population at risk for OA development (i.e. anterior cruciate reconstructed patients).[34] While the vGRF provides a measure of gross impact force during gait, OA often times affects mainly the medial tibiofemoral compartment. Quantification of the knee adduction moment (KAM)[35] and KAM loading rate[36] during gait are used as a more specific gait variable to estimate the magnitude and rate of medial compartment loading, respectively. Alterations in both the KAM and KAM loading rate are observed in patients with OA, and have been linked to disease progression.[35,36] Understanding how these specific knee gait biomechanics affect both the structure and biology of the cartilage will be important in determining who may be at risk and which prevention interventions may best slow the progression of the disease.

Interaction Between the Structural, Biological, and Mechanical Components of Cartilage Health

While each of component of cartilage health are extremely important in isolation, using an multifaceted systems model approach to examine the interaction between the individual components may uncover earlier declines in joint health. For example, the

medial-to-lateral cartilage thickness ratio was positively associated with KAM in healthy individuals, while medial-to-lateral cartilage thickness ratio and KAM was negatively associated in individuals with OA.[37] This indicates that healthy cartilage may be positively conditioned to the cyclical loading of gait, while OA cartilage responds negatively to load. Additionally, COMP has been described as mechano-sensitive as it plays a role in transducing mechanical forces in the cartilage, and there is evidence that subjecting the cartilage to a mechanical stimulus (i.e. gait) is effective at elevating the sensitivity of COMP to provide an indicator of cartilage health.[38] Using a stimulus-response study design, OA subjects completed a 30 min walk (i.e. mechanical component) that stimulated a change in COMP concentration (i.e. biological component), and this change in COMP was significantly correlated with five year longitudinal declines in cartilage thickness (i.e. structural component).[31] Similar to the purpose of a cardiac stress test, in which controlled exercise is used to produce a physiological response that is used to reveal underlying pathology that is not observed at rest, determining changes in both cartilage structure and biology following a mechanical stimulus may reveal early declines in cartilage health.

1.2 Statement of Purpose

Therefore, if we plan to effectively monitor joint health in patients at risk for developing OA, we need to better understand the relative state and interaction of the mechanical, biological, and structural components of cartilage health in individuals without knee injury or joint related conditions. Establishing “healthy ranges” for these primary components of joint health will serve as benchmarks that will hopefully be able

to be used in an attempt to prevent OA development in patients following acute injury. Thus, the following specific aims have been developed to establish the relative state and interaction of the mechanical, biological, and structural components of joint health in healthy individuals.

1.3 Specific Aims

Specific Aim 1 - Cartilage Ultrasound and Biomechanics

The purpose of specific aim 1 was to compare the acute response and recovery of US measures of cartilage health (i.e. thickness, area, and echo-intensity) between a walking, drop-landing, and control condition in healthy participants. Additionally, we sought to determine the associations between a change in US measures of cartilage health and lower extremity loading biomechanics during the walking and drop-landing condition.

We hypothesized that cartilage thickness and area will decrease, while echo-intensity will increase following the walking and drop-landing condition when compared to the control condition. We hypothesized that the deformation created by the drop-landing condition will take longer to recover when compared to the walking cartilage deformation recovery. Additionally, we hypothesized that lower extremity loading biomechanics will be associated with greater changes in the US outcomes following the walking and drop-landing condition.

Specific Aim 2 - Cartilage Metabolism Biomarkers and Biomechanics

The purpose of specific aim 2 was to compare the acute serum COMP response between walking, drop-landing, and control in healthy individuals. Secondarily, we sought to determine the association between the COMP response and lower extremity loading biomechanics during the walking and drop-landing conditions.

We hypothesized that we would see an increase in COMP concentration following the walking and drop-landing conditions when compared to the control condition, as well as a larger magnitude COMP response in the drop-landing compared to the walking condition. Additionally, we hypothesized that lower extremity loading biomechanics would be associated with a greater COMP response following the walking and drop-landing conditions.

Specific Aim 3 - Cartilage Ultrasound and Cartilage Metabolism Biomarkers

The purpose of specific aim 3 was to determine the association between baseline US measures of cartilage health (i.e. thickness, area, and echo-intensity) and baseline serum COMP. The second purpose of this study was to determine the association between the change in US measures of cartilage health and the serum COMP response following walking and drop-landing in healthy individuals.

We hypothesized that there will be a significant association between baseline US measures of cartilage health and baseline COMP concentrations. We hypothesized that a greater change in US measures of cartilage health will be associated with a greater serum COMP response following walking and drop-landing.

1.4 Operational Definitions

1. *Loading Protocol* – a loading protocol will be considered the walking and drop-landing protocols.
2. *Cartilage Health* – The health of the joint is dependent on the interaction between three main components: structural, biological, and mechanical.
3. *Structural Component of Cartilage Health* – The structural component of cartilage health relates to cartilage morphology as measured by different imaging modalities. Within this study, the structural component of cartilage health is defined using ultrasound.
4. *Biological Component of Cartilage Health* – The biological component of cartilage health is defined by measures of cartilage metabolism. Within this study the biological component of cartilage health is quantified using serum concentrations of COMP.
5. *Mechanical Component of Cartilage Health* - The mechanical component of cartilage health describes the joints ability to withstand and cope with forces applied to the joint, most notably how the joint functions during walking. The mechanical component of cartilage health will be quantified by the magnitude and rate of the vertical ground reaction force, internal knee extension moment, internal knee abduction moment, and the internal knee adduction moment during walking and drop-landing.
6. *Cartilage Echo-intensity* – The calculated grey scale of the cartilage area measurements. This measure is theorized to indicate cartilage composition.

CHAPTER II

2.1 Pathogenesis of Osteoarthritis Development

Epidemiology of Knee Osteoarthritis

Knee osteoarthritis (OA) affects 29 million Americans at an annual cost of $165 billion.[39,40] OA is a leading cause of disability by deteriorating quality of life[41,42] and leading to comorbidities such as obesity, depression, and cardiovascular disease.[43,44] The World Health Organization reports that OA is the 4th leading cause of years of life lost due to disability.[45] Currently, osteoarthritis treatment is palliative rather than preventative, with joint replacements being the primary end-stage treatment for OA.[46] Joint replacements are utilized to alleviate pain, but no interventions have been established to prevent OA development or progression. One reason for the lack of an effective prevention strategy is because OA is not diagnosed until later stages of joint breakdown has occurred. Since the majority of OA phenotypes are idiopathic (i.e. uncertain cause and timing of the disease origin), this complicates early detection and treatment at the earliest stages of disease development. Therefore, further research is necessary to better identify early changes in joint health, which will hopefully allow us to prevent the progression of knee OA and reduce the associated disability.

Post-traumatic Osteoarthritis Pathogenesis

While the majority of OA develops idiopathically, approximately 12% of OA is considered to occur post-traumatically (PTOA) as a result of a previous acute joint

injury.[47] Due to the known “inciting event” that triggers PTOA development, researchers and clinicians are able to monitor patients’ joints following acute injury to better understand early PTOA pathogenesis.[48] Since approximately one-third of patients following anterior cruciate ligament (ACL) injury and reconstruction (ACLR) develop knee OA within the first decade following acute injury, these patients serve as a good model for PTOA development.[49] While the median age of individuals with idiopathic OA is 55 years of age,[50] ACL injuries occur primarily in patients between the ages of 15 – 24[51] Thus, patients following an ACLR are ideal for the prospective study of PTOA development because of 1) their significant patient population (~250,000 occurring annually[52]), 2) they are already seeking medical attention prior to the development of OA, and 3) PTOA is a rapidly advancing form of OA that may allow for shorter follow times to determine progression. Therefore, utilizing ACLR patients as a model for PTOA development, we are able to monitor changes in cartilage health to determine very early risk factors that may lead to the future development of OA.

Knee OA is described as a disease of the entire synovial joint with signs such as cartilage breakdown, bone remodeling and sclerosis, meniscal damage, and synovial hypertrophy.[2] However, the breakdown of cartilage is considered a hallmark signs of OA development,[2] and knowledge of the basic anatomy of cartilage and how the structures relate to the function of the tissue will be important in understanding the pathogenesis of OA.

Anatomy of Articular Cartilage

Articular cartilage is a dynamic tissue that plays an important role in the protection of synovial joints. While healthy cartilage may only be ~2mm thick,[53] cartilage is imperative for distributing load and minimizing stresses placed on the subchondral bone, as well as providing a low friction environment within synovial joints.[29] Cartilage is both avascular and aneural. Due to a lack of blood supply, cartilage receives its nutrients from mechanical movement of synovial fluid in and out of the structure. The absence of a nerve supply is important because degeneration can occur to the tissue the occurrence of pain. Combined, this makes the tissue susceptible to early damage without many warning signs. Additionally, once damage has occurred to the tissue, cartilage has very limited healing capacity.

Figure 1 depicts the main functional components of cartilage. Fluid is the main component of articular cartilage; constituting between 60-80% of the entire structure.[54] Water is a contributor to the mechanical strength of the tissue due to its interaction with the extracellular matrix.[29] The chondrocytes are the main types of cell type constituting only 5-10% of articular cartilage, but are responsible for production and maintenance of the entire extracellular matrix.

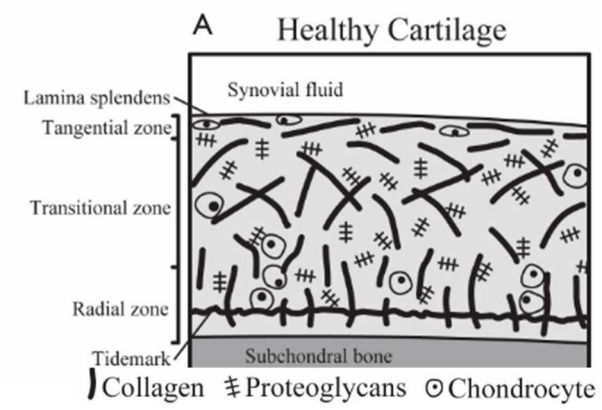


Figure 1. Anatomy of Articular Cartilage; Matzat 2013

The extracellular matrix is comprised mainly of collagen and proteoglycans. Collagen accounts for 25% of the entire structure of articular cartilage and the bulk of the extracellular matrix, with type II collagen being the most prevalent (95% of total cartilage collagen).[54] Proteoglycans are negatively charged hydrophilic molecules that consist of aggregates of glycosaminoglycans (GAGs) bonded together by link proteins. The major proteoglycan in articular cartilage is aggrecan, which consists of the following GAGs: chondroitin sulfate and keratin sulfate.[29] Collagen restricts the hydration of these hydrophilic molecules to 40-60% and variation from this homeostatic balance can change the mechanical qualities of the cartilage and lead to damage. As cartilage is compressed during movement the water is propelled out of the cartilage, which forces the like charged GAGs to come in close proximity to each other.[29] The closely oriented GAGs repel each other and create the majority of the compressive strength seen in articular cartilage.

Since healthy cartilage is imperative to sustaining the health of the entire joint, monitoring cartilage structure for subtle declines in cartilage health is important at determining patients at risk for future OA development.

2.2 Structural Component of Cartilage Health

Problems with Current OA Management

The structural component of cartilage health is quantified by imaging modalities that provide information regarding the structural integrity of cartilage.[8] Monitoring structural changes in cartilage is one of the primary factors used to diagnose OA, and the amount of structural cartilage degradation is utilized to grade the severity of the disease. However, radiographs (i.e. X-rays) are currently used to diagnose OA with a semi-quantitative grading scale (i.e. the Kellgren and Lawrence [KL] Grade[55]) that is less than adequate for assessing early cartilage alterations.[56] The first issue with radiographical imaging of OA is that X-rays only indicate changes in bone (i.e. osteophyte formation, sclerosis, reduced joint space), and indirectly imply declines in cartilage health (Figure 2). Joint space narrowing is determined by a decline in the space between the tibia and femur, and is theorized to indicate cartilage breakdown, however, this measurement can easily be confounded by meniscal cartilage legions and meniscal extrusion.[57] Another issue with radiographs is that evidence of cartilage damage does not appear on X-rays until after significant irreversible deterioration has occurred at the joint.[58,59] Thus, there has been much debate of whether the inability to discover efficacious therapies to prevent OA progression is due to the inadequacies of radiographical imaging outcome measures.[60] Therefore, utilizing imaging modalities capable of assessing early changes in cartilage health will be important for determining patients at highest risk of OA development and allowing for early treatment to prevent disease progression.

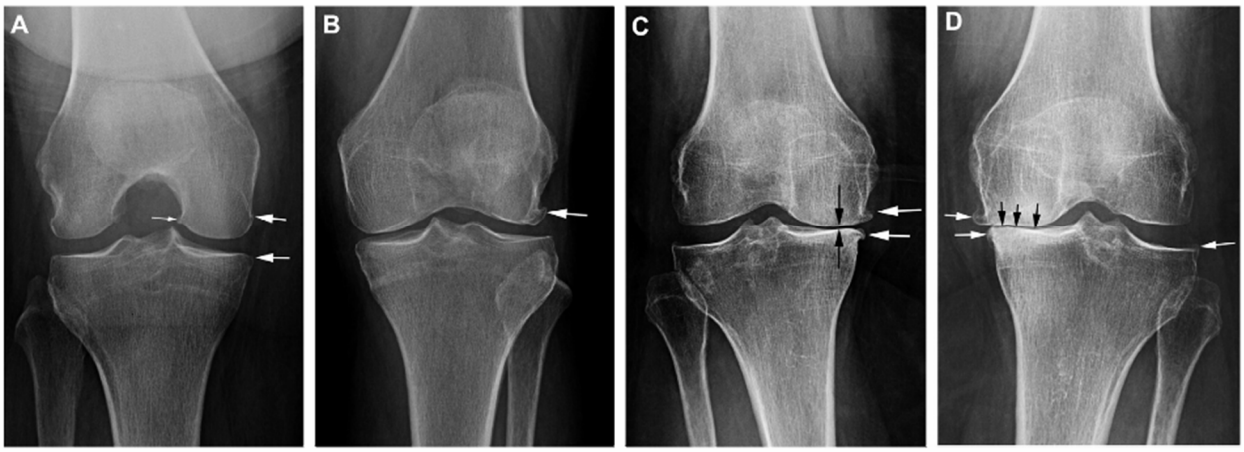


Figure 2. Radiographic Signs of Osteoarthritis. Hayashi 2016

Magnetic Resonance Imaging and Cartilage Health

Magnetic resonance imaging (MRI) has emerged as the gold standard for non-invasively monitoring early structural cartilage changes.[61] Unlike radiography, MRI is capable of direct imaging of many of the joint structures involved in OA pathogenesis such as the cartilage, meniscus, bone marrow, tendon and ligaments.[62] There are multiple semi-quantitative MRI assessments that provide a global OA score: the Whole Organ Magnetic Resonance Imaging Score (WORMS) and the Knee Osteoarthritis Scoring System (KOSS).[63] Additionally, a more recent ACL specific OA scoring system (ACLOAS[64]) takes in to account baseline structural damage following acute injury. While these scoring systems are a powerful tool at providing a global view of the different aspects of OA, they lack a specific direct quantification of cartilage structure.

Quantitative MRI is capable of providing objective three-dimensional measurements of cartilage structure.[61] Figure 4 displays the basic procedures involved in quantifying cartilage structure. Step 1 (Figure 3A) is acquiring the MR image. Step 2 (Figure 3B) is termed segmentation, which involves tracing the articular cartilage of interest through serial images of the entire knee joint (i.e. femoral, tibial, patellar). One

measure commonly used to classify cartilage structure is the volume of the cartilage. Another similar measurement used is the mean thickness of the cartilage, which is defined as the ratio of the previously mentioned cartilage volume divided by the underlying subchondral bone area.[65]

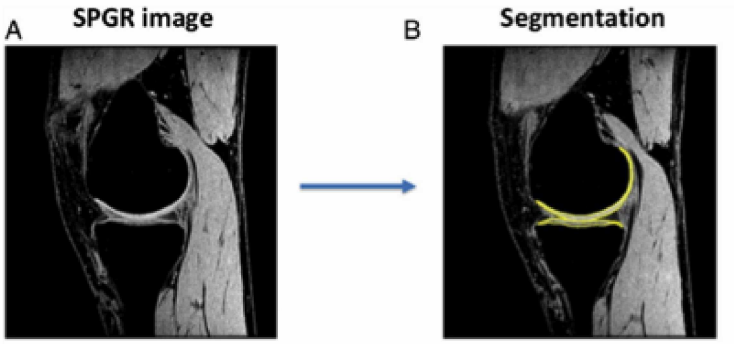
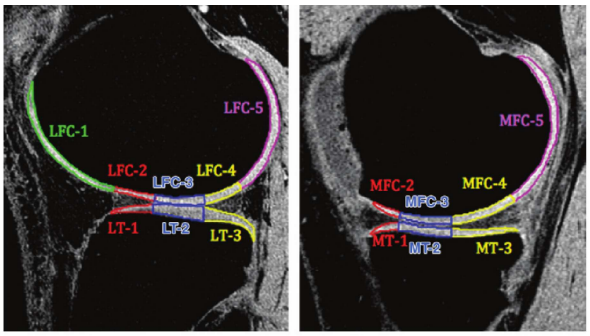


Figure 3.Morphological MRI Processing; Gupta 2014

Instead of utilizing entire joint volumetric measurements of cartilage, many researchers separate the knee into subcompartments in order to get a better spatial distribution of the morphological cartilage alterations. Usually the knee is divided into functional compartments: medial tibiofemoral (medial femoral condyle and medial tibial surface), lateral tibiofemoral (lateral femoral condyle and lateral tibial surface), and the patellar compartments. However, these compartments are more commonly subdivided with the femoral condyles being separated into five different subcomponents (FC-1 through FC-5) and the tibias separated into three compartments (T-1 through T-3) (Figure 4).[66-68] Division of these subcompartments is determined by the position of the meniscus. FC-3 and T-2 are regions of the femoral and tibial cartilage that are in contact at the middle of the knee. FC-2 and T-1 are the regions above and below the anterior horn of the menisci, while FC-4 and T-3 are above and below the posterior horn of the menisci. Finally FC-1 and FC-5 are the anterior and posterior non-weightbearing sections of the femoral cartilage, respectively. However, researchers have been even

more thorough, by dividing the MR images according to the Outerbridge Scoring sheet and dividing a single knee in to 49 separate compartments (9 patella, 18 tibia, 18 femoral condyle, and 4 trochlea);[69] providing even more complexity into the spatial alterations in cartilage structure.



a. b.
Figure 4. Subcompartmentalization of Cartilage; Li 2011

Cartilage degradation (i.e. thinning of cartilage structure) has been extensively studied and validated as a marker of disease progression in patients with diagnosed knee OA. Systematic reviews have established the concurrent and predictive validity of MRI,[70] as well as its responsiveness and reliability in monitoring changes in cartilage structure.[71] Additionally, members of the Osteoarthritis Research Society International working with the US Food and Drug Administration have recommended that MRI is now recommended for clinical OA trials assessing cartilage structure.[72] This evidence indicates that a decline in cartilage volume/thickness measured with MRI provides insight into the progression of the disease. Yet, all of the patients utilized in these studies already have radiographic evidence of the disease. Therefore, monitoring the changes in MRI cartilage structure following ACLR may provide evidence of early structure changes following may provide a structural link between acute injury and PTOA development.

Evidence of MRI Cartilage Alterations Following ACL Injury and Reconstruction – A Model for Structural Post-traumatic Osteoarthritis Development

There is mounting evidence that cartilage degradation following ACL injury and reconstruction is eminent with approximately one-third of patients presenting with radiographic OA within the first decade following injury.[49] However, it remains unclear how early cartilage breakdown begins following injury. This section will outline the current evidence regarding alterations in MRI measured cartilage thickness and volume following injury/surgery.

Within 1 Year of Injury

The earliest quantification of cartilage structure following ACL injury is at a mean of 3.4 months following injury, and provides insight in the early cartilage alterations following injury.[67] The total cartilage volume from all subjects was significantly less than the their contralateral knee. Further sub-compartment analyses indicate that these overall decreases were driven by a decrease in both the lateral femur and tibia cartilage volume, while the medial compartments were no different than the contralateral limb. This initial decline in the lateral joint may be contributed to the initial impact of injury; since the valgus/internal rotation mechanism of injury seen in the majority of ACL tears results in large compressive forces being placed on the posterior lateral tibia and femur.[73] The contralateral limb was utilized as a control rather than the knee of a healthy control participant because of the large inter-subject variability noted between participants. Van Ginckel et al found that ACLR patients at 6 months following reconstruction presented with similar cartilage volume when compared to a healthy

control group.[74] These studies within the first year following ACL injury indicate that there may be within subject declines in cartilage volume, but comparing these declines may be masked when comparing to a healthy control group.

Between 1 and 2 years following injury

The first longitudinal evaluation of cartilage structure compares the volume and thickness at three months post injury to outcomes at one year following injury and highlights the spatially different cartilage responses to ACL injury.[75] While a majority of the sub-compartments indicate a decline in both volume and thickness (femoral trochlea demonstrating the greatest decline), other compartments resulted in increased thickness of cartilage (medial femoral cartilage demonstrating greatest increase). This thickening of cartilage initially appears contradictory to diagnosed MRI studies, as cartilage decline is the end stage of OA. However, there is evidence that the earliest stages of OA actually result in an increase in cartilage thickness.[76,77] Apparently, OA is not a one-way road to cartilage loss, as there appears to be spatial differences in cartilage adaptation with some cartilage exhibiting cartilage thinning, while some cartilage thickening.[77] This thickening is theorized to be due to two potential mechanisms: cartilage matrix hypertrophy or cartilage swelling. Cartilage matrix hypertrophy is though to be a protective mechanism to the altered stresses being placed on the joint. Cartilage swelling is theorized to be due to the influx of water due to the injured extracellular matrix.[77] Utilizing the same subjects as the previous longitudinal investigation of cartilage structure following ACL injury, a two-year follow up was used determine if these trends in cartilage thinning/thickening continued.[78] There was a

continuing trend observed with an increase in the thickness of the central medial femur and thinning of the femoral trochlea. However, there was also additional thinning of the posterior medial femur and posterior lateral femur between year one and year two following injury. Interestingly, the magnitude of cartilage morphology changes over this year is comparable to (or greater) than the annual change observed in patients with diagnosed OA, potentially demonstrating signs of rapidly progressing PTOA.

Greater than 5 years Following ACL Injury

There are conflicting results in the structural response of the cartilage at least half a decade following the initial ACL injury. One study discovered that at an average of seven years following reconstruction there are no observed side-to-side differences in subcompartment cartilage thickness.[79] However, another study provides longitudinal evidence of an overall increase in cartilage thickness over the first 5 years following injury (increase of 31μm/year).[80] This study provided additional evidence that changes within cartilage thickness are variable depending on subcompartment, with the medial femur and tibial being the most affected compartments (Figure 5).[80] Additionally, this was the first article to include a new measure termed the total subregional tibiofemoral cartilage thickness change score, which summed the absolute value of the cartilage thickness changes over all 16 of the subregions. This value indicated a large absolute change in the cartilage thickness, once again highlighting that OA might not be a one-way road to cartilage thinning.

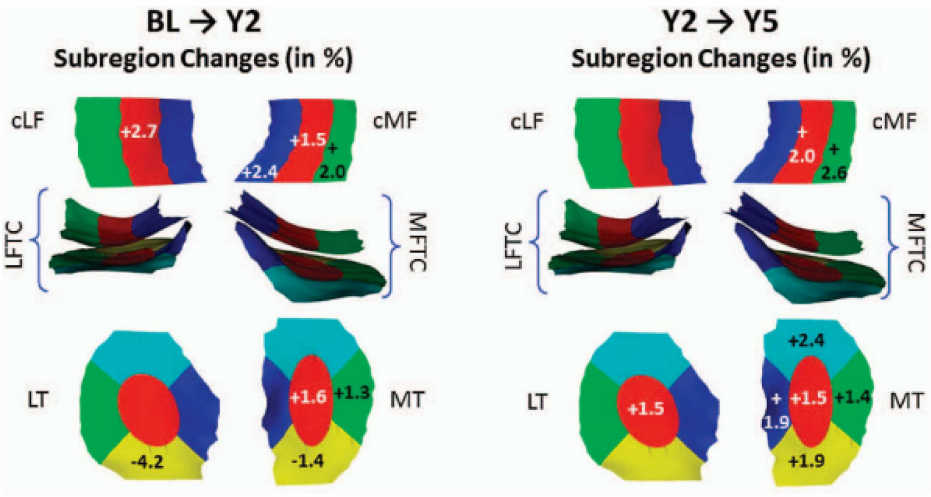


Figure 5. Baseline to Year 2 (Left) and Year 2 to Year 5 (Right) thickness changes Eckstein 2014

Despite all the MRI's ability to provide a valid and reliable tool for specifically visualizing cartilage, MRIs are extremely expensive, the availability of machines is very low, and extensive specialized training is needed to operate. Thus, limiting the ability of MRIs to provide routine use in the clinic to monitor cartilage health. Therefore, additional imaging modalities are needed to provide the same sensitivity to visualize cartilage, while also allowing for accessible clinical use.

Musculoskeletal Ultrasound Imaging and Cartilage Health

Musculoskeletal ultrasound has recently become very popular within research laboratories and clinics due to the potential for ultrasound to be a robust imaging modality to monitor joint changes following acute injury.[81] First, ultrasound is a safe, radiation-free and non-invasive technique that allows for accurate measurement of cartilage thickness, as well as other aspects of the joint indicative of OA.[16] Additionally, the equipment is widely available due to its cost-effectiveness compared to the other imaging modalities. Due to the accessibility of ultrasound, this technique has the

potential to be used as a bedside procedure for both researchers and clinicians, allowing for a quick and accurate measurement of joint structure that can easily occur during a more inclusive orthopedic assessment. Also, with the other imaging modalities, the patient is required to sit in an uncomfortable machine/position for an extended period of time to only get an image in one plane; however, the ultrasound allows for a multiregional evaluation in a very short period of time.

Validity and Reliability of Ultrasound Measured Cartilage Thickness

Naredo et al conducted a validity study by comparing ultrasound measured cartilage thickness to anatomical measured thickness.[17] Following an ultrasound thickness measurement in a cadaver knee, the knee was dissected and the cartilage thickness was assessed via a cross-section view of the same location.[17] There was high agreement between the ultrasound and cross-sectional cadaveric measurements of cartilage thickness for the medial and lateral condyles (ICC= 0.732 – 0.883), indicating that the ultrasound measurement is a valid tool for measuring anatomical cartilage thickness. However, this anatomical measurement of cartilage thickness is highly laboratory based, so there is also a need to compare ultrasound to MRI, which is considered the *in vivo* "gold-standard" measurement of cartilage thickness. In a study comparing ultrasound and MRI measured cartilage thickness, there was an observed strong association between the two imaging modalities (ρ=0.82).[82]

In addition to validity, the reliability between raters and the reliability between different sessions needs to be established to ensure that this can be reproduced by different people and at different time points. Naredo et al established excellent reliability

between raters (ICC=0.86-0.94); however, this study was completed on human cadavers, and may not represent the ability to reproduce the measure on a patient population.[17] Bevers et al demonstrated good reproducibility of cartilage thickness in OA patients (κ=0.62-0.68), as well as good reproducibility for other measures of OA ultrasound like protrusion of medial meniscus, infrapatellar bursitis, effusion, and Baker's Cyst.[83] Additionally, Abraham et al demonstrated that in a clinic setting, there was good reliability between two different raters separated by two weeks (ICC=0.50-0.67).[84]

Ultrasound has demonstrated the ability to produce a reliable and valid quantification of the femoral articular cartilage thickness, and multiple systematic reviews have described the use of ultrasound to quantify declines in cartilage thickness in patients with OA.[81,85,86] However, no studies have been conducted examining participants following ACLR, indicating that more work is needed to determine if ultrasound is sensitive enough to determine early changes in cartilage thickness in people at risk for PTOA development. Due to the importance of cartilage in attenuating excessive energy at the knee during gait, ultrasound may be able to provide us with invaluable information as we are treating patients at high risk for developing OA. As there is a huge push in OA care to move from palliation to prevention, a readily available bed-side tool that can accurately monitor the progression of the disease will be beneficial in determining the effectiveness of treatments aimed to slow disease progression.

Limitations of the Structural Component of Cartilage Health

While the structural component of cartilage health plays a very important role in the diagnosis and progression of OA, overt structural damage is occurring following a long-term latent period of compositional breakdown of cartilage. Thus, coupling structural measurements with biological outcome measures that provide insight into the cartilage metabolism and composition will be important into understanding very early changes in cartilage breakdown.

2.3 Biological Component of Cartilage Health

The biological component of cartilage health is defined by factors that alter cartilage metabolism and composition that ultimately leads to cartilage degradation. In early stages of OA, the extracellular matrix of the cartilage begins to breakdown and the function of the cartilage declines with gross alterations in cartilage structure.[22] Figure 6B depicts some of the common early changes observed in cartilage that occur prior to gross degradation of the structure: initial depletion of proteoglycans, disorganization of collagen fibers, and increased water content.[22] These changes decrease the ability of the cartilage to withstand stress, which results in increased strain on the tissue and eventually the advanced degeneration (Figure 6C). The two main ways to quantify the biological component of cartilage health are measuring the concentration of cartilage metabolism biomarkers and utilizing novel compositional imaging modalities.

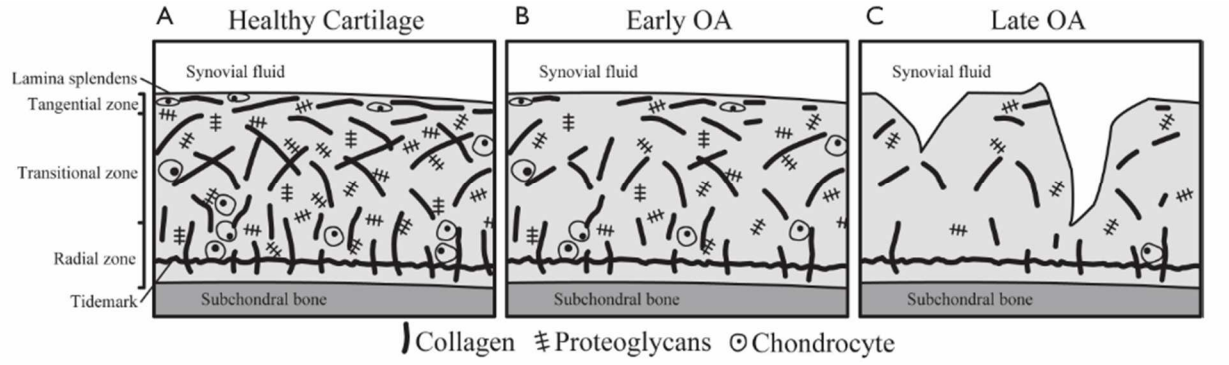


Figure 6. Microscopic Stages of OA; Matzat 2013

Biomarkers of Osteoarthritis

Biomarkers reflect dynamic alterations in joint metabolism allowing insight into joint remodeling and disease progression.[14] Utilizing samples of either blood, urine and synovial fluid, a biomarker can either quantify an operator of joint damage/synthesis or products of joint damage/synthesis.[21] OA has been described as a disease on a continuum that begins with a prolonged asymptomatic period that is characterized by molecular changes to the joint that are unable to be detected with structural imaging modalities (Figure 7).[87] By monitoring alterations in biomarkers of cartilage metabolism, we may be able to identify individuals at risk for OA development.[88] Knowledge of the individuals at most risk for OA development will allow for more targeted prevention programs, in hopes of normalizing cartilage metabolism prior to the development of radiographic OA.[48]

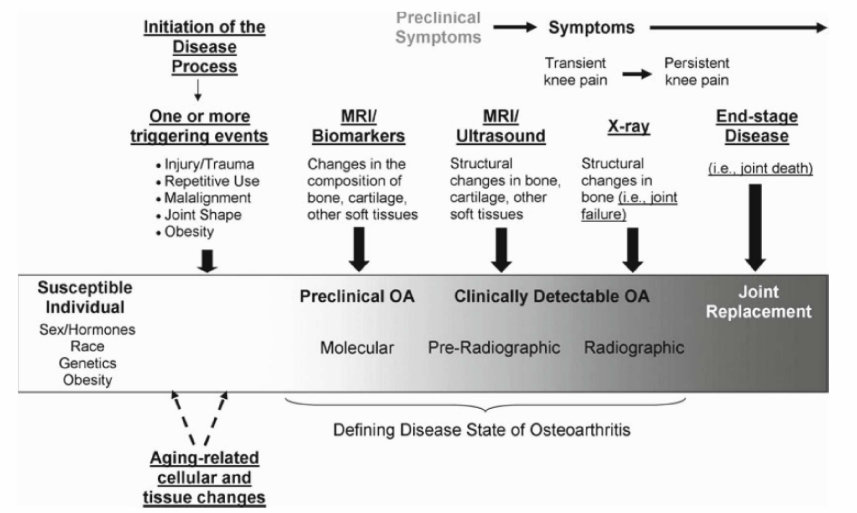


Figure 7. Continuum of OA Stages; Kwoh 2012

The OA Research Society International has established a classification system for OA biomarkers, BIPEDS, which is intended to allow for a central language and structure with which to communicate knowledge and advances related to OA biomarkers.[87] The acronym BIPEDS (Burden of Disease, Investigative, Prognostic, Efficacy of Intervention, Diagnostic, and Safety) is used to describe the six potential categories an OA biomarker may belong to, which is theorized to aid in the validation of future OA biomarkers (Figure 8).

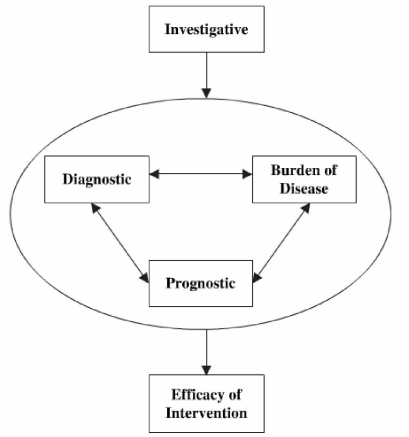


Figure 8. Hypothetical Development of OA Biomarkers; Bauer 2006

Burden of Disease[14]

These biomarkers are used to indicate severity of the disease, which is useful in categorizing the different stages of the disease. They can only be used to represent the extent of the disease at the time of assessment, and are not able to denote whether there is any potential for progression of the disease.

Investigative[14]

This category is for novel biomarkers that have the potential for utilization with an OA population, but there is not enough evidence for the biomarker to fit into a particular category. These will be used along with validated biomarkers to hopefully in the future be able to provide a greater understanding of the metabolic processes within OA.

Prognostic[14]

This class will be used to indicate likelihood of progression and potentially the rate at which OA will develop. Also these biomarkers could also indicate who may be at risk for developing the disease. These will be important for the development of potential drug therapies because if we know who will be progressing faster we can use these prognostic biomarkers as an inclusion criteria so that we will know sooner whether or not the drug has an affect, which will save both time and money.

Efficacy of Intervention[14]

These are investigating for drug therapies and investigating whether or not the drug is reaching the desired target and if it is having the desired effect. Helps understand the pharmacodynamics and pharmacokinetics of the drug interventions.

Diagnostic[14]

A diagnostic biomarker is indicative of whether or not the disease is present within an individual, but not necessarily the severity of OA. It also possesses the strength to identify people who may be at risk for OA. This is a more promising means of diagnosing an injury than the current radiographic gold standard because the biomarkers will be more sensitive than the imaging technique at initial discovery of the disease and tracking changes over time. These will also be able to identify true controls to use during future OA studies.

Safety[87]

Safety biomarkers could be used in preclinical and clinical applications to monitor the health of joint tissues, in an attempt to detect pathological changes and cytotoxicity.

Cartilage Oligomeric Matrix Protein as a Biomarker of Osteoarthritis

Cartilage breakdown is one of the earliest signs of OA; therefore, utilizing a biomarker that is capable of detecting early alterations in cartilage metabolism will be pivotal in detecting early declines in cartilage health. Cartilage oligomeric matrix protein (COMP) is an essential structural and functional component of the cartilage extracellular

matrix, and by monitoring concentrations of COMP in serum, we are able to monitor alterations in cartilage metabolism.[89] COMP has been established as one of the best candidates as a marker to monitor the progression of OA.[90] Multiple studies have demonstrated that serum COMP was able to distinguish between an OA and control group, with the OA group presenting with significantly greater concentrations of serum COMP than individuals without OA.[91-95] Additionally, COMP levels were also able to distinguish between severity of OA, with greater KL Grades presenting with greater concentrations of serum COMP.[91] The change between serum concentration at baseline and one[96] and three year[25] follow ups have demonstrated to be a longitudinal predictor of joint space narrowing over 5 years. Also, using the Outerbridge Score (i.e. an arthroscopic cartilage grading scale), serum COMP is significantly positively correlated with greater cartilage damage, indicating that greater COMP concentrations reflects greater cartilage damage.[27] Therefore, COMP appears to reflect changes in molecular cartilage metabolism that if left unchanged, will eventually lead to greater concentration and greater breakdown. Thus, monitoring concentrations of serum COMP following acute injury may allow for us to determine who may be at risk for future OA development.

Osteoarthritis-Related Biomarkers Following ACL Injury and Reconstruction - A Model for Biological Post-traumatic Osteoarthritis Development

Since OA has been described as a disease on a continuum and acute injuries have been cited as an inciting event that begins the progression to PTOA,[87,97] we can monitor changes in biomarker concentrations following acute injuries in hopes of gaining

a better understanding of the very early pathogenesis of OA. In the previous paragraph we eluded that serum COMP is one of the better OA biomarkers for detecting early changes in cartilage metabolism; however, serum COMP has yet to be investigated in patients following ACL injury or reconstruction. Yet, there is evidence that following ACL injury there is a significant increase in synovial fluid concentrations of COMP when compared to healthy controls, indicating that there is potentially early alteration in cartilage metabolism following acute injury.[98] While not much research has been done utilizing COMP following ACL injury, there is evidence that there are alterations in cartilage metabolism and inflammation following both ACL injury and reconstruction.[99] Currently, synovial fluid concentrations of cartilage extracellular matrix degradation (i.e. type II collagen and proteoglycans) are the most consistently increased OA biomarker following injury and reconstruction. Additionally, following ACLR there is an increased early inflammatory cytokine response in the synovial fluid that may contribute to altered tissue turnover in the joint.[99] However, more research needs to be done to link early increases in OA-related biomarkers following ACL injury to future development of OA development.

Cartilage Compositional Imaging as a Measure of Cartilage Biology

Compositional MRI reveals biochemical and microstructural changes in cartilage that provide insight into cartilage composition.[20] Since the composition is responsible for the functional strength of cartilage, alterations in cartilage composition will result in a declined ability of the tissue to transmit load to the underlying subchondral bone. Similar to biomarkers of cartilage breakdown, compositional MRI focuses on determining early

molecular changes in the cartilage that are potentially reversible, and may help to identify individuals at risk for OA development prior to overt joint damage (Figure 7).[100] T1rho MRI is one of these compositional imaging modalities that probes at the slow motion interactions between motion-restricted water molecules and the extracellular matrix.[101] Since the hydration of the proteoglycans of the extracellular matrix is so important to the function of the cartilage, this measure of the motion of the water within the tissue (i.e. T1rho relaxation times) provides us with a quantification of the proteoglycan density of the structure. Initial *in vitro* studies provided early evidence that increases in T1rho relaxation times were correlated with depletion of proteoglycan.[101] Additionally, *in vivo* studies have provided evidence that patients with OA have significantly higher relaxation times when compared to control participants, as well as the ability of T1rho to distinguish between stages of cartilage degradation.[102] While T1rho has demonstrated a good ability to detect cartilage declines in patients with established OA, the real utility of T1rho will be to discover early declines in cartilage health in patients following acute injury, prior to severe degradation.

Declines in Cartilage Composition Following ACL Injury

One-third of patients following ACL injury and reconstruction will develop radiographic OA within the first decade following injury. Therefore, if we are able to monitor changes in cartilage health following acute injury, we may be able to better identify patients at risk for OA development, which will allow for more targets prevention strategies. At one year following ACLR, T1rho relaxation times are increased in the medial tibia and medial femoral condyle why compared to the uninjured contralateral

knees, indicating a decrease in proteoglycan density early following surgery.[68] At two years following ACLR, the declines in cartilage health persisted as the ACLR individuals presented with greater T1rho relaxation times in the posterolateral tibia and medial femur when compared to a healthy control group.[103] One of the theories behind the early declines in cartilage health is that the initial mechanical trauma sustained during the injury may alter the metabolism of the cartilage and ultimately lead to the cartilage breakdown.[75] Bone marrow lesions are present in up to 80% ACL injured knees and are theorized to be due to the translational impact during the injury itself. Interestingly, the cartilage overlying bone marrow lesions immediately presents with increased T1rho relaxation times; as well as increased relaxation times at one-year following injury, despite resolution of the bone marrow lesion.[104] Therefore, it appears that compositional imaging modalities are capable of detecting very early changes in cartilage health following acute injuries; however, due to the expense, the use of T1rho MRI may not be as clinically feasible as ultrasound.

Use of Ultrasound as a Cartilage Compositional Imaging Modality

Currently, ultrasound has not been used as a cartilage compositional imaging modality, but has been used as a compositional imaging modality for other musculoskeletal tissues. Ultrasound echo-intensity has been used extensively in skeletal muscle to discriminate between healthy and pathologic tissues.[105] Ultrasound echo-intensity (i.e. the greyness of the image) is dependent on the amount of returning sound waves that return to the ultrasound head, and this echo-intensity is used to discriminate between tissues.[28] Muscle echo-intensity increases with age, which is

theorized to be due to increased fat and fibrous tissue infiltration into the tissue.[106] Due to the different composition of fat and fibrous tissue, the echo-intensity of the muscle will be altered. Using this notion that a change in echo-intensity is due to a change in tissue composition, we believe that we will be able to detect composition changes in cartilage composition utilizing ultrasound echo-intensity. Early changes in cartilage composition will result in an influx of water due to disruption of the extracellular matrix, and this increase in water content will be reflected by a change in ultrasound echo-intensity. Therefore, we believe that utilizing cartilage ultrasound echo-intensity will allow accessible monitoring of cartilage composition following acute injury.

2.4 Mechanical Component of Cartilage Health

The mechanical component of cartilage health deals with the ability of cartilage to withstand and cope with forces applied to the joint, most notably how the knee functions during walking. Many believe that OA is a disease that is both initiated and progressed due to one or many sources of increased load at the joint.[9] Whether this increased load is due to acute injury,[97] obesity,[107] or an abnormal anatomical malalignment,[108] all of these will ultimately increase the stress placed on the cartilage and will eventually result in breakdown of the tissue. It has been suggested that the mechanics that occur at the knee during walking conditions healthy cartilage, and small alterations in the mechanics of walking can drastically affect the knee health due to the repetitive cyclic loading that occurs with this task.[109] Gait biomechanics have been described as one of the most important risk factors for the development and progression of knee OA,[6] and altering the way loads are distributed across the knee during gait has been a common therapeutic

target when attempting to slow the progression of the disease. Determining how the mechanics are associated with both cartilage structure and biology will be important to understanding how mechanics are involved in the early pathogenesis of the disease.

One quantifier of mechanical loading during walking gait in the lower extremity is the vertical ground reaction force (vGRF), which is simply the force applied to the body by the ground. While the vGRF may not be specific to the knee and may affect many joints within the lower extremity, it is theorized that greater vGRF is indicative of greater mechanical loading at the knee.[34] Greater vGRF in ACL transected dogs has been associated with greater depletion of cartilage proteoglycans, indicating that greater mechanical load of the knee resulted in osteoarthritic changes in the knees.[110] Additionally, patients with OA demonstrate greater bilateral vGRF during walking when compared to healthy control participants.[111] With respect to PTOA, patients soon after ACLR presented with greater magnitude of vGRF during both walking and running; indicating that if left unchanged, this elevated impact loading during gait may be a gait deviation responsible for the greater risk of early cartilage breakdown.[34]

In addition to the increase in magnitude of the vGRF, increases in the rate of loading is theorized to be just as detrimental to the cartilage.[112] Animal studies suggest that higher loading rates are more important than the magnitude of loading as faster loading affects the viscoelastic properties of cartilage and decreases the ability of cartilage to dampen loads.[32] Specifically, when testing rabbits *in vivo*, higher loading rates led to greater cartilage degradation than in animals with lower loading rates, even though the lower loading rates were subjected to greater magnitudes of load.[113] Additionally, in both patients with OA[114] and following ACLR,[34] these individuals present

with an elevated vGRF loading rate when compared to healthy individuals. Therefore, monitoring both the magnitude and loading rate of the vGRF will provide us characteristics of the mechanical loading during gait. In addition to the vGRF being an important indicator of mechanical loading itself, vGRF also influences many other kinetic biomechanical variables that are important to joint loading linked to OA development (i.e. external knee adduction moment).

Current literature on gait biomechanics in individuals with knee OA focus on the external knee adduction moment (KAM), as this is a surrogate measure of medial compartment knee joint compressive loading. Greater KAM has been associated with medial compartment knee OA,[114] as well as greater OA severity[115] and disease progression (Figure 9).[35] The risk of progression of knee OA increased 6.46 times with every 1% increase in KAM.[35] Healthy cartilage appears to be conditioned to KAM, as the medial to lateral femoral compartment cartilage thickness ratio is positively associated with an increase in KAM, indicating that increased medial compartment loading leads to an increase in cartilage thickness (Figure 10, blue line).[116] However, in patients with knee OA this association between KAM and cartilage thickness is reversed, with an increased KAM being negatively associated with medial to lateral cartilage thickness (Figure 10, red line). In addition to the association with cartilage thickness, an increased baseline KAM predicts declines in cartilage thickness after five years, indicating that this increased load during gait is very important for declining future cartilage health.[117] Similarly to the vGRF, determining the loading rate of KAM will provide information on the rate of medial compartment loading during gait. Using the semi-quantitative Whole-Organ Magnetic Resonance Imaging Score as a measure of

cartilage degeneration, the KAM loading rate was significantly positively correlated to cartilage degeneration in transfemoral amputees.[118] Interestingly, in the same study, the peak KAM (when controlling for KAM rate) was not significantly related to cartilage degeneration, providing initial evidence that the rate of medial compartment loading may be more influential for cartilage health than magnitude of loading.

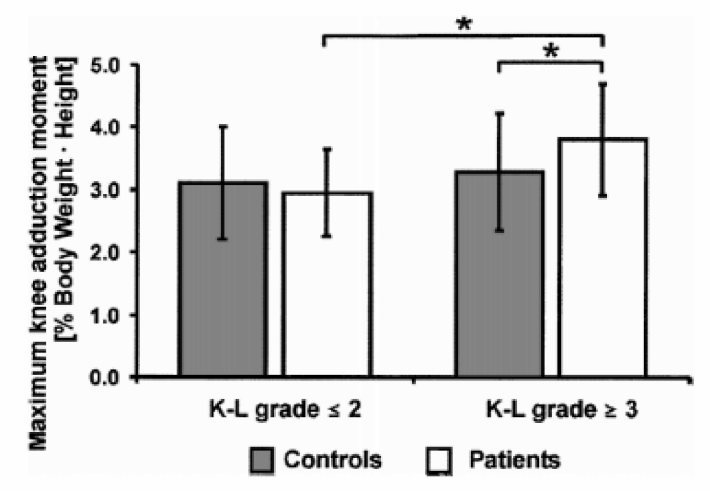


Figure 9: Knee adduction moment and OA Severity. Mundermann 2005

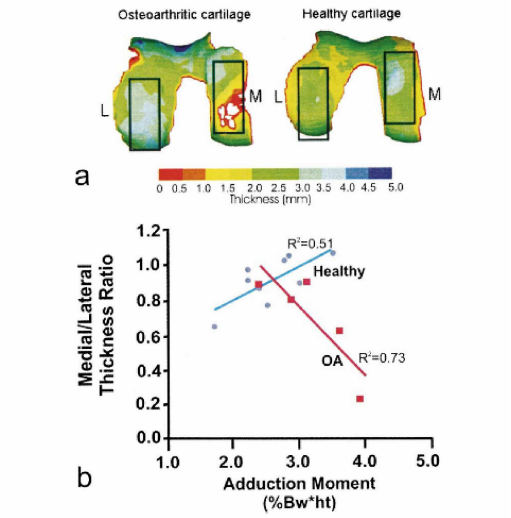


Figure 10: Cartilage response to the knee adduction moment during gait. Andriacchi 2004

While the individual components of cartilage health (i.e. structural, biological, and mechanical) are important for the development of OA, this complex disease does not

originate from a single factor. Rather, the interactions between the components drives the multifactorial etiology of the disease.[11] Therefore, an inter-disciplinary systems-based approach is needed to determine how the continuously shifting balance of these factors ultimately leads to OA development.[119] Using a systems-based approach involves a stimulus-response model by introducing the "system" (i.e. participant) to a mechanical stimulus (i.e. walking/drop-landing) and determining how this affects multiple aspects of cartilage health (i.e. structure and biology) (Figure 11). Thus, the following sections discuss how previous work has used this approach to determine the interaction of mechanics on cartilage structure and biology.

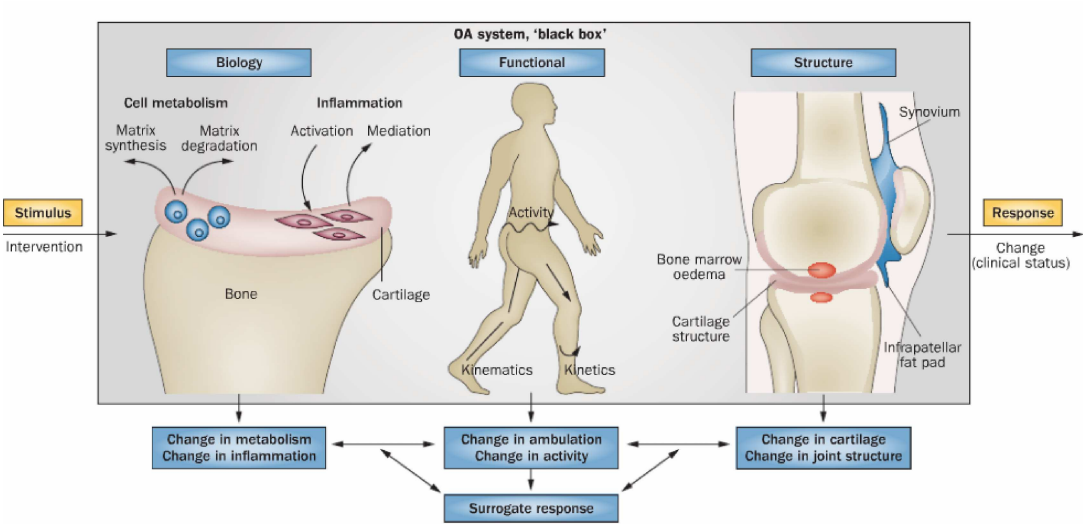


Figure 11: Utilizing a system-based stimulus-response model to determine the interaction of components of cartilage health.

2.5 Interaction Between Mechanics and Cartilage Structure – Cartilage Deformation Following Acute Dynamic Loading

While static measures of morphology provide an approximate estimation of the cartilage anatomy, they may not provide the most robust functional assessment of cartilage.[30] The cartilage is responsible for facilitating the transmission of loads to underlying subchondral bone, while providing a low friction surface between articulating

bones.[29] Alterations in how the cartilage deforms in response to load will alter these shock absorption capabilities, and potentially result in damage to the cartilage and surrounding structures. Cartilage deformation is directly linked to the biochemical composition of the extracellular matrix and may be a more sensitive marker of early declines in cartilage health than the baseline structural measures like volume and thickness. As discussed previously, the earliest alterations in cartilage are the disorganization of the collagen network and depletion of proteoglycans. These alterations in the cartilage structure decreases the ability of the cartilage to withstand normal loads, but this occurs without a gross change in the morphological characteristics of the cartilage. Figure 12 displays the different levels of OA depending on the type of imaging modality used to assess the cartilage.[30] While static cartilage thickness measures are more sensitive of detecting early changes in cartilage than radiography, the use of functional imaging procedures (i.e. stimulus-response model[119]) are more capable of detecting early changes in cartilage composition.[120]

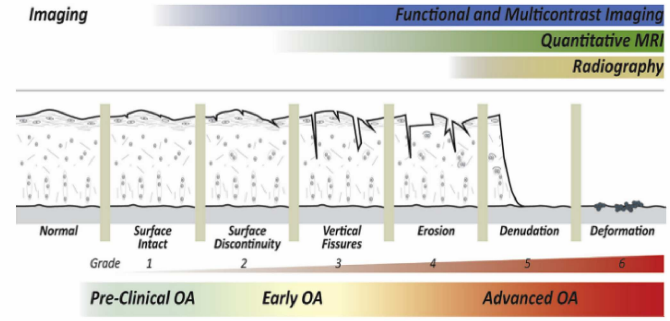


Figure 12. Pre-clinical stages of OA. Neu 2014

To understand unhealthy cartilage loading, we first need to gather an understanding of how normal healthy cartilage responds to various dynamic loads. Eckstein et al produced one of the earliest studies investigating how different dynamic loads affected patellar, tibial, and femoral cartilage deformation.[120] Using trained healthy

volunteers, a “dose-dependent” response to loading was observed, with more intense loads leading to the most cartilage deformation. The dynamic activities that created the most patellar deformation were running and deep knee bends, while the isometric squats and walking resulted in very limited deformation.[120] Interestingly, there were not much cartilage deformation observed in the tibiofemoral joint during these loading protocols, but this may be due to the short transient loads applied during this study (i.e. 200 meter run and 30 deep knee bends). Further studies investigating more sustained loading will be needed to understand the true physiological effects of loading on femoral cartilage.

Another early study regarding exercise and knee cartilage deformation sought to determine how MRI volume would respond to a 1 hour run; as well as how multiple biomechanical and electromyographic variables during running associated with the change in MRI volume.[121] Overall cartilage volume was decreased by 3% following the run, with decreases in the femur and lateral tibia being the greatest changes; however, no changes were observed in the medial tibia. The only biomechanical variable that associated with greater cartilage deformation was time in co-contraction of the knee extensors and flexors. This indicating that greater co-contraction requires greater contraction of the agonist and resulting in a soft tissue force applied to the knee, a force large enough to greatly deform the cartilage at the joint. However, in this study the participants were highly trained, and higher straining status may be related to the cartilage better able to withstand the loading.

The previous studies provided evidence of how cartilage deforms following activity in highly trained individuals,[120,121] but not much research had sought to

determine how more recreational runners would respond to load. Boocock et al utilized recreational runners during a loading procedure that was loading dependent (i.e. 5000 steps) versus time-dependent (i.e. 30 minute run), as they believed this would better control the load being applied to the knee between participants.[122] These recreational runners presented with greater cartilage deformation than previously observed in highly trained runners, even though the recreational runners ran for approximately have the duration of the trained runners. Providing early evidence that the composition of the cartilage in trained runners may be conditioned to respond to load better than observed in recreational runners.

More recently, Niehoff et al[123] completed a study to compare the change in cartilage volume following a running and drop-landing protocol; in an attempt to compare a high frequency/small amplitude and a small frequency/high amplitude task, respectively. These authors provide a biomechanical justification for the magnitude and duration of each of the protocols in an attempt to normalize the total kinetic energy between the two interventions. The running intervention resulted in a mean of 4,262 footfalls during the 30 minute running intervention which resulted in a loading frequency of 1.2 Hz per leg, while the 100 drop landings in 30 minutes equated to a loading frequency of 0.06 Hz; or approximately 20 times lower than the running intervention. The kinetic energy of each touchdown in the landing was estimated at 0.73 (height of the box in meters) x BW, while the per strike kinetic energy at footstrike when running was estimated as 0.035 x BW; or approximately 21 times greater than the running intervention. This provides a strong justification that a similar quantity of load was applied during the different interventions. Figure 13 displays that both the running and

drop landing produced significant decreases in cartilage volume when compared to baseline measurements. However, the results indicate that running produced a greater deformation than drop landing, with the greatest observed in the lateral tibia.

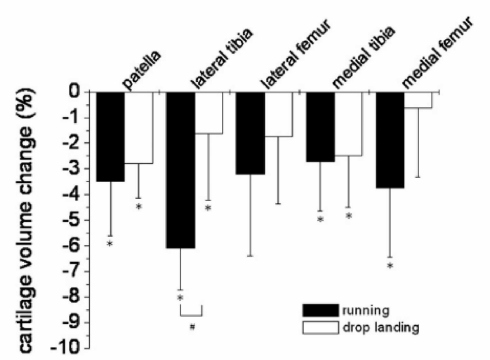


Figure 13. Comparison of Cartilage Deformation following a drop landing and running. Niehoff 2011

The previous studies[120,122-124] have provided some interesting insights into the healthy cartilage response to dynamic loads; however, one study has provided an investigation regarding the acute deformation in cartilage of ACLR patients compares to healthy controls.[74] This is important as this may provide insights into how cartilage adapts early following an acute injury, prior to the development of PTOA. The ACLR patients were only 6 months out from surgery and completed the same 30 minutes running protocol as the healthy controls to determine how the cartilage of each group responded to loading. Interestingly, baseline MRI revealed no differences in cartilage volume between the ACL and healthy participants; however, there were differences observed in between groups using a compositional MRI technique (i.e. T2 mapping). This indicates that while the ACL participants presented with similar cartilage structure when compared to control participants, there were declines in the cartilage composition at baseline. Following baseline MRI, both the healthy and ACLR subjects completed a similar 30 minute run in an attempt to stress the articular cartilage immediately followed

by four successive MRIs separated by 15 minutes. This study design allows for the investigation of both the acute deformation following loading, but also the temporal sequence of cartilage reformation following load. When compared to baseline, the ACLR participants presented with significant deformation of the medial femur, lateral femur, and lateral tibia cartilage; however, the deformation observed was no different that the deformation observed in the healthy individuals. Yet, the serial MRIs following the run demonstrated that the ACLR participants presented with slower recovery of cartilage volume following load. This delay in cartilage recovery following load may potentially be due to the differences in cartilage composition (i.e. T2 mapping) measured at baseline, indicating that the disruption of cartilage composition results in in the tensile strength of the tissue and impaired its ability to reform following load. These results provide evidence for measuring a wide range of outcome measures encompassing both cartilage biology and structure to gain a better understanding of the interaction of each component of cartilage health.

2.6 Interaction Between Mechanics and Cartilage Biology – Change in COMP Concentration and Compositional Imaging Following Acute Dynamic Loading

Similar to the response of cartilage structure to mechanical loading, determining how cartilage biology responds to mechanical loading may provide underlying declines in cartilage health that are not apparent utilizing only baseline measurements. Not only is COMP important for maintaining the properties and integrity of cartilage, but COMP has been described as mechano-sensitive as it plays a role in transducing mechanical forces in the cartilage.[38] Thus, subjecting the cartilage to a mechanical stimulus (i.e.

walking/running/drop-landing) is effective at elevating the sensitivity of COMP to provide an indicator of cartilage health.[38]

In a study of healthy individuals, 30 minutes of walking (i.e. average of 3507 steps) on an outdoor track resulted in an immediate increase in serum COMP that returned to baseline levels within 30 minutes.[125] Interestingly, there was an additional increase in COMP at 5.5 hours following the walking indicating a potential delayed response of cartilage turnover in healthy individuals. In a similar study design utilizing older participants both with and without OA, the authors reported a similar initial increase in COMP between the aged matched older adults.[126] While two different studies, the healthy and older individuals serum was processed in the same laboratory with the same enzyme-linked immunoassay, and interestingly the older individuals did not present with the same 5.5 hour increase in COMP concentration following the 30 minutes walking. The authors suggest that the exercise influence on cartilage turnover may be dependent on age, potentially indicating that the younger population is more metabolically active than the older individuals.

Utilizing compositional imaging techniques, we can determine the affect of loading on cartilage composition. Previously, researchers investigated how axial compression device was used to simulate axial compression of the knee to determine how compression of 50% of body weight would affect T2 values during imaging.[127] This study determined that during loading, the knee was affected differentially with T2 decreases observed in the femoral loading occurring primarily in the medial compartment, while the T2 decreases were observed in both the lateral and medial tibia. Additionally, a 30 min jog in healthy individuals was discovered to significantly

decrease femoral T2 (i.e. collagen cartilage composition) preferentially near the articular surface of the cartilage,[128] while an additional study determined similar reductions in T1rho following a similar jogging procedure.[129] Collectively, these studies indicate that compositional imaging techniques are sensitive enough to detect cartilage composition response to mechanical load.

In addition to the cartilage composition responses to acute loading, cartilage composition is associated with knee biomechanics during drop-landing[130] and walking[131]. Peak KAM during a drop-landing in healthy individuals was positively associated with the medial/lateral T1rho relaxation time ratio, indicating that increased medial compartment loading (i.e. greater KAM) was associated with lesser medial compartment proteoglycan density.[130] Similarly, when ACLR participants were separated into a group with low KAM and high KAM, the group with higher KAM had elevated T1rho relaxation times than the low KAM group.[131] Thus, providing data that individuals that have undergone ACLR and have greater medial compartment loading are at increased risk for knee OA.

Collectively, these studies indicate that mechanics and cartilage biology are associated. Combining the use of COMP concentration with compositional imaging may provide interesting evidence combining complementary measures of cartilage biology and determining the interaction between cartilage metabolism and cartilage composition.

2.7 Interaction Between Mechanics, Biology, and Structure – Providing the Link Between the Main Components of Cartilage Health

As mentioned throughout this document, the biological, structural, and mechanical components are intricately related to the risk of future OA development. The maintenance of healthy cartilage is dependent on each of the three main components of cartilage health being maintained within a normal homeostatic range (Figure 14).[12] To best describe the complex interaction between the components of cartilage health, the maintenance of healthy cartilage has been compared to a slot machine (Figure 15).[11] With healthy cartilage, there is a large "homeostatic envelope" for each of the components to fit within, and as activity occurs the cartilage is not at risk for injury. However, this homeostatic range can expand or contract based on the interaction between the cartilage components, and whenever either of the components falls outside of this range, activity has the possibility of creating cartilage damage. Thus, studying this complex interactions within healthy cartilage will be imperative for understanding a healthy "homeostatic envelope", which can then be used to determine which people outside of these healthy ranges that may be at risk for OA development.

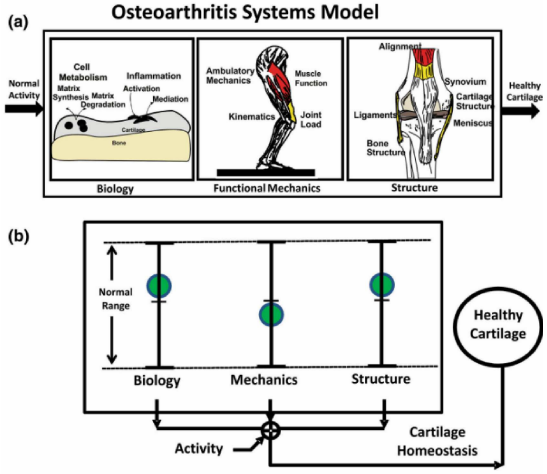


Figure 14: Maintenance of Healthy Cartilage. Andriacchi 2015

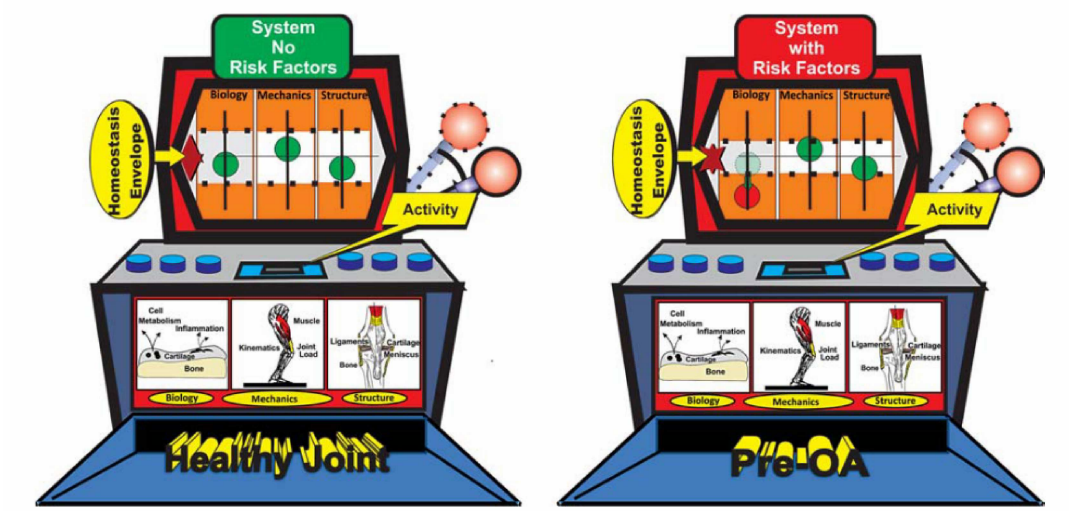


Figure 15: Maintenance of Cartilage Health displayed as a Slot Machine. If the homeostatic envelope is wide enough for all components of cartilage health to fit within, the joint is not at risk when performing activity. However, if the homeostatic window becomes smaller or either component moves outside of a homeostatic range, the cartilage is at risk for degradation when activity occurs. Chu 2015

Using the previously mentioned stimulus response model research design, we are able to investigate this the complex interactions between each of the components of cartilage health. This stimulus response model for determining cartilage health has been equated to a cardiac stress test.[31] Using a controlled exercise stimulus (i.e. mechanical loading) to produce a physiological response, we are able to reveal underlying pathology in the tissue that cannot be observed utilizing only baseline measurements. Earlier sections describe how an acute mechanical stimulus (i.e. walking/running/drop-landing) affected cartilage structure or biology separately, but understanding the relationship between the change in cartilage structure and change in biology will be important for gaining a holistic view of how the system responds.[119] Kersting et al demonstrated that in healthy individuals following a one hour run a change in total cartilage volume was negatively correlated with a change in COMP concentration (R=-0.487), indicating that as more deformation occurred in the cartilage there was an increase in COMP.[121] To further these efforts, Niehoff et al utilized both a running and

drop-landing protocol in healthy individuals to determine how differing mechanical stimuli affected this relationship between a change in cartilage biology and structure.[124] Contrary to the earlier study,[121] running and drop-landing both created significant changes in structure (i.e. cartilage deformation) and biology (i.e. COMP concentration), but only the drop-landing protocol created a significant correlation between change in cartilage volume and COMP concentration (r=0.774).[124] Thus, indicating that differing mechanical stimuli may differentially affect this relationship between cartilage structure and biology. The study that demonstrates the most promise of the utility of this stimulus response model in regards to cartilage health has discovered that the change in COMP following 30 minute walk in participants with OA significantly predicted declines in cartilage thickness over five years.[31] Interestingly, the change in cartilage thickness over five years was not predicted by baseline COMP concentrations, but the change in COMP due to the walking was what uncovered this relationship with cartilage thinning. Thus, a biological response (i.e. COMP change) to a mechanical stimulus (i.e. walking) may aid in the early detection of future structure (i.e. cartilage thickness) declines.[31]

Therefore, the combination of all of the evidence leads us to believe that we have developed a project that will fill critical gaps in the previous literature by determining the differential affects of mechanical loading protocols on altering cartilage structure and biology in healthy individuals. While the use of ultrasound imaging in this stimulus response framework may provide a novel utilization of ultrasound as a compositional imaging modality.

CHAPTER III

3.1 Overview: Aims 1-3

Aim 1 investigated how ultrasonographic (US) measures of cartilage health (i.e. thickness, area, and echo-intensity) respond and recover to a walking, drop-landing, and a control condition, as well as determining the association between lower extremity loading measures and changes in US measures of cartilage health following walking and drop-landing. Aim 2 investigated how serum cartilage oligomeric matrix protein (COMP) responds a walking, drop-landing, and control condition, as well as determining the association between lower extremity loading measures and the acute COMP response following walking and drop-landing. Lastly, Aim 3 determined the association between baseline US measures of cartilage health and baseline serum COMP, as well as the association between the change in US measures of cartilage health and the acute COMP response following walking and drop-landing.

3.2 Participants: Aims 1-3

Forty three healthy individuals (22 males and 21 females) between the ages of 18 and 35 that presented with a body mass index (BMI) <30 kg/m^2 were recruited for this project.[123] All participants reported participating in at least 30 minutes of physical activity at least three times per week. Participants reported no history of the following general orthopedic conditions: congenital or degenerative joint condition, orthopedic

implants, current joint pain (quantified as less than 2 on a 10cm visual analog scale), cartilage injury of any joint, lower extremity fracture, or upper extremity fracture within the last year. Additionally, participants reported no major ligamentous or cartilage injury of the knee or hip joints, as well as reported no cartilage injury at the ankle or demonstrate chronic ankle instability. Chronic ankle instability was defined as demonstrating: [1) previous significant ankle sprain, 2) history of ankle joint 'giving way', and 3) Foot and ankle ability measure index [FAAM] < 90, FAAM sport [FAAMs] index < 80).]

We conducted an *a priori* power analysis using data from medial femoral compartment thickness changes following 30 minutes of walking (pre avg= 2.23mm, post avg=2.09mm, pooled SD=0.42mm, effect size: *d*=0.33) published in a previous study.[132] We estimated that we would need 33 participants to determine statistical differences, with 80% power and an α level of 0.05, if the smallest effect we found in the current study across the three loading conditions and five total time points was similar to previously published research *(d*=0.33).[133] As larger effects are observed in COMP change following various activities,[123,124] we decided to power the study on changes in cartilage thickness. Previously, large effects in COMP concentrations have been found in healthy participants following running (d=1.59[124]), as well as moderate effects following running (d=0.49 [123]) and jump-landing (d=0.53 [123]). Due to the time commitment of three separate three-hour data collection sessions, we over-sampled by 30% to ensure that we would achieve adequate statistical power in our final analyses if 30% of the initial sample were to drop-out of the study.

3.3 Experimental Procedures and Data Analysis: Aims 1-3

Research Design

A femoral cartilage US assessment and an ante-cubital blood draw were performed in healthy individuals before and after a walking, drop-landing, and control conditions to determine the acute structural and metabolic cartilage response to the each condition. Additional US images were recorded at 15, 30, and 45 minutes following the cessation of each condition to determine the cartilage recovery. We assessed walking and drop-landing biomechanics at the beginning of each of respective condition to determine how lower extremity loading measures in each condition related to the acute structural and metabolic cartilage responses. We utilized a repeated measures design in which each participant completed all conditions during independent data collection sessions separated by at least one week at the same time of day to control for diurnal variation in serum COMP[134] and femoral cartilage thickness.[135,136] The order of the conditions was counterbalanced. Participants were instructed to limit their physical activity on the days that data collection occurred.

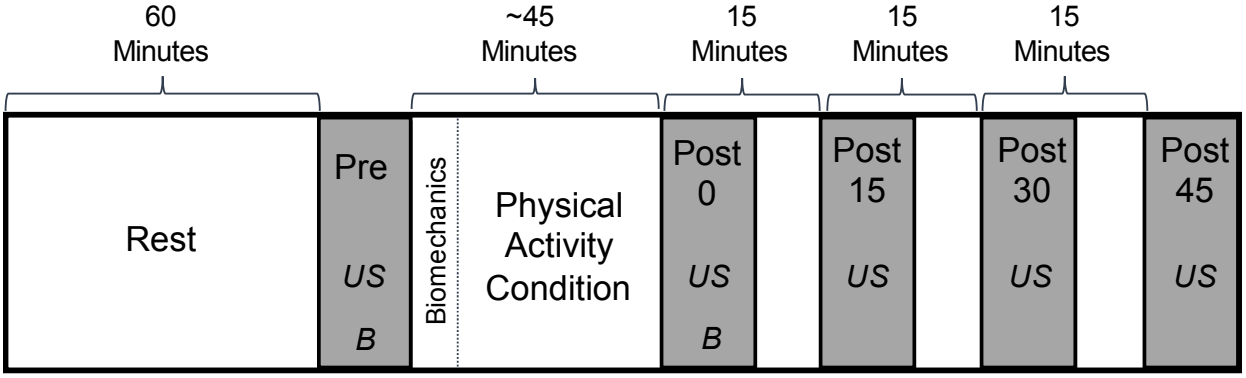


Figure 16: Within study design for the walking, drop-landing, and control conditions. Ultrasounds performed at all time points. Blood draw only performed at pre and post0.

Screening Session

Prior to testing, participants were required to come to the laboratory for an initial session to determine their habitual walking speed and their corresponding comfortable step frequency, which was used to standardize the amount of time used for each condition. Habitual over-ground walking speed was initially determined in our motion capture laboratory utilizing two sets of infrared timing gates (TF100, TracTronix, Lenexa, KS, USA). Participants were instructed to walk at a self-selected speed described as “comfortably walking on the sidewalk” through the 6-meter capture area.[137-139] After completing five familiarization trials, we recorded the time of the next five walking trials to determine their average habitual walking speed. Next, the speed on the treadmill (4Front, WOODWAY, Waukesha, WI, USA) was increased to the habitual walking speed of each participant and 60 seconds of walking was continued for the purpose of treadmill familiarization. After treadmill familiarization, study personnel manually counted the steps of each participant for one minute in order to determine the time each participant would need to reach 5000 steps.[122] The calculated time for each participant was used for each condition.

Data Collection Overview

On the day of each data collection session, we began by collecting a urine sample to test urine specific gravity via refractometry to confirm that each participant was not dehydrated (i.e. urine specific gravity < 1.025) prior to testing.[140] Following hydration testing, participants were seated on a padded plinth with their back against a

wall in a long-sit position with their knees in full extension[133] for one hour to unload the femoral articular cartilage, permit fluid rebound, and minimize effect of the preceding activity on the cartilage. Next, the US cartilage assessment occurred immediately prior to the blood sample collection at both time points in each session. The participants were then immediately transferred across the laboratory with a wheel chair to begin the walking or drop-landing condition activity condition or remain on the padded plinth for the control condition. The participants wore the same pair of their own personal athletic footwear for all sessions. Immediately following cessation of the condition, the participants were transferred back to the padded plinth with wheel chair to begin the posttest US assessment followed by the posttest blood sample collection. Due to the proximity of the walking and drop-landing conditions and the accessibility of US, posttest US images and blood sample collection were obtained within five minutes following each condition.[133]

Ultrasonographic Assessment of the Femoral Articular Cartilage

Ultrasonographic Image Acquisition

US images were obtained in the dominant limb, which was defined as the self-reported limb that the participant preferred to use for kicking a ball.[132] Participants were positioned with their back against a wall and the dominant limb was positioned at 140° of knee flexion using a manual goniometer (Figure 2).[133] A tape measure was secured to the treatment table and used to record the distance between the wall and the posterior calcaneus in order to standardize positioning for each participant during the posttest and throughout all data collection sessions. A single investigator performed all

femoral cartilage US imaging using a LOGIQe US system (General Electric Co., Fairfield, CT, USA) with a 12MHz linear probe. The probe was placed transversely in line with the medial and lateral femoral condyles above the superior edge of the patella (Figure 2) and rotated to maximize the reflection of the articular cartilage surface, as previously reported.[17,141,142] A transparency grid was placed over the US screen to aid in reproducibility of the US image.[133] Once the intercondylar notch was centered on the grid, the locations of the lateral and medial femoral condyles at the edges of the screen were recorded. This probe positioning was replicated during subsequent US assessments to ensure similar probe placement between assessments. Three images were recorded, with the US probe being removed and repositioned, on the knee between each recorded image at baseline and immediately after the walking and drop-landing condition.

Ultrasonographic Imaging Processing

A single investigator manually segmented the US images using ImageJ software (National Institutes of Health, Bethesda, MD, USA). All three of the femoral cartilage US images from each time point were processed and averaged for the following outcome measures:

Cartilage Thickness

Femoral cartilage thickness was assessed at the midpoints of the medial femoral condyle, lateral femoral condyle, and intercondylar notch as the straight-line distance in millimeters (mm) between the cartilage-bone interface to the synovial space-cartilage

interface (Figure 18a).[17,133,141,142] Strong intra-session reliability for the cartilage thickness assessment has previously been established within our laboratory ($ICC_{2,k}$ = 0.966).[133]

Cartilage Area and Echo-intensity

The femoral cartilage was then segmented by individually outlining the cartilage of the medial and lateral femoral condyles to obtain the size (i.e. cartilage area [mm^2]) and the greyness (i.e. cartilage echo-intensity) of the cartilage (Figure 18b). The medial and lateral areas were separated based on the location of the intercondylar thickness measure. Echo-intensity evaluates the average gray scale brightness of each pixel segmented on a scale from 0 (i.e. black; more water content) to 255 (i.e. white; lesser water content). US echo-intensity (i.e. grey-scale brightness) has primarily been used as a measure of "muscle quality",[28,143] with the average echo-intensity representing the relative water content of muscle. Since cartilage is approximately 60-80% fluid and acute cartilage deformation is in part due to fluid exudation,[29,144] we may be able to use US echo-intensity to monitor acute changes in cartilage water content that occur with loading during activity.

Additionally, a percent change score from baseline to each posttest time point was calculated to determine the acute cartilage response for each US measure following both conditions (Equation 1). A greater negative percent change of thickness and area indicates greater cartilage deformation. A greater negative percent change in echo-intensity is theorized to indicate an increase in cartilage water content.

Equation 1: Percent change (%Δ) = $(\frac{\text{mean}_{\text{post}} - \text{mean}_{\text{pre}}}{\text{mean}_{\text{pre}}})*100$

Figure 17. Femoral Cartilage Ultrasonography Setup and Participant Positioning.

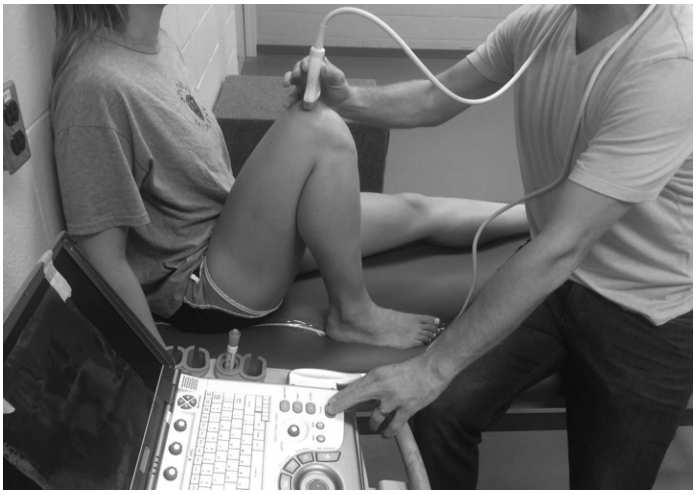
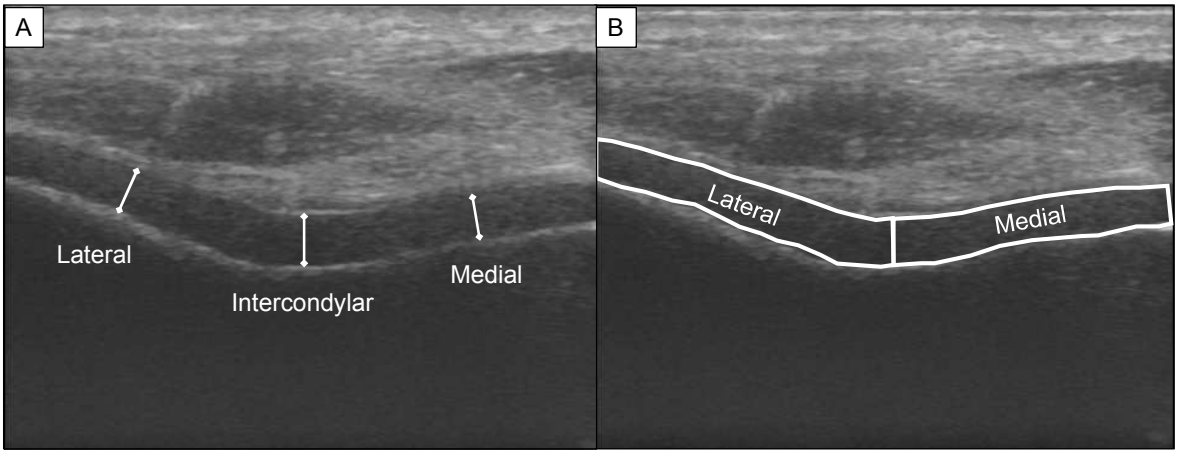


Figure 18. Femoral Cartilage Ultrasonography Outcome Measures. A) Cartilage Thickness; B) Cartilage Compartmental Area



Quantifying Cartilage Metabolism

Blood Sample Collection

For both the baseline and posttest time points, the participants were positioned supine on the padded plinth, and five milliliters of blood were collected from the antecubital vein in a serum separator tube vacutainer. Blood samples were placed on ice until centrifuged at 4°C for 15minutes at 4000rpm. Serum was pipetted equally into

two cryovials and stored at -80°C until a batch analysis that occurred after all participants were collected.

Analysis of Serum Cartilage Oligomeric Matrix Protein

Serum was assessed for cartilage oligomeric matrix protein (COMP) using commercially available enzyme-linked immunosorbent assays (Human COMP PicoKine ELISA; Boster Biological Technology; Pleasanton, CA, USA). Blood samples were analyzed in triplicate. The COMP assay detection sensitivity was <10pg/mL, and the intra-assay variability was 2.35%. Serum samples for each participant were analyzed on a single plate to control for differences caused by inter-assay variation. For data analysis, we utilized resting concentration as well as calculating a percent change score to determine the COMP response from baseline to posttest for each condition (Equation 1).

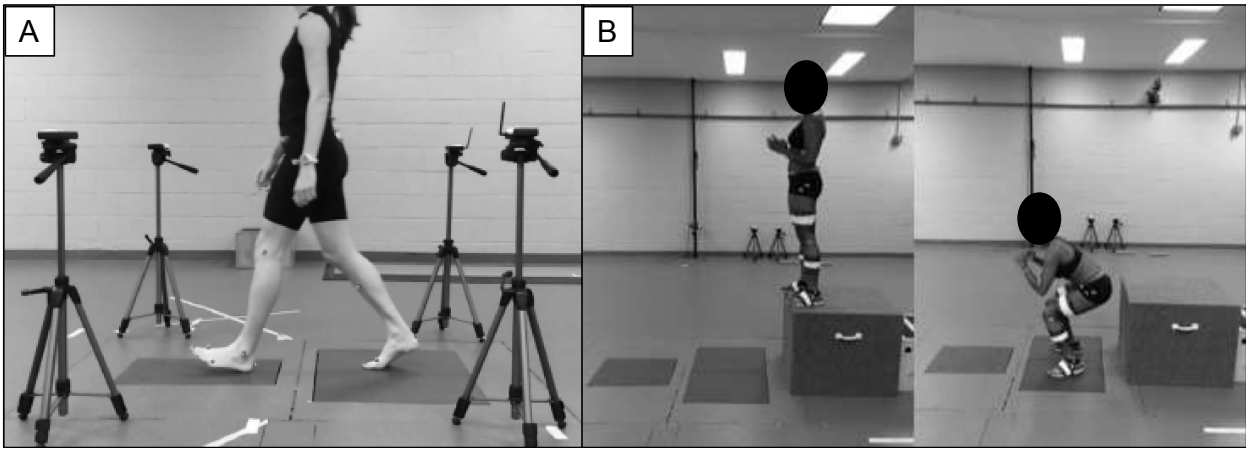
Biomechanical Assessment of Lower Extremity Loading

Participant Preparation for Biomechanical Assessment

Identical marker setups were used for the walking and drop-landing biomechanical assessments. A modified retroreflective marker cluster/bony landmark setup was used for data collection. Marker clusters were secured bilaterally at the middle 1/3rd of the anterior lateral thigh, middle 1/3rd of the anterior lateral shank, middle of the dorsum of the foot, and over the sacrum. Additional bony landmark markers were affixed with double sided tape at the L4/L5, manubrium, and bilaterally over the anterior superior iliac spine, greater trochanter, medial/lateral femoral epicondyle, medial/lateral

malleoli, and acromion processes. Medial epicondyles and medal malleoli markers were removed during dynamic trials. A static trial was taken at the beginning of each session for marker identification. Marker positions were collected using a 10-camera three-dimensional motion capture system with a sampling frequency of 120Hz with Vicon Nexus v1.4.1 motion capture software (Vicon Motion Systems, Centennial, CO) and lowpass filtered at 10Hz.[139] The cameras were interfaced with three total Bertec force plates (40cm x 60cm, FP406010, Bertec Corporation, Columbus, OH) collecting at 1200Hz and lowpass filtered at 75Hz.[139] Two of the force plates were positioned side by side and used to collect drop-landing data (Figure 19a), while two of the force plates were staggered to allow for bilateral limb collection during a single walking trial (Figure 19b).[139]

Figure 19. Walking and Drop-landing Biomechanical Assessments.



Walking Biomechanical Assessment

The participants walked shod through the capture area at their habitual walking speed (Figure 19a). Five practice trials were performed to familiarize the participants with the walking task. Five test trials were recorded in which: 1) both limbs individually landed on a single force plate, 2) maintained forward eye contact and were not “aiming”

for the force plates, 3) and maintained a consistent gait speed (±5% of the habitual walking speed calculated in the screening session).[137,138] Immediately following the collection of the last test trial, the participants were returned to the wheelchair and transferred across the laboratory to begin the walking condition.

Drop-Landing Biomechanical Assessment

A 62cm platform was positioned behind the side-by-side force plates to allow for simultaneous collection of both limbs during a single drop-landing trial (Figure 3b). A separate step was positioned behind the 62cm platform and was utilized by the participants to ascend onto the platform. For each drop-landing trial, the participants ascended onto the platform and were instructed to drop down from the platform and perform a comfortable double-legged landing with each limb on a separate force plate. No specific instructions were provided to the participants on how to perform the double-legged landing. After each drop-landing, participants would walk around the box and ascend up the step in order to prepare for the next drop-landing trial. Drop-landings trials 5 through 10 were recorded and analyzed for the biomechanical assessment.

Analysis of Lower Extremity Loading

Marker Identification and Processing

Individual trials of the biomechanics collected during the walking and drop-landing conditions will be labeled to identify all of the retro-reflective markers within the Vicon Nexus motion capture software (version 1.8.5, Vicon Systems). Once labeled, the marker trajectory data synchronized with the ground reaction force and will be exported

from Vicon Nexus and further processed using Motion Monitor software (version 8.0, Innovative Sports Training).

The Motion Monitor software used the marker trajectories from Vicon Nexus to construct a three-dimensional segment-link model for each participant. The lower extremity and trunk segments for each participant was modeled as rigid bodies using the individual and cluster markers. A minimum of 3 non-collinear points was used to model each segment as follows:

- Left foot: Left foot cluster, left medial malleolus, left calcaneus
- Right foot: Right foot cluster, right medial malleolus, right calcaneus
- Left shank: left shank cluster, left lateral malleolus, left medial malleolus, left lateral epicondyle, left medial epicondyle
- Right shank: right shank cluster, right lateral malleolus, right medial malleolus, right lateral epicondyle, right medial epicondyle
- Left thigh: left thigh cluster, left lateral epicondyle, left medial epicondyle, left greater trochanter
- Right Thigh: right thigh cluster, right lateral epicondyle, right medial epicondyle, right greater trochanter
- Pelvis: sacral cluster, right anterior superior iliac spine, left anterior superior iliac spine
- Trunk: right acromion process, left acromion process, sternal notch, L4-L5

Joint Center Calculations and Alignment

Once we have defined the rigid body segments from the static calibration file, joint centers were defined between the rigid body segments. The ankle joint centers were defined as the midpoint between the lateral and medial malleolus markers. The knee joint centers were defined as the midpoint between the lateral and medial epicondyle markers. The hip center were estimated with the Bell Method using the left and right anterior superior iliac spine markers.[145] Once joint centers were established, each segment's local coordinate system was aligned to the global axis system, with the anterior-posterior axis of each segment aligned with the world x-axis, the medial-lateral axis of each segment aligned with the world y-axis, and the superior-inferior axis of each segment aligned with the world z-axis.

Kinematic Calculations

Knee joint motion was defined as the motion of the shank segment relative to the thigh segment using a Euler angle rotation sequence of Y [(+) flexion / (-) extension] and X' [(+) varus / (-) valgus].[146]

Kinetic Calculations

Ground reaction force and interpolated segment kinematic data was used to derive net internal knee moments using inverse dynamics procedures.[147] Negative internal sagittal plane moment was used to quantify the internal knee (EXT) extension moment. The internal frontal plane moment was used to quantify the internal knee varus (VAR) and valgus (VAL) moments. The internal knee varus and valgus moments were corrected so that a more positive value indicates increased moment for both variables.

Biomechanics Data Reduction

All dependent variables were identified during the first 50% of the stance phase for the walking trials. The stance phase of walking was defined as the time between initial ground contact (ground reaction force > 20N) and to toe-off (ground reaction force <20N). For the drop-landing trials, all dependent variables were identified during the first 100ms following initial ground contact.

Peak magnitude of the following lower extremity loading biomechanical variables was assessed during walking and drop-landing: vertical ground reaction force (vGRF), EXT moment, VAL moment, and VAR moment. Instantaneous loading rate of the vGRF (vGRF-LR), EXT, VAL, and VAR moments were calculated as the peak of first derivative of the force-time and moment-time curves.[139] Peak vGRF (xBW) and vGRF-LR (xBW/s) were normalized to participants' body weight.[138] Peak moments (xBW*Ht) and moment loading rates (xBW*Ht/s) were normalized to the product of participants' height and weight.[139]

Walking, Drop-Landing, and Control Conditions

Walking Condition

The participants were positioned on the treadmill and the speed was increased to the habitual walking speed determined during the screening session. This speed was maintained for the time calculated during the screening session to reach 5,000 steps.

Drop-Landing Condition

The drop-landing biomechanical assessment occurred concurrently with the drop-landings used for the drop-landing condition. For the drop-landing condition, the participants continued performing drop-landings until they completed 120 total drop-landings. We selected the amount of drop-landings from the 62cm platform in order to match the high magnitude loading condition utilized in a previous study utilizing a similar drop-landing protocol.[123] The 120 drop-landing trials were evenly distributed over the same period of time utilized in the other conditions.

Control Condition

During the control condition, participants remained on the treatment table following the baseline blood sample collection in a long-sit position for the same period of time utilized in the other conditions.

3.6 Statistical Analysis: Aims 1-3

All statistical analyses were performed using SPSS (version 21.0; IBM Corporation) with an *a priori* α level of $P < 0.05$.

Aim 1: Cartilage Ultrasound and Biomechanics

The first purpose of Aim 1 was to compare the acute response and recovery of US cartilage outcome measures between a walking, drop-landing, and control condition in healthy participants. Separate one-way repeated measures analysis of variance (RM-ANOVA) were used to determine differences in the baseline values for each cartilage

US measure between the walking, drop-landing, and control condition. Separate 4 x 3 (time x condition) RM-ANOVAs were used to compare the percent change scores between the conditions for acute cartilage response and recovery at each time point. Outliers were defined as > two standard deviations away from the mean for each US measure at each time point. Next, a participant was removed from an individual US outcome measure RM-ANOVA if more than two time points were defined as outliers during a single condition. After outlier removal, a Shapiro-Wilk test was used to confirm normal distribution for each outcome measure. If there was a significant interaction effect for any of the RM-ANOVAs, we utilized Bonferroni corrected ($p = 0.05/12 = 0.004$) paired samples t-tests to determine the specific differences between conditions at each time point for all of the US measures.

The second purpose of Aim was to determine the associations between the acute cartilage response and lower extremity loading biomechanics during the walking and drop-landing condition. Separate Pearson product moment correlations were used to determine the association between the acute change (i.e. post0 percent change score) for each US measure and each lower extremity loading biomechanical variable during the walking and drop-landing condition. For correlational analysis between each acute change in US measure and lower extremity loading variable, outliers greater than two standard deviations away from the mean for either measure were removed for that individual analysis. Following outlier removal, if the data were still found to be non-normal with the Shapiro-Wilk test, a Spearman Rank-Order correlation was used to determine the association between the acute change in each US measure and each lower extremity loading biomechanical measure. Correlational analyses involving the

vGRF, vGRF loading rate, and EXT moment were conducted for all US measures. Since greater VAL moment is theorized to increase medial compartment loading, correlational analyses involving the VAL moment were only conducted on the medial and intercondylar US measures. Similarly, correlational analyses involving the VAR moment were only conducted on the lateral and intercondylar US measures. Associations were classified as negligible (0.0 – 0.30), low (0.31 – 0.50), moderate (0.51 – 0.70), high (0.71 – 0.90), and very high (0.90 – 1.00).[148] Additionally, we included the 95% confidence intervals around each Pearson r and Spearman ρ to ensure that the intervals of each association did not cross zero.[149]

Aim 2: Cartilage Biomarkers and Biomechanics

The first purpose of Aim 2 was to compare the acute serum COMP response between walking, drop-landing, and control in healthy individuals. A one-way repeated measures analysis of variance (RM-ANOVA) was used to determine if the baseline COMP concentration was similar between the walking, drop-landing, and control condition. A separate one-way RM-ANOVA was used to compare acute COMP response between each condition. Outliers were defined as > two standard deviations away from the mean of COMP response in any of the conditions. After outlier removal, a Shapiro-Wilk test was used to confirm normal distribution for COMP response. If there were significant differences in COMP response, we utilized paired samples t-tests and a Bonferroni correction ($p = 0.05/3 = 0.017$) to determine specific differences in COMP response between conditions.

The second purpose of Aim 2 was to determine the association between the COMP response and lower extremity loading biomechanics during the walking and drop-landing conditions. Separate Pearson product moment correlations were used to determine the association between COMP response and each measure of lower extremity loading during the walking and drop-landing condition. For the correlational analysis between each COMP response and lower extremity loading variable, outliers > two standard deviations away from the mean for either measure were removed for that individual analysis. Following outlier removal, if the data were still found to be non-normal with the Shapiro-Wilk test, a Spearman Rank-Order correlation was used to determine the association between COMP response and each lower extremity loading measure. Associations were classified as negligible (0.0 – 0.30), low (0.31 – 0.50), moderate (0.51 – 0.70), high (0.71 – 0.90), and very high (0.90 – 1.00).[148] Additionally, we included the 95% confidence intervals around each Pearson r and Spearman ρ to ensure that the intervals of each association did not cross zero.[149]

Aim 2 Post-Hoc: Comparison of Increased and Decreased COMP Responders following Walking and Drop-Landing

During our initial analysis comparing COMP response between conditions, we identified a heterogeneous COMP response to both the walking and drop-landing conditions, as some participants demonstrated an increased (i.e. posttest increased above baseline) or a decreased (i.e. posttest decreased below baseline) COMP response.

Our first *post hoc* test sought to determine if participants demonstrated a similar COMP response between the walking and drop-landing sessions. We created a binary COMP response outcome measure to indicate whether a participant's post-activity COMP concentration increased or decreased compared to the baseline COMP concentration for walking and drop-landing. We used a chi square analysis to determine if the frequency of being an increased COMP responder in the walking condition was similar to the frequency of being an increased COMP responder in the drop-landing condition. This analysis will determine if being an increased COMP responder (i.e. post COMP > baseline COMP) in the walking condition is associated with being an increased COMP responder in the drop-landing condition.

With our second *post hoc* test, we used a Pearson product moment correlation to determine whether the magnitude of COMP response following walking was associated with the magnitude of COMP response following drop-landing.

Lastly, we utilized separate independent t-tests to determine if lower extremity loading measures were different between the increased and decreased COMP responders in both the walking and drop-landing conditions.

Aim 3: Cartilage Ultrasound and Cartilage Biomarkers

The purpose of Aim 3 was to determine the association between baseline US measures of cartilage health (i.e. thickness, area, and echo-intensity) and baseline serum COMP. The second purpose of Aim 3 was to determine the association between the change in US measures of cartilage health and the serum COMP response following walking and drop-landing in healthy individuals. Separate Pearson product

moment correlations were used to determine the association between baseline US measures and baseline COMP concentration, as well as determining the association between the percent change in each US measure and the COMP response following the walking and drop-landing conditions. For each correlational analysis between COMP response and each US measure, outliers > two standard deviations away from the mean for either measure were removed for that individual analysis. Following outlier removal, if the data were still found to be non-normal with the Shapiro-Wilk test, a Spearman Rank-Order correlation was used to determine the association between COMP and each US outcome measure. Associations were classified as negligible (0.0 – 0.30), low (0.31 – 0.50), moderate (0.51 – 0.70), high (0.71 – 0.90), and very high (0.90 – 1.00).[148] Additionally, we included the 95% confidence intervals around each Pearson r and Spearman ρ to ensure that statistically significant associations did not cross zero.[149]

Aim 3 Post-hoc Test: Sex-Specific Associations between Ultrasonographic Measures of Femoral Cartilage and Cartilage Oligomeric Matrix Protein

As previous reports have indicated sex differences in cartilage volume[150] and resting concentrations of serum COMP,[151] we performed separate *post hoc* associations individually for males and females to determine the association between cartilage structure and metabolism for each sex individually.

CHAPTER 4: RESULTS

Specific Aim 1 Results - Cartilage Ultrasound and Biomechanics

To compare the acute response and recovery of US measures of cartilage health (i.e. thickness, area, and echo-intensity) between a walking, drop-landing, and control condition in healthy participants. Additionally, we sought to determine the associations between change in US measures of cartilage health and lower extremity loading biomechanics during the walking and drop-landing condition.

Comparison of Ultrasonographic Cartilage Outcome Measure Response and Recovery between Conditions

Femoral Cartilage Thickness

Baseline medial cartilage thickness was not different between the conditions ($F_{2,78}$=1.08, p=0.346, n=40). There was a significant time by condition interaction effect for medial thickness deformation ($F_{6,234}$=18.60, p<0.001). When compared to the control condition, medial thickness deformation was significantly greater following walking at post0 (t_{39}=7.48, p<0.001) and post15 (t_{39}=3.05, p=0.004), as well as following drop-landing at post0 (t_{39}=12.41, p<0.001), post15 (t_{39}=7.36, p<0.001), and post30 (t_{39}=3.24, p=0.002). The magnitude of medial thickness deformation was greater in the drop-landing condition compared to the walking condition at post0 (t_{39}=3.81, p<0.001) and post15 (t_{39}=4.49, p<0.001), but was not statistically significant at post30 (t_{39}=2.96, p=0.005) and post45 (t_{39}=2.74, p=0.009).

Baseline intercondylar cartilage thickness was not different between the conditions ($F_{2,76}$=1.51, p=0.228, n=39). There was a significant time by condition interaction effect for the intercondylar thickness deformation ($F_{6,234}$=18.60, p<0.001). Intercondylar thickness deformation was significantly greater at post0 following walking (t_{38}=4.20, p=0.004) and jumping (t_{38}=3.05, p<0.001) when compared to the control condition, but was not different at any other time points. Intercondylar thickness deformation following walking and drop-landing did not significantly differ at any time point.

Baseline lateral thickness was significantly different between the conditions ($F_{2,72}$=3.18, p=0.048, n=37). However, multiple comparisons t-tests indicate no differences in lateral thickness between conditions, and the largest baseline lateral thickness mean difference (walking vs. drop-landing = 0.05mm) observed between conditions was within the intersession MDC we have previously established (MDC = 0.19mm).[132] When compared to the control condition, the lateral thickness deformation was significantly greater following walking at post0 (t_{36}=7.26, p<0.001) and post15 (t_{36}=4.02, p<0.001), as well as following drop-landing at post0 (t_{36}=10.44, p<0.001) and post15 (t_{36}=4.88, p<0.001). Lateral thickness deformation was not different between the walking and drop-landing at any time point.

Femoral Cartilage Area

Baseline medial cartilage area was statistically different between conditions ($F_{2,78}$=11.18, p<0.001, n=40). However, the largest baseline medial cartilage area difference (walking vs. drop-landing = 1.01mm^2) observed between conditions was

within the intersession medial cartilage area MDC we established (MDC = 2.69mm^2, Table 2). There was a significant time by condition interaction effect for medial cartilage area ($F_{6,234}$=11.91, p<0.001). When compared to the control condition, medial cartilage area deformation was significantly greater during the walking condition at post0 (t_{39}=8.87, p<0.001) and post15 (t_{39}=4.68, p<0.001), as well as following drop-landing at all post time points (post0: t_{39}=10.12, p<0.001; post15: t_{39}=9.11, p<0.001; post30: t_{39}=3.79, p=0.001; post45: t_{39}=4.04, p<0.001). Additionally, the magnitude of medial area deformation was greater in the drop-landing condition compared to the walking condition at the post15 (t_{39}=3.60, p=0.001) time point, but this difference was not statistically significant at post0 (t_{39}=2.78, p=0.008), post30 (t_{39}=2.91, p=0.006), post45 (t_{39}=2.60, p=0.013).

Baseline lateral cartilage area was statistically different between conditions ($F_{2,74}$=4.13, p=0.020, n=38). However, the largest baseline lateral cartilage area difference (walking vs. drop-landing = 1.42mm^2) observed between conditions was within the intersession lateral cartilage area MDC we established (MDC = 2.54mm^2, Table 2). There was a significant time by condition interaction effect for lateral cartilage area ($F_{6,234}$=11.91, p<0.001). When compared to the control condition, lateral cartilage area deformation was significantly greater during the walking condition at post0 (t_{37}=5.53, p<0.001) and post15 (t_{37}=3.88, p<0.001), as well as following drop-landing at post0 (t_{37}=6.47, p<0.001), post15 (t_{37}=5.532, p<0.001), and post30 (t_{37}=3.19, p=0.003). Lateral cartilage deformation was not different between the walking and drop-landing conditions at any post time points.

Femoral Cartilage Echo-intensity

Baseline medial cartilage echo-intensity was not statistically different between the conditions ($F_{2,72}$=0.22, p=0.806, n=37). There was a statistically significant time by condition interaction effect for medial cartilage echo-intensity percent change ($F_{6,216}$=2.215, p=0.043), but none of the t-tests indicated any differences between the conditions at any of the posttest time points.

Baseline lateral cartilage echo-intensity was not statistically different between the conditions ($F_{2,72}$=0.747, p=0.477, n=37). There was not a statistically significant interaction effect between time and condition ($F_{6,216}$=1.81, p=0.098). Therefore, t-tests were not utilized to identify specific group differences at each time point.

Association between Ultrasonographic Cartilage Outcome Measure Response and Lower Extremity Loading during the Walking and Drop-Landing Conditions

Femoral Cartilage Thickness

During walking, lesser peak EXT moment associated with greater medial compartment deformation (r=-0.37, p=0.022; Table 4). However, the other walking lower extremity loading measures were not significantly associated with medial (r range = -0.24 – 0.19), intercondylar (r/ρ range = -0.20 – 0.16), and lateral (r/ρ range = -0.16 – 0.09) thickness deformation.

Drop-landing lower extremity loading measures were not significantly associated with medial (r/ρ range = -0.18 – 0.32; Table 4), intercondylar (r/ρ range = -0.11– 0.12), or lateral (r/ρ range = -0.31– 0.01) thickness deformation.

Femoral Cartilage Area

Walking lower extremity loading measures were not significantly associated with medial (r range = -0.21 – 0.24, Table 4) and lateral (r/ρ range = -0.16– 0.26) compartment area deformation.

Drop-landing lower extremity loading measures were not significantly associated with medial cartilage area deformation (r/ρ range = -0.19 – -0.08; Table 4). During drop-landing, greater peak vGRF(r=-0.34, p=0.036) and vGRF loading rate (r=-0.42, p=0.008) were significantly associated with greater lateral compartment area deformation. However, the other drop-landing lower extremity loading measures were not significantly associated with lateral compartment area deformation (r/ρ range = -0.20 – 0.17).

Femoral Cartilage Echo-intensity

During walking, greater vGRF loading rate was moderately associated with a decrease in medial compartment echo-intensity (r=-0.51, p=0.001; Table 4). However, the other walking lower extremity loading measures were not significantly associated with a change in medial compartment echo-intensity (r range = -0.21 – 0.14). Walking lower extremity loading measures were not associated with a change in lateral compartment echo-intensity (r/ρ range = -0.25 – 0.08).

Drop-landing lower extremity loading measures were not statistically associated with a change in medial compartment echo-intensity (r/ρ range = -0.22 – 0.14; Table 4). During drop-landing, greater peak VAR moment was significantly associated with an increase in lateral compartment echo-intensity (ρ=0.34, p=0.04). However, the other

drop-landing lower extremity loading measures were not statistically associated with a change in lateral compartment echo-intensity (r/ρ range = -0.27 – 0.21).

Specific Aim 2 Results - Cartilage Breakdown Biomarkers and Biomechanics

The first purpose of aim 2 was to compare the acute serum COMP response between walking, drop-landing, and control in healthy individuals. Secondarily, we sought to determine the association between the COMP response and lower extremity loading biomechanics during the walking and drop-landing conditions.

Comparison of COMP Response between Walking, Drop-Landing, and Control Conditions

Baseline COMP concentration was not different between the walking, drop-landing, or control conditions ($F_{2,64}$=1.71, p=0.189, Table 6.). There was a significant difference in COMP response between the conditions ($F_{2,64}$=14.58, p<0.001). COMP response was greater in the walking (t_{32}=-4.291, p<0.001) and drop-landing (t_{32}=4.331, p<0.001) conditions when compared to the control condition. COMP response was not different between the walking and drop-landing condition (t_{32}=-0.535, p=0.001).

Association between COMP Response and Lower Extremity Loading during the Walking and Drop-landing Conditions

Greater walking valgus moment loading rate was associated with a decreased COMP response (r=-0.48, p=0.005, n=33; Table 6). However, no other walking lower extremity loading measure was significantly associated with the COMP response

following walking (r/ρ range = -0.24 – 0.30). There were no significant associations between drop-landing lower extremity loading measures and COMP response (r/ ρ range = -0.30 – 0.26).

Post-Hoc: Comparison of Increased and Decreased COMP Responders following Walking and Drop-Landing

For this analysis, we only excluded individuals with outliers in the walking or drop-landing COMP response (walking, n=2); thus, 36 individuals were included in the following analyses. 12 and 10 participants presented with a decreased COMP response, while 24 and 26 participants presented with an increased COMP response following the walking and drop-landing conditions, respectively (Figure 26).

Being an individual with increased COMP response in the walking condition does mean that you will be an individual with an increased COMP response in the drop-landing condition ($\chi_1 = 0.277$, $p = 0.599$). However, the magnitude of COMP response following walking was moderately associated with the magnitude of COMP response following drop-landing ($r = 0.55$, $p = 0.001$). There were no significant differences in our lower extremity loading measures when comparing the increased COMP responders to the decreased COMP responders following the walking or drop-landing condition.

Specific Aim 3 Results - Cartilage Ultrasound and Cartilage Breakdown Biomarkers

To determine the association between baseline US measures of cartilage health (i.e. thickness, area, and echo-intensity) and baseline serum COMP. The second purpose of this study was to determine the association between the change in US

measures of cartilage health and the serum COMP response following walking and drop-landing in healthy individuals.

Association between Ultrasonographic Measures of Femoral Cartilage and Cartilage Oligomeric Matrix Protein

Within the entire cohort, there were no significant associations between baseline COMP and any US measure (r range= -0.23 – 0.22; Table 11).

For the walking condition, greater medial femoral compartment area deformation is significantly associated with a decreased COMP response (r=0.36, p=0.036; Table 12). However, no other US measure percent change was significantly associated with the acute COMP response following walking (Table 12). For the drop-landing condition, all associations between each US measure percent change and the acute COMP response were negligible and non-significant (r/ρ range = -0.15 – 0.22).

Post-hoc Test: Sex-Specific Associations between Ultrasonographic Measures of Femoral Cartilage and Cartilage Oligomeric Matrix Protein

In males, greater baseline COMP concentration is significantly associated with lesser medial cartilage echo-intensity (i.e. more water content; r = -0.52, p = 0.023; Table 11). In females, greater baseline COMP is significantly associated with lesser lateral cartilage area (r = -0.57, p = 0.014). While not all associations are statistically significant, the directions for all associations between cartilage structure measures and COMP for the males were positive (r range = 0.05 – 0.39), while all these same

associations for females were negative (r range = -0.39 - -0.57). Additionally, the directions for all associations between cartilage echo-intensity measures and COMP for the males were negative (r range = -0.21 - -0.52), while all these same associations for the females were positive (r range = 0.29 – 0.36).

In males, increased COMP response was significantly associated with lesser medial cartilage area deformation (r = 0.48, p = 0.036; Table 12). In females, increased COMP response in females was significantly associated with lesser medial cartilage thickness deformation (r = 0.46, p = 0.46). However, no other significant associations were observed for females and no significant associations were reported in males.

CHAPTER 5 - MANUSCRIPT 1

Acute Femoral Cartilage Deformation and Recovery Following Walking and Drop-Landing and its Association with Lower Extremity Biomechanics in Healthy Individuals

OVERVIEW

Context: Understanding how cartilage structure responds and recovers to acute physical activity is needed in order for future research to determine how people at risk for cartilage disease differ in response to mechanical loads during physical activity. Additionally, determining if magnitude and rate of lower extremity loading during walking and drop-landing are associated with cartilage deformation may be important in better understanding the role of lower extremity loading on cartilage mechanics. **Objective:** To compare the acute response and recovery of ultrasonography (US) cartilage outcome measures (i.e. thickness, area, and echo-intensity) between a walking, drop-landing, and control condition in healthy participants. Additionally, we sought to determine the associations between the acute cartilage response and lower extremity loading measures during the walking and drop-landing condition. **Design:** Repeated measures crossover study. **Setting:** Research laboratory. **Patients or Other Population:** 43 healthy individuals with no history of lower extremity injury. **Interventions:** A femoral cartilage US assessment was performed in healthy individuals before and after a walking, drop-landing, and control condition to determine the acute cartilage response

and recovery at 15, 30, and 45 minutes following each condition. Additionally, we assessed walking and drop-landing biomechanics at the beginning of each of respective condition to determine how lower extremity loading measures in each condition related to the acute cartilage US changes. We utilized a repeated measures design in which each participant completed all conditions during independent data collection sessions separated by at least one week. **Main Outcome Measures:** Femoral articular cartilage was assessed with US to determine the thickness, area, and echo-intensity. Percent change scores from pre to all post activity time points were used for analysis. Peak magnitude and loading rate of the vertical ground reaction force, and internal knee extension, valgus, and varus moments were assessed during walking and drop-landing. Acute cartilage response and recovery were analyzed with a 3x4 (condition x time) ANOVA. Associations between the change in US measures and lower extremity loading measures were assessed with Pearson product moment correlations or Spearman rank order correlations. **Results:** Acute deformation of the medial and lateral compartments was observed immediately following both the walking and drop-landing conditions when compared to the control condition. Following walking, the magnitude of medial and lateral cartilage deformation remained significant until at least 15 minutes post loading compared to the control condition. Following drop-landing, significant cartilage area deformation persisted for 45 and 15 minutes in the medial and lateral compartments, respectively, compared to the control condition. The femoral cartilage deformation was not accompanied by concurrent alterations in cartilage echo-intensity. The majority our US cartilage structure measures were not associated with lower extremity loading biomechanics during both walking and drop-landing. However, we did observe a weak

association between greater lateral compartment area deformation and greater vGRF and vGRF loading rate during drop-landing. **Conclusions:** US provides a reliable and precise modality for detecting the *in vivo* cartilage deformation and recovery response following walking and drop-landing, but the majority of US measures are not associated with lower extremity biomechanics.

INTRODUCTION

Osteoarthritis (OA) is one of the most common joint diseases worldwide, affecting an estimated 10% of men and 18% of women.[1] A hallmark feature of knee OA is a decline in articular cartilage health.[2] Cartilage injury or chronic metabolic changes that alter cartilage tissue structure can influence how the articular cartilage responds to mechanical joint loading,[109] which may lead to further deleterious changes in cartilage health.[152] Understanding how the cartilage of healthy individuals responds and recovers to acute physical activity is needed in order for future research to determine how individuals at risk for OA development may respond differently to mechanical loads experienced during physical activity.

Assessing cartilage thickness and volume provides an estimate of cartilage structure, however, the earliest stages of cartilage health decline are due to changes in cartilage composition without overt declines in cartilage structure measures.[22] Quantifying acute cartilage thickness deformation following physical activity is theorized to provide a surrogate assessment of cartilage composition, as cartilage deformation is governed by the composition of the tissue.[153] Thus, assessing cartilage deformation in response to an acute loading condition may be a more sensitive measure of cartilage

health when compared to assessing resting cartilage structure. Yet, only a few investigations have reported the magnitude of instantaneous[154] (i.e. during the stance phase) and acute[133,155] (i.e. change following a walking bout) femoral cartilage deformation in healthy individuals following walking. As the cartilage of individuals with knee OA demonstrates greater deformation following walking compared to healthy individuals, greater deformation following activity may indicate greater alterations in cartilage composition.[156] Not only is the magnitude of acute deformation important, but also the rate of recovery of cartilage deformation following activity as it provides a measure of cartilage resiliency.[74,144] Specifically, in individuals with altered cartilage composition (i.e. anterior cruciate ligament reconstructed [ACLR]) initial cartilage deformation following thirty minutes of running was not different when compared to the deformation observed in healthy control individuals, yet the time to recovery of cartilage thickness was slower in the ACLR individuals.[74] However, the recovery of cartilage thickness following walking in healthy individuals is yet to be determined.

Healthy knee cartilage structure is theorized to adapt to the specific lower extremity loading applied during walking.[37] For example, greater medial-to-lateral femoral cartilage thickness ratio measured with MRI is associated with greater peak internal knee abduction moment (i.e. theorized to increase dynamic medial joint loading) during walking in healthy individuals.[116] Previous investigations in acute patellar cartilage deformation have discovered that there is a dose-dependent relationship response for cartilage deformation, where more strenuous tasks result in greater deformation.[120] However, no studies have determined if biomechanics of lower extremity loading are associated with the acute change in femoral cartilage that occur

after a bout of walking or if an activity with higher magnitude loading (i.e. drop-landing) results in greater deformation when compared to walking. The peak vertical ground reaction force (vGRF) is a commonly used biomechanical variable that estimates the overall lower extremity loading that has previously been associated with cartilage metabolism.[138] Additionally, the peak internal knee abduction moment and internal knee adduction moment are theorized to provide information regarding the medial and lateral compartment dynamic loading that occurs at the knee,[157] respectively, while the internal knee extension moment has recently emerged as a contributor to knee joint loading.[158] The peak magnitude of these lower extremity loading measures are important in cartilage deformation; however, due to the viscoelastic nature of cartilage, the rate of loading is also important in influencing the magnitude of acute cartilage deformation.[112] Therefore, determining if magnitude and rate of lower extremity loading are associated with cartilage deformation may be important in better understanding the role of lower extremity loading on cartilage mechanics.

Magnetic resonance imaging (MRI) is the current gold standard for *in vivo* knee cartilage imaging to assess cartilage deformation.[159] Ultrasonography (US) has emerged as a valid and reliable technique[17] to assess femoral cartilage thickness that can detect cartilage deformation acutely following physical activity.[133] We have developed a novel method for measuring US cross-sectional area of the medial and lateral compartments of the femur that may provide a more representative measure of cartilage deformation than the previously established US cartilage thickness measurement.[133] Additionally, the US area measurement permits assessment of cartilage echo-intensity, which may represent the relative water content.[28,143] Cartilage

is approximately 60-80% water, and the interaction of water with the cartilage matrix is important in cartilage resisting compression.[29] Therefore, the purpose of this study was to compare the acute response and recovery of US cartilage outcome measures between walking, drop-landing, and control conditions in healthy participants. Additionally, we sought to determine the associations between the acute change in US cartilage measures and lower extremity loading measures during the walking and drop-landing condition. We hypothesized that cartilage thickness and area would decrease while echo-intensity would increase following the walking and drop-landing condition compared to the control condition. We also hypothesized that the deformation created by the drop-landing condition would take longer to recover compared to the walking cartilage deformation recovery, and that lower extremity biomechanics indicative of greater knee loading would be associated with greater changes in the US outcomes following the walking and drop-landing condition.

METHODS

Design

In this study, a femoral cartilage US assessment was performed in healthy individuals before and after walking, drop-landing, and control conditions to determine the acute cartilage response and recovery at 15, 30, and 45 minutes following each condition (Figure 20). Additionally, we assessed walking and drop-landing biomechanics at the beginning of each of respective conditions. We utilized a repeated measures design in which each participant completed all conditions during independent data collection sessions separated by at least one week (11.5±8.7 days between sessions)

at the same time of day (0.26±0.71 hours difference in time of day) to control for diurnal variations in femoral cartilage thickness (Figure 20).[135,136] The order of the conditions was counterbalanced. Participants were instructed to limit their physical activity on the days that data collection occurred.

On the day of each data collection session, we began by collecting a urine sample to test urine specific gravity via refractometry to confirm that each participant was not dehydrated (i.e. urine specific gravity < 1.025) prior to testing.[140] Following hydration testing, participants were seated on a padded plinth with their back against a wall in a long-sit position with their knees in full extension[133] for one hour to unload the femoral articular cartilage, permit fluid rebound, and minimize the effect of preceding activity on the cartilage. This was followed by the baseline US cartilage assessment. The participants were then immediately transferred across the laboratory using a wheel chair to begin setup for the biomechanical assessment during the walking/drop-landing condition or remained on the treatment table for the control condition. Next, the participants completed the activity condition (i.e. walking, drop-landing, or control). The participants wore the same pair of their own personal athletic footwear for all three sessions. Immediately following cessation of the activity condition, the participants were transferred back to the padded plinth and initial posttest (post0) US cartilage images were obtained within five minutes following the end of the condition. The participants remained seated on the padded plinth and additional US images were obtained at 15 (post15), 30 (post30), and 45 (post45) minutes post each condition.

Participants

We recruited a convenience sample of healthy individuals between the ages of 18 and 35 who self-reported participating in at least 30 minutes of physical activity at least three times per week. We excluded individuals with a history of ligamentous or cartilage injury to the knee or hip, cartilage injury to the ankle, congenital or degenerative joint condition, orthopedic implant, lower extremity fracture, or upper extremity fracture. Additionally, those with current joint pain (quantified as greater than 2 on a 10cm visual analog scale) were excluded from participation. We conducted an *a priori* power analysis using data from medial femoral compartment thickness changes following 30 minutes of walking (pre avg= 2.23mm, post avg=2.09mm, pooled SD=0.42mm, effect size: d=0.33) published in a previous study.[133] We estimated that we would need 33 participants to determine statistical differences in medial femoral thickness,[133] with 80% power and an α level of 0.05, if the smallest effect we found in the current study across the three loading conditions and five total time points was similar to previously published research (d=0.33).[133] Due to the time commitment of three separate three-hour data collection sessions, we over-sampled by 30% to ensure that we would achieve adequate statistical power in our final analyses if 30% of the initial sample were to drop-out of the study.

Screening Session

Prior to testing, participants came to the laboratory for an initial session to determine their habitual walking speed and determine their corresponding comfortable step frequency, which was used to standardize the amount of time used for each of the

conditions. Habitual over-ground walking speed was initially determined in our motion capture laboratory utilizing two sets of infrared timing gates (TF100, TracTronix, Lenexa, KS, USA). Participants were instructed to walk at a self-selected speed described as “comfortably walking on the sidewalk” through the 6-meter capture area.[137-139] After completing five familiarization trials, we recorded the time of the next five walking trials to determine their average habitual walking speed. Next, the speed on the treadmill (4Front, WOODWAY, Waukesha, WI, USA) was increased to the habitual walking speed of each participant and 60 seconds of walking was continued for the purpose of treadmill familiarization. After treadmill familiarization, study personnel manually counted the steps of each participant for one minute in order to determine the time each participant would need to reach 5000 steps (46.09±4.15 minutes).[122] This calculated time for each participant was used for all three conditions.

Ultrasonographic Assessment of the Femoral Articular Cartilage

Ultrasonographic Image Acquisition

US images were obtained in the dominant limb, which was defined as the self-reported limb that the participant preferred to use for kicking a ball.[133] Participants were positioned with their back against a wall and the knee of the dominant limb was positioned at 140° of flexion using a manual goniometer (Figure 21).[133] A tape measure was secured to the padded plinth and used to record the distance between the wall and the posterior calcaneus in order to standardize positioning for each participant during the posttest and throughout all data collection sessions. A single investigator performed all femoral cartilage US imaging using a LOGIQe US system (General Electric Co.,

Fairfield, CT, USA) with a 12MHz linear probe. The probe was placed transversely in line with the medial and lateral femoral condyles above the superior edge of the patella (Figure 21) and rotated to maximize the reflection of the articular cartilage surface, as previously reported.[17,141,142] A transparency grid was placed over the US screen to aid in reproducibility of the US image.[133] Once the intercondylar notch was centered on the grid, the locations of the lateral and medial femoral condyles at the edges of the screen were recorded. The probe positioning was replicated during subsequent US assessments to ensure similar probe placement between assessments. Three images were recorded, with the US probe being removed and repositioned on the knee between each recorded image at baseline, post0, post15, post30, and post45 after each condition.

Ultrasonographic Imaging Processing

A single, unblinded investigator manually segmented the US images using ImageJ software (National Institutes of Health, Bethesda, MD, USA). All three of the femoral cartilage US images from each time point were processed and averaged for all US outcome measures.

Cartilage Thickness

Femoral cartilage thickness was assessed at the midpoints of the medial femoral condyle, lateral femoral condyle, and intercondylar notch as the straight-line distance in millimeters (mm) between the cartilage-bone interface to the synovial space-cartilage interface (Figure 22a).[17,133,141,142] Strong intra-session reliability for the cartilage

thickness assessment has previously been established within our laboratory ($ICC_{2,k}$ = 0.966).[133]

Cartilage Area and Echo-intensity

The femoral cartilage was then be segmented by individually outlining the cartilage of the medial and lateral femoral condyles to obtain the size (i.e. cartilage area [mm^2]) and the grey-scale value (i.e. cartilage echo-intensity) of the cartilage (Figure 22b). The medial and lateral areas were separated based on the location of the intercondylar thickness measure. Echo-intensity evaluates the average gray scale brightness of each pixel segmented on a scale from 0 (i.e. black) to 255 (i.e. white). US echo-intensity has primarily been used as a measure of "muscle quality",[28,143] with the average echo-intensity representing the relative water content of muscle. Cartilage is approximately 60-80% fluid and acute cartilage deformation is in part due to fluid exudation.[29,144] Thus, US echo-intensity may be able to monitor acute changes in cartilage water content that occur during physical activity.

Additionally, a percent change score from baseline to each posttest was calculated to determine the acute cartilage response for each US measure following each condition (Equation 1). A greater negative percent change of thickness and area indicates greater cartilage deformation. A greater negative percent change in echo-intensity is theorized to indicate an increase in cartilage water content.

Equation 1: Percent change (%Δ) = $(\frac{\text{mean}_{\text{post}} - \text{mean}_{\text{pre}}}{\text{mean}_{\text{pre}}})*100$

Biomechanical Assessment of Lower Extremity Loading

Participant Preparation for Biomechanical Assessment

A modified retroreflective marker cluster/bony landmark setup was used for data collection for both walking and drop-jump landings.[139] Marker clusters were secured bilaterally at the middle 1/3rd of the anterior lateral thigh, middle 1/3rd of the anterior lateral shank, middle of the dorsum of the foot, and over the sacrum. Additional bony landmark markers were affixed with double sided tape at the L4/L5, manubrium, and bilaterally over the anterior superior iliac spine, greater trochanter, medial/lateral femoral epicondyle, medial/lateral malleoli, and acromion processes. Marker positions were collected using a 10-camera three-dimensional motion capture system with a sampling frequency of 120Hz with Vicon Nexus v1.4.1 motion capture software (Vicon Motion Systems, Centennial, CO) and lowpass filtered at 10Hz.[139] The cameras were interfaced with three total Bertec force plates (40cm x 60cm, FP406010, Bertec Corporation, Columbus, OH) collecting at 1200Hz and lowpass filtered at 75Hz.[139] Two of the force plates were positioned side by side and used to collect drop-landing data (Figure 23a), while two of the force plates were staggered to allow for bilateral limb collection during a single walking trial (Figure 23b).[139]

Walking Biomechanical Assessment

The participants walked shod through the capture area at their habitual walking speed (Figure 23a). Five practice trials were performed to familiarize the participants with the walking task. Five test trials were recorded in which: 1) both limbs individually landed on a single force plate, 2) maintained forward eye contact and were not “aiming”

for the force plates, 3) and maintained a consistent gait speed (±5% of the habitual walking speed calculated in the screening session.[137,138] Immediately following the collection of the last test trial, the participants were returned to the wheelchair and transferred across the laboratory to begin the walking condition.

Drop-Landing Biomechanical Assessment

A 62cm platform was positioned behind the side-by-side force plates to allow for simultaneous collection of both limbs during a single drop-landing trial (Figure 23b). A separate step was positioned behind the 62cm platform and was utilized by the participants to ascend onto the platform. For each drop-landing trial, the participants ascended onto the platform and were instructed to drop down from the platform and perform a comfortable landing with each limb on a separate force plate. No specific instructions were provided to the participants on how to perform the double-legged landing. After each drop-landing, participants would walk around the box and ascend up the step in order to prepare for the next drop-landing trial. The drop-landing biomechanics assessment was conducted on trials 5 through 10.

Analysis of Lower Extremity Loading

Individual trials from the walking and drop-landing biomechanical assessments were labeled to identify all of the retro-reflective markers within the Vicon Nexus motion capture software (version 1.8.5, Vicon Motion Systems, Oxford, UK). Once labeled, the marker trajectory data, synchronized with the ground reaction force, was exported from Vicon Nexus and further processed using The Motion Monitor software (version 8.0,

Innovative Sports Training, Chicago, IL, USA). A custom LabVIEW program (National Instruments Corp., San Antonio, TX) was used to measure our biomechanics variables during the early loading phase of walking and drop-landing. For the walking trials, the loading phase was defined as the first 50% of stance phase; defined as the interval from heel strike (vGRF > 20N) to toe-off (vGRF < 20N). The loading phase of drop-landing was defined as the first 100ms following ground contact (vGRF > 20N). Peak vGRF was determined during the loading phase of the walking and drop-landing conditions. Peak internal knee extension (EXT) moment, peak internal knee valgus (VAL) moment, and internal knee varus (VAR) moment moments during the loading phase were calculated using inverse dynamics as previously performed in our laboratory.[139] Greater EXT moments were depicted as negative values, while more positive VAL and VAR moments are indicative of greater moment.[160] Instantaneous loading rate of the vGRF (vGRF-LR), EXT, VAL, and VAR moments were calculated as the peak of first derivative of the force-time and moment-time curves.[139] Peak vGRF (xBW) and vGRF-LR (xBW/s) were normalized to participants' body weight.[138] Peak moments (xBW*Ht) and moment loading rates (xBW*Ht/s) were normalized to the product of participants' height and weight.[139]

Walking, Drop-Landing, and Control Conditions

Walking Condition

The participants were positioned on the treadmill and the speed was increased to the habitual walking speed determined during the screening session. This speed was

maintained for the time calculated during the screening session to reach 5,000 steps (46.09±4.15 minutes).

Drop-Landing Condition

The drop-landing biomechanical assessment occurred concurrently with the drop-landings used for the drop-landing condition. For the drop-landing condition, the participants continued performing drop-landings until they completed 120 total drop-landings. We selected the amount of drop-landings from the 62cm platform in order to match the high magnitude loading condition utilized in a previous study utilizing a similar drop-landing protocol.[123] The 120 drop-landing trials were evenly distributed over the same period of time utilized in the other conditions (46.09±4.15 minutes).

Control Condition

During the control condition, participants remained on the treatment table following the baseline blood sample collection in a long-sit position for the same period of time utilized in the other conditions (46.09±4.15 minutes).

Statistical Analysis

Intra-session and Inter-session Reliability, Precision, and Minimal Detectable Change of Ultrasonographic Assessed Femoral Cartilage Compartmental Area and Echo-intensity

Prior to our primary analyses, separate intraclass correlation coefficients ($ICC_{2,k}$) were calculated between the control condition baseline and post0 assessment of medial and lateral femoral cartilage compartment area and echo-intensity to establish the intra-

session session reliability. ICCs were classified as weak (<0.5), moderate (0.5 – 0.69), or strong (≥0.7).[161] Standard error of the measurement (SEM) was calculated between the control condition time points to establish the measurement's precision (Equation 2).[162] Minimal detectable change (MDC) was calculated between the two control condition time points to determine the change in US variables that may be expected due to measurement error (Equation 3).[163]

Equation 2[162]: Standard error of measurement (SEM) = $\mathrm{SD}\sqrt{1-\mathrm{ICC}}$

Equation 3[163]: Minimal detectable change (MDC) = $1.645 \times SEM \times \sqrt{2}$

Separate $ICC_{2,k}$, SEM, and MDC were calculated for the baseline cartilage area and echo-intensity from the control and jumping sessions to establish each measurement's inter-session reliability, precision, and minimal detectable change, respectively. All statistical analyses were performed using SPSS (version 21.0; IBM Corporation) with an *a priori* α level of $P < 0.05$.

Comparison of Ultrasonographic Cartilage Outcome Measure Response and Recovery between Conditions

Separate one-way repeated measures analysis of variance (RM-ANOVA) were used to compare the baseline values for each cartilage US measure between the walking, drop-landing, and control condition. Separate 4 x 3 (time x condition) RM-ANOVAs were used to compare the percent change scores between the conditions for acute cartilage response and recovery at each time point. Outliers were defined as >

two standard deviations away from the mean for each US measure at each time point. Next, a participant was removed from an individual US outcome measure RM-ANOVA if more than two time points were defined as outliers during a single condition. After outlier removal, a Shapiro-Wilk test was used to confirm normal distribution for each outcome measure. If there was a significant interaction effect for any of the RM-ANOVAs, we utilized Bonferroni corrected ($p = 0.05/12 = 0.004$) paired samples t-tests to determine the specific differences between conditions at each time point for all of the US measures.

Association between Ultrasonographic Cartilage Outcome Measure Response and Lower Extremity Loading during Conditions

Separate Pearson product moment correlations were used to determine the association between the acute change (i.e. post0 percent change score) for each US measure and each lower extremity loading biomechanical variable during the walking and drop-landing condition. For correlational analysis between each acute change in US measure and lower extremity loading variable, outliers greater than two standard deviations away from the mean for either measure were removed for that individual analysis. Following outlier removal, if the data were still found to be non-normal with the Shapiro-Wilk test, a Spearman Rank-Order correlation was used to determine the association between the acute change in each US measure and each lower extremity loading biomechanical measure. Correlational analyses involving the vGRF, vGRF loading rate, and EXT moment were conducted for all US measures. Since greater VAL moment is theorized to increase medial compartment loading, correlational analyses

involving the VAL moment were only conducted on the medial and intercondylar US measures. Similarly, correlational analyses involving the VAR moment were only conducted on the lateral and intercondylar US measures. Associations were classified as negligible (0.0 – 0.30), low (0.31 – 0.50), moderate (0.51 – 0.70), high (0.71 – 0.90), and very high (0.90 – 1.00).[148] Additionally, we included the 95% confidence intervals around each Pearson r and Spearman ρ to ensure that the intervals of each association did not cross zero.[149]

RESULTS

Participants

Forty-three total participants were included in this study (Table 1); however, due to participant dropout between data collection sessions, not every participant completed each data collection session (walking n = 42, drop-landing n = 41, and control n = 43). The two dropouts in the walking condition were due to unrelated injuries that occurred between their 2nd and 3rd sessions, and the one drop-landing dropout was due to the participant being unwilling to complete the drop-landing condition. Forty participants completed all three sessions and are included in the RM-ANOVAs. The correlational analyses included the maximum amount of participants for each condition and outcome measure.

Reliability, Precision, and Minimal Detectable Change of Ultrasonographic Assessed Femoral Cartilage Compartmental Area and Echo-intensity

Overall, we demonstrated adequate intra- and inter-session reliability, precision, and small minimal detectable changes for all of our novel US measures of compartmental area and echo-intensity (Table 2).

Comparison of Ultrasonographic Cartilage Outcome Measure Response and Recovery between Conditions

Baseline as well as both the absolute and percent change scores for each posttest time point for the conditions are presented in Table 3.

Femoral Cartilage Thickness

Baseline medial cartilage thickness was not different between the conditions ($F_{2,78}$=1.08, p=0.346, n=40). There was a significant time by condition interaction effect for medial thickness deformation ($F_{6,234}$=18.60, p<0.001). When compared to the control condition, medial thickness deformation was significantly greater following walking at post0 (t_{39}=7.48, p<0.001) and post15 (t_{39}=3.05, p=0.004), as well as following drop-landing at post0 (t_{39}=12.41, p<0.001), post15 (t_{39}=7.36, p<0.001), and post30 (t_{39}=3.24, p=0.002). The magnitude of medial thickness deformation was greater in the drop-landing condition compared to the walking condition at post0 (t_{39}=3.81, p<0.001) and post15 (t_{39}=4.49, p<0.001), but was not statistically significant at post30 (t_{39}=2.96, p=0.005) and post45 (t_{39}=2.74, p=0.009).

Baseline intercondylar cartilage thickness was not different between the conditions ($F_{2,76}$=1.51, p=0.228, n=39). There was a significant time by condition interaction effect for the intercondylar thickness deformation ($F_{6,228}$=3.37, p=0.003). Intercondylar thickness deformation was significantly greater at post0 following walking (t_{38}=4.20, p=0.004) and jumping (t_{38}=3.05, p<0.001) when compared to the control condition, but was not different at any other time points. Intercondylar thickness deformation following walking and drop-landing did not significantly differ at any time point.

Baseline lateral thickness was significantly different between the conditions ($F_{2,72}$=3.18, p=0.048, n=37). However, multiple comparisons t-tests indicate no differences in lateral thickness between conditions, and the largest baseline lateral thickness mean difference (walking vs. drop-landing = 0.05mm) observed between conditions was within the intersession MDC we have previously established (MDC = 0.19mm).[132] There was a significant time by condition interaction effect for the lateral thickness deformation ($F_{6,216}$=15.174, p<0.001). When compared to the control condition, the lateral thickness deformation was significantly greater following walking at post0 (t_{36}=7.26, p<0.001) and post15 (t_{36}=4.02, p<0.001), as well as following drop-landing at post0 (t_{36}=10.44, p<0.001) and post15 (t_{36}=4.88, p<0.001). Lateral thickness deformation was not different between the walking and drop-landing at any time point.

Femoral Cartilage Area

Baseline medial cartilage area was statistically different between conditions ($F_{2,78}$=11.18, p<0.001, n=40). However, the largest baseline medial cartilage area

difference (walking vs. drop-landing = 1.01mm^2) observed between conditions was within the intersession medial cartilage area MDC we established (MDC = 2.69mm^2, Table 2). There was a significant time by condition interaction effect for medial cartilage area ($F_{6,234}$=11.91, p<0.001). When compared to the control condition, medial cartilage area deformation was significantly greater during the walking condition at post0 (t_{39}=8.87, p<0.001) and post15 (t_{39}=4.68, p<0.001), as well as following drop-landing at all post time points (post0: t_{39}=10.12, p<0.001; post15: t_{39}=9.11, p<0.001; post30: t_{39}=3.79, p=0.001; post45: t_{39}=4.04, p<0.001). Additionally, the magnitude of medial area deformation was greater in the drop-landing condition compared to the walking condition at the post15 (t_{39}=3.60, p=0.001) time point, but this difference was not statistically significant at post0 (t_{39}=2.78, p=0.008), post30 (t_{39}=2.91, p=0.006), post45 (t_{39}=2.60, p=0.013).

Baseline lateral cartilage area was statistically different between conditions ($F_{2,74}$=4.13, p=0.020, n=38). However, the largest baseline lateral cartilage area difference (walking vs. drop-landing = 1.42mm^2) observed between conditions was within the intersession lateral cartilage area MDC we established (MDC = 2.54mm^2, Table 2). There was a significant time by condition interaction effect for lateral cartilage area ($F_{6,228}$=5.82, p<0.001). When compared to the control condition, lateral cartilage area deformation was significantly greater during the walking condition at post0 (t_{37}=5.53, p<0.001) and post15 (t_{37}=3.88, p<0.001), as well as following drop-landing at post0 (t_{37}=6.47, p<0.001), post15 (t_{37}=5.532, p<0.001), and post30 (t_{37}=3.19, p=0.003). Lateral cartilage deformation was not different between the walking and drop-landing conditions at any post time points.

Femoral Cartilage Echo-intensity

Baseline medial cartilage echo-intensity was not statistically different between the conditions ($F_{2,72}$=0.22, p=0.806, n=37). There was a statistically significant time by condition interaction effect for medial cartilage echo-intensity percent change ($F_{6,216}$=2.215, p=0.043), but none of the t-tests indicated any differences between the conditions at any of the posttest time points.

Baseline lateral cartilage echo-intensity was not statistically different between the conditions ($F_{2,72}$=0.747, p=0.477, n=37). There was not a statistically significant interaction effect between time and condition ($F_{6,216}$=1.81, p=0.098). Therefore, t-tests were not utilized to identify specific group differences at each time point.

Association between Ultrasonographic Cartilage Outcome Measure Response and Lower Extremity Loading during the Walking and Drop-Landing Conditions

Descriptive statistics for the lower extremity loading variables during walking and drop-landing can be found in Table 1.

Femoral Cartilage Thickness

During walking, lesser peak EXT moment associated with greater medial compartment deformation (r=-0.37, p=0.022; Table 4). However, the other walking lower extremity loading measures were not significantly associated with medial (r range = -0.24 – 0.19), intercondylar (r/ρ range = -0.20 – 0.16), or lateral (r/ρ range = -0.16 – 0.09) thickness deformation.

Drop-landing lower extremity loading measures were not significantly associated with medial (r/ρ range = -0.18 – 0.32; Table 4), intercondylar (r/ρ range = -0.11– 0.12), or lateral (r/ρ range = -0.31– 0.01) thickness deformation.

Femoral Cartilage Area

Walking lower extremity loading measures were not significantly associated with medial (r range = -0.21 – 0.24, Table 4) or lateral (r/ρ range = -0.16– 0.26) compartment area deformation.

Drop-landing lower extremity loading measures were not significantly associated with medial cartilage area deformation (r/ρ range = -0.19 – -0.08; Table 4). During drop-landing, greater peak vGRF(r=-0.34, p=0.036) and vGRF loading rate (r=-0.42, p=0.008) were significantly associated with greater lateral compartment area deformation. However, the other drop-landing lower extremity loading measures were not significantly associated with lateral compartment area deformation (r/ρ range = -0.20 – 0.17).

Femoral Cartilage Echo-intensity

During walking, greater vGRF loading rate (r=-0.51, p=0.001; Table 4) was moderately associated with a decrease in medial compartment echo-intensity. However, the other walking lower extremity loading measures were not significantly associated with a change in medial compartment echo-intensity (r range = -0.21 – 0.14). Walking lower extremity loading measures were not associated with a change in lateral compartment echo-intensity (r/ρ range = -0.25 – 0.08).

Drop-landing lower extremity loading measures were not statistically associated with a change in medial compartment echo-intensity (r/ρ range = -0.22 – 0.14; Table 4). During drop-landing, greater peak VAR moment was significantly associated with an increase in lateral compartment echo-intensity (ρ=0.34, p=0.04). However, the other drop-landing lower extremity loading measures were not statistically associated with a change in lateral compartment echo-intensity (r/ρ range = -0.27 – 0.21).

DISCUSSION

This is the first study to assess both the acute cartilage deformation and recovery following a walking and drop-landing task. We demonstrated that our novel US compartmental area and echo-intensity technique is both reliable and precise within and between separate testing sessions. Acute deformation of the medial and lateral compartments was observed immediately following both the walking (i.e. low magnitude) and drop-landing (i.e. high magnitude) conditions when compared to the control (i.e. no loading) condition. Greater acute medial cartilage deformation was observed following drop-landing when compared to walking. The medial and lateral cartilage area remained deformed up to 15 minutes after the end of the walking condition when compared to the control condition. Additionally, significant deformation was observed in the medial and lateral cartilage area up to 45 and 15 minutes following the drop-landing condition, respectively, when compared to the control condition. These results indicate that the high magnitude drop-landing condition resulted in greater acute cartilage deformation as well as longer time to recover when compared to the walking condition. Even though significant deformation in the cartilage structure was observed

following both walking and drop-landing, this structural response was not accompanied by concurrent alterations in cartilage echo-intensity changes following the conditions. The majority our US cartilage structure measures were not associated with lower extremity loading measures during both walking and drop-landing; however, we did observe a weak association between greater lateral compartment area deformation and greater vGRF and vGRF loading rate during drop-landing.

Only a few studies have quantified *in vivo* femoral cartilage deformation created by walking.[133,154,155] Utilizing a combination of dual fluoroscopic imaging and magnetic resonance imaging (MRI), previous authors have quantified the amount of cartilage deformation occurring throughout the entire stance phase of gait.[154] Medial femoral deformation ranged from 8% to 23%, while lateral femoral deformation ranged from 7% to 16% depending on the specific phase of the gait cycle.[154] Total volumetric deformation (i.e. deformation of the entire compartment) was only found to be 1.2% and 1.3% in the medial and lateral femoral compartments, respectively.[155] However, we have previously observed an acute anterior medial thickness deformation of 6.7% after 30 minutes of walking,[133] and specific local MRI acute deformation has observed a 7.3% maximal deformation occurring in the anteromedial portion of the medial femoral condyle and a 3.3% maximal deformation in the lateral femoral condyle following 20 minutes of walking.[155] This indicates that volumetric MRI assessments of cartilage deformation may underestimate the magnitude of deformation. Interestingly, the medial cartilage deformation observed in this study (area = 7.09%, thickness = 7.11%) was similar to what has been found in these previous studies,[133,155] even though participants in the current study incurred a greater number of steps compared to previous studies

(~5,000 steps; ~46 minutes). Therefore, it is possible that cartilage deformation in response to cyclic loading may reach a certain threshold in which further deformation does not occur.[159]

Only one other investigation has utilized a similar drop-landing protocol as we used in the current study,[123] and they observed non-significant cartilage volume deformation in the medial (i.e. 0.66%) and lateral (i.e. 1.6%) femoral condyles as compared to the deformation we observed in the medial (i.e. area=10.05%, thickness=9.30%) and lateral (i.e. area=6.95%, thickness=6.8%) femoral condyles. The discrepancy between cartilage deformation measurements in our study and the overall volumetric cartilage deformation reported following walking[155] and drop-landing[123] again highlight that volumetric measurements of the entire femoral condyle may underestimate the site-specific deformation that occurs locally on specific aspects of the femur. Therefore, the cartilage deformation observed in this study is due to our US cartilage assessment providing a specific estimate of the anterior femur cartilage deformation. Greater magnitude cartilage deformation assessed with US may also be due to the accessibility and quick image acquisition of US. Thus, US may allow for earlier imaging of the cartilage following activity conditions when compared to the time needed for an MRI assessment. We are able to collect bilateral US images within less than five minutes following the end of the activity conditions, whereas previous studies have reported that participant setup and image acquisition can take as much as 13 minutes to capture a single knee.[155]

This is the first study that has assessed femoral cartilage recovery following an acute bout of walking and drop-landing. We found that drop-landing results in greater

initial medial cartilage deformation, and cartilage thickness takes longer time to recover following drop-landing when compared to the walking condition. These results reinforce the results of an earlier study of patellar cartilage[120] that indicate the presence of a dose-dependent relationship of cartilage deformation in which more intense physical activity results in greater cartilage deformation. While no previous research has investigated the recovery of cartilage deformation following an acute bout of walking or drop-landing, a previous study compared tibiofemoral cartilage deformation following running between a small cohort of healthy individuals and patients following anterior cruciate ligament reconstruction (ACLR).[74] The overall magnitude of acute deformation did not differ between healthy individuals and patients following ACLR, but the cartilage of ACLR patients took longer to recover compared to the healthy individuals.[74] Thus, assessing cartilage recovery following physical activity may provide a measure of cartilage resiliency that could be important to understanding how cartilage is recovering to acute loading.

Even though we observed significant cartilage deformation following both the walking and drop-landing protocols, these structural changes were not accompanied with concurrent alterations in cartilage echo-intensity. Since cartilage deformation is in part due to fluid exudation,[29] we hypothesized that we would detect water content changes using cartilage echo-intensity. Previously, T2 MRI Mapping (i.e. compositional imaging technique indicative of water content[164]) has been used to determine how different types of acute activity acutely alter cartilage water content.[127,128,165] Using a static loading technique inside an MRI scanner, the amount of femoral cartilage thickness deformation was not associated with concurrent changes in T2 relaxation

time.[127] Additionally, following a 30 minute jog, a ~7% decrease in femoral cartilage thickness was not accompanied by an overall change in mean T2 relaxation time.[166] However, there was a significant change in the mean T2 relaxation time of the superficial femoral cartilage,[166] indicating that activity may non-uniformly affect cartilage water content depending on the depth of the cartilage. Therefore, depending on the activity condition, region of cartilage, and depth within the cartilage there may be a differential association between the structural deformation and change in cartilage water content. While this is the first investigation to assess acute changes in cartilage echo-intensity following acute loading, our results may indicate that this technique may not be sensitive enough to detect acute changes throughout the entire thickness of cartilage. However, future work that assesses echo-intensity at different depths of cartilage thickness may allow for more specific determination of cartilage echo-intensity changes following acute activity.

This study was the first to attempt to associate acute cartilage deformation with specific measures of lower extremity loading during a walking and drop-landing task. Greater drop-landing peak vGRF and vGRF loading rate are significantly associated with greater lateral compartment area deformation, while lesser walking EXT moment associated with greater medial compartment thickness deformation. However, the more important finding is that there were no other significant associations between the multiple lower extremity loading variables and cartilage structure alterations following walking or drop-landing. Our lower extremity loading variables focused on the early loading phase of walking and drop-landing, but future investigations should also evaluate lower extremity loading variables occurring in later phases of the gait cycle or

knee kinematics (i.e. joint movement), as both of these variables affect cartilage loading.[154,167] Thus, the majority of our discrete measures of lower extremity loading during a single gait cycle did not associate with the cumulative cartilage deformation throughout the activity conditions, but future studies are needed to determine other biomechanical parameters that are most associated with acute cartilage deformation.

While this is the first study to determine the acute cartilage recovery following walking and drop-landing, as well as determining the association between acute cartilage response and lower extremity loading, there are limitations that should be considered. Due to restraints of the US imaging technique, we are only able to provide an assessment of a small area of the anterior femoral cartilage, and are unable to differentially describe how walking and drop-landing affects the central and posterior regions of femoral cartilage. While this limits the scope of cartilage we are able to image, this does allow us to provide a more precise indication of how loading affects anterior femoral cartilage compared to the traditional volumetric cartilage assessments utilizing MRI. An unblinded reader conducted US image analysis, and future studies should consider utilizing blinded readers unaware of the loading condition. Our lower extremity biomechanics outcomes focused solely on discrete peaks in lower extremity loading during the early phases of loading. Knee moments have demonstrated to predict joint contact force;[157] however, joint moments do not provide direct quantification of compression force that occurs at the joint surface. Future studies should consider lower extremity loading variables throughout the entire gait cycle, joint kinematics, or measures of joint contact forces that may affect *in vivo* cartilage mechanics.

In conclusion, US provides a reliable and precise modality for detecting the *in vivo* cartilage deformation and recovery response following walking and drop-landing. Following walking, medial and lateral cartilage deformation remains significant until at least 15 minutes post loading, while drop-landing induced cartilage area deformation persists for 45 and 15 minutes in the medial and lateral compartments, respectively. The femoral cartilage deformation was not accompanied with concurrent alterations in cartilage echo-intensity. Additionally, the majority of our lower extremity loading biomechanics were not significantly associated with the acute alterations in cartilage US measures.

Table 1. Demographics

	Mean	SD
n	43 (22 male, 21 female)	
Age (years)	21.44	3.10
Height (meters)	1.72	0.09
Mass (kilograms)	68.82	11.39
Tegner	5.91	1.23
Walking Biomechanics Descriptive Data		
Walking Speed (meters/sec)	1.24	0.18
Walking Distance (kilometers)	3.45	0.37
Peak vGRF (xBW)	1.13	0.08
vGRF Loading Rate (xBW/s)	20.76	3.68
Peak VAL Moment (xBW*Ht)	0.030	0.009
VAL Moment Loading Rate (xBW*Ht/s)	1.165	0.613
Peak VAR Moment (xBW*Ht)	0.0043	0.0032
VAR Moment Loading Rate (xBW*Ht/s)	0.505	0.153
Peak EXT Moment (xBW*Ht)	-0.033	0.015
EXT Moment Loading Rate (xBW*Ht/s)	-1.791	0.654
Drop-Landing Biomechanics Descriptive Data		
Peak vGRF (xBW)	2.92	0.78
vGRF Loading Rate (xBW/s)	194.03	67.51
Peak VAL Moment (xBW*Ht)	0.141	0.148
VAL Moment Loading Rate (xBW*Ht/s)	12.695	10.959
Peak VAR Moment (xBW*Ht)	0.021	0.017
VAR Moment Loading Rate (xBW*Ht/s)	9.980	7.328
Peak EXT Moment (xBW*Ht)	-0.230	0.061
EXT Moment Loading Rate (xBW*Ht/s)	-16.15	3.37

vGRF = vertical ground reaction force, VAL = valgus, VAR = varus, EXT = extension
BW = body weight, HT = height, s = seconds, SD = standard deviation

Table 2. Reliability, Precision, and Minimal Detectable Change

	Intra-session			Inter-session		
	ICC	SEM	MDC	ICC	SEM	MDC
Medial Area (mm^2)	0.993	0.68	1.58	0.978	1.16	2.69
Medial Echo-intensity	0.934	1.41	3.29	0.949	1.29	3.01
Lateral Area (mm^2)	0.985	0.91	2.24	0.978	1.09	2.54
Lateral Echo-intensity	0.943	1.00	2.11	0.828	1.91	4.44

mm = millimeters, ICC = intraclass correlation coefficient, SEM = standard error of the measurement, MDC = minimal detectable change

Table 3. Ultrasonography Outcome Measure Percent Change Scores Following Loading Conditions

Outcome	Compartment	Condition	Baseline Absolute		Post0 %Δ		Post0 Absolute		Post15 %Δ		Post15 Absolute		Post30 %Δ %Δ		Post30 %Δ Absolute		Post45 %Δ %Δ		Post45 %Δ Absolute	
			mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Thickness (mm)	Medial (n=40)	Walking	2.22	0.39	-7.11*^	3.00	2.07	0.38	-4.21*^	2.89	2.13	0.39	-2.09^	3.28	2.18	0.39	-0.07^	3.65	2.22	0.39
		Drop-landing	2.21	0.40	-10.05*	3.80	1.99	0.36	-7.57*	4.03	2.04	0.36	-4.70*	4.89	2.11	0.39	-2.57	4.10	2.15	0.38
		Control	2.23	0.40	-0.95	3.81	2.21	0.4	-2.24	3.98	2.18	0.39	-1.43	4.69	2.2	0.38	-1.71	4.29	2.19	0.38
	Intercondylar (n=39)	Walking	2.28	0.46	-2.46*	6.64	2.22	0.48	-2.56	5.26	2.22	0.46	-1.26	6.07	2.25	0.47	-0.52	6.36	2.26	0.48
		Drop-landing	2.25	0.47	-3.96*	6.54	2.16	0.43	-2.59	6.02	2.19	0.45	-1.29	6.72	2.22	0.48	-0.12	7.20	2.24	0.45
		Control	2.24	0.47	1.06	4.97	2.26	0.48	-0.36	4.81	2.23	0.46	-0.54	4.57	2.23	0.47	-0.08	5.15	2.24	0.49
	Lateral (n=37)	Walking	2.13	0.34	-5.18*	3.74	2.03	0.35	-3.26*	3.00	2.07	0.33	-1.13	2.76	2.11	0.31	-0.31	2.81	2.13	0.34
		Drop-landing	2.09	0.31	-6.8*	3.84	1.94	0.29	-4.41*	4.14	1.99	0.29	-2.37*	4.28	2.04	0.31	-1.63	4.23	2.05	0.31
		Control	2.10	0.34	1.13	4.24	2.13	0.36	-0.49	4.13	2.1	0.36	-0.27	3.83	2.1	0.38	-1.88	3.26	2.07	0.35
Area (mm^2)	Medial (n=40)	Walking	41.97	7.51	-7.09*	4.10	39.02	7.26	-3.37*^	3.94	40.52	7.20	-0.69	4.74	41.61	7.22	0.61	6.06	42.18	7.48
		Drop-landing	42.96	7.50	-9.30*	4.49	38.97	6.95	-6.30*	3.97	40.22	6.92	-3.21*	3.14	41.55	7.09	-2.00*	4.35	42.10	7.42
		Control	41.54	8.11	-0.66	3.24	41.24	8.07	-0.25	3.56	41.36	7.80	-0.13	4.22	41.41	7.77	1.34	3.99	41.99	7.83
	Lateral (n=38)	Walking	44.38	7.15	-6.03*	3.85	41.75	7.16	-3.42*	4.63	42.84	7.08	-1.51	4.31	43.64	6.87	-0.66	5.15	44.09	7.55
		Drop-landing	44.71	7.29	-6.95*	4.92	41.55	6.83	-4.23*	4.31	42.79	7.10	-2.09*	4.59	43.70	6.98	-2.03	4.59	43.78	7.26
		Control	43.71	7.42	-0.62	4.20	43.39	7.42	0.15	3.95	43.7	7.26	0.37	4.03	43.81	7.35	-0.44	3.73	43.44	7.11
Echo-intensity	Medial (n=37)	Walking	64.23	5.66	-1.86	3.76	62.94	4.97	-0.32	3.50	63.95	5.16	1.10	4.04	64.85	5.34	0.88	4.88	64.66	5.06
		Drop-landing	63.96	5.99	-1.78	3.98	62.81	6.19	-0.10	4.21	63.86	6.14	0.43	4.47	64.18	6.10	0.70	4.88	64.36	6.21
		Control	64.24	5.45	-0.79	4.15	63.69	5.56	-0.11	4.90	64.13	5.83	-0.17	5.05	64.11	6.02	-0.35	4.88	64.00	6.06
	Lateral (n=37)	Walking	58.59	4.67	-0.66	4.47	58.11	4.13	-0.11	4.32	58.47	4.58	0.23	3.21	58.67	4.37	0.60	4.14	58.87	4.39
		Drop-landing	59.13	5.09	-2.02	3.30	57.87	4.58	-0.05	4.20	59.01	4.52	0.35	4.57	59.25	4.65	0.18	4.90	59.14	4.69
		Control	58.65	4.11	-0.88	3.24	58.12	4.23	0.25	4.73	58.76	4.44	-0.13	4.39	58.55	4.45	-0.51	5.63	58.33	4.98

*different than control (p≤0.004), ^different than drop-landing (p≤0.004), n = sample size, mm = millimeter, sd = standard deviation, %Δ = percent change

Table 4. Association between Ultrasonographic Cartilage Outcome Measure Response and Lower Extremity Loading during Physical Activity Conditions

			Walking								Drop-Landing							
			vGRF	vGRF LR	KEM	KEM LR	VAL	VAL LR	VAR	VAR LR	vGRF	vGRF LR	KEM	KEM LR	VAL	VAL LR	VAR	VAR LR
Medial Condyle	Thickness	r/ρ	**-0.11**	**-0.24**	**0.09**	**-0.37***	**0.19**	**0.15**	--------	--------	**0.32**	**0.06**	*-0.01*	**0.15**	*-0.18*	*-0.11*	--------	--------
		p	**0.473**	**0.136**	**0.588**	**0.022**	**0.244**	**0.386**	--------	--------	**0.057**	**0.736**	*0.963*	**0.394**	*0.286*	*0.526*	--------	--------
		n	**42**	**41**	**40**	**39**	**39**	**38**	--------	--------	**37**	**38**	*38*	**34**	*37*	*38*	--------	--------
	Area	r/ρ	**0.07**	**0.12**	**-0.18**	**0.02**	**0.24**	**-0.21**	--------	--------	**-0.12**	**-0.08**	*0.25*	**-0.32**	*-0.19*	*-0.08*	--------	--------
		p	**0.676**	**.462**	**0.274**	**0.914**	**0.142**	**0.209**	--------	--------	**0.473**	**0.615**	*0.129*	**0.066**	*0.264*	*0.640*	--------	--------
		n	**42**	**41**	**40**	**39**	**39**	**38**	--------	--------	**38**	**38**	*38*	**34**	*37*	*38*	--------	--------
	Echo-Intensity	r/ρ	**-0.02**	**-0.51***	**0.04**	**-0.20**	**-0.21**	**0.14**	--------	--------	**-0.22**	**-0.12**	*-0.10*	**-0.10**	*0.08*	*0.14*	--------	--------
		p	**0.920**	**0.001**	**0.819**	**0.227**	**0.217**	**0.401**	--------	--------	**0.173**	**0.451**	*0.553*	**0.571**	*0.657*	*0.383*	--------	--------
		n	**41**	**40**	**39**	**38**	**38**	**37**	--------	--------	**39**	**39**	*39*	**35**	*38*	*39*	--------	--------
Lateral Condyle	Thickness	r/ρ	**-0.15**	**-0.15**	**-0.03**	**-0.19**	--------	--------	*0.09*	**-0.16**	**-0.07**	**-0.15**	*0.19*	**0.07**	--------	--------	*0.01*	*-0.31*
		p	**0.350**	**0.360**	**0.838**	**0.261**	--------	--------	*0.583*	**0.327**	**0.690**	**0.361**	*0.242*	**0.696**	--------	--------	*0.971*	*0.055*
		n	**42**	**41**	**40**	**39**	--------	--------	*40*	**40**	**39**	**39**	*39*	**35**	--------	--------	*40*	*39*
	Area	r/ρ	**0.07**	**0.26**	**-0.09**	**0.25**	--------	--------	*0.01*	**-0.16**	**-0.34***	**-0.42***	*0.11*	**-0.20**	--------	--------	*0.17*	*-0.03*
		p	**0.674**	**0.097**	**0.565**	**0.132**	--------	--------	*0.930*	**0.339**	**0.036**	**0.008**	*0.508*	**0.264**	--------	--------	*0.309*	*0.855*
		n	**42**	**41**	**40**	**39**	--------	--------	*40*	**40**	**38**	**38**	*38*	**34**	--------	--------	*40*	*39*
	Echo-intensity	r/ρ	**-0.09**	**-0.25**	**0.02**	**-0.18**	--------	--------	*-0.08*	**-0.15**	**-0.27**	**0.07**	*-0.27*	**-0.07**	--------	--------	*0.34**	*0.21*
		p	**0.593**	**0.122**	**0.881**	**0.266**	--------	--------	*0.634*	**0.368**	**0.107**	**0.686**	*0.106*	**0.686**	--------	--------	*0.040*	*0.203*
		n	**42**	**41**	**40**	**39**	--------	--------	*40*	**40**	**37**	**37**	*37*	**33**	--------	--------	*38*	*37*
Intercondylar Thickness		r/ρ	**-0.20**	**-0.10**	**0.17**	**-0.17**	**0.04**	**0.16**	*-0.17*	**0.02**	**0.12**	**0.09**	*0.09*	**-0.04**	*-0.06*	*0.05*	*0.01*	*-0.11*
		p	**0.221**	**0.557**	**0.288**	**0.301**	**0.803**	**0.332**	*0.305*	**0.917**	**0.463**	**0.574**	*0.608*	**0.835**	*0.745*	*0.748*	*0.939*	*0.512*
		n	**41**	**40**	**39**	**38**	**38**	**37**	*39*	**39**	**39**	**39**	*39*	**35**	*38*	*39*	*40*	*39*

Figure 20. Study design. A femoral cartilage ultrasonographic assessment was performed at each time point.

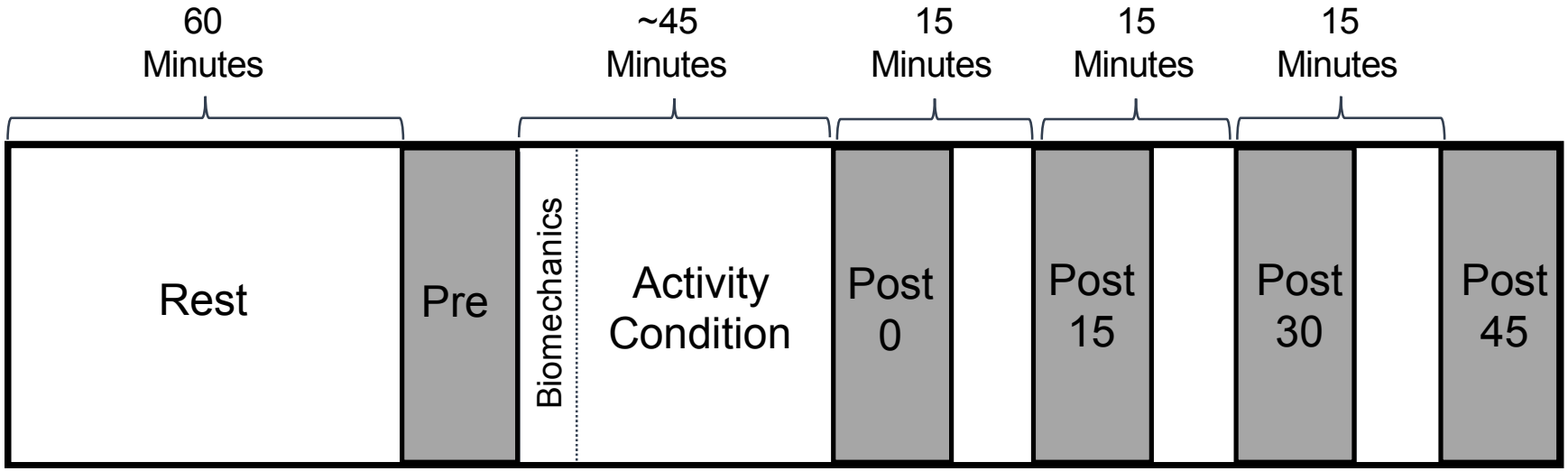


Figure 21. Femoral Cartilage Ultrasonography Setup and Participant Positioning.

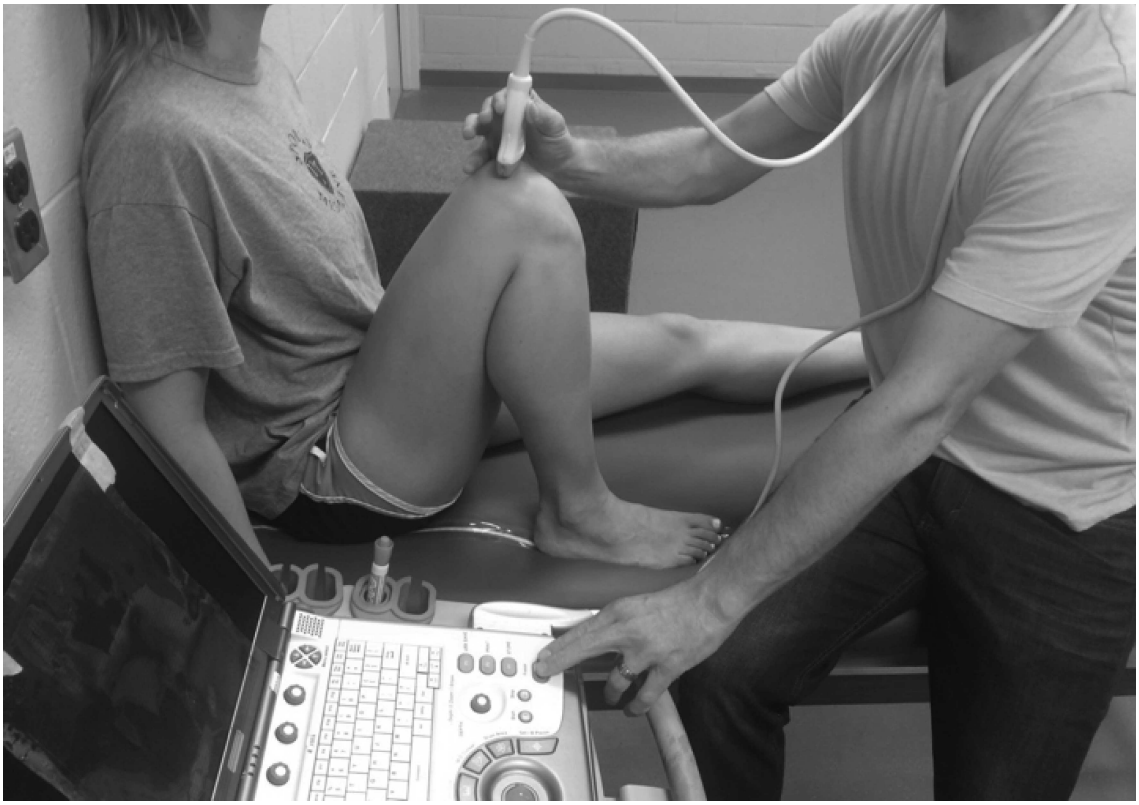


Figure 22. Femoral Cartilage Ultrasonography Outcome Measures. A) Cartilage Thickness; B) Cartilage Compartmental Area

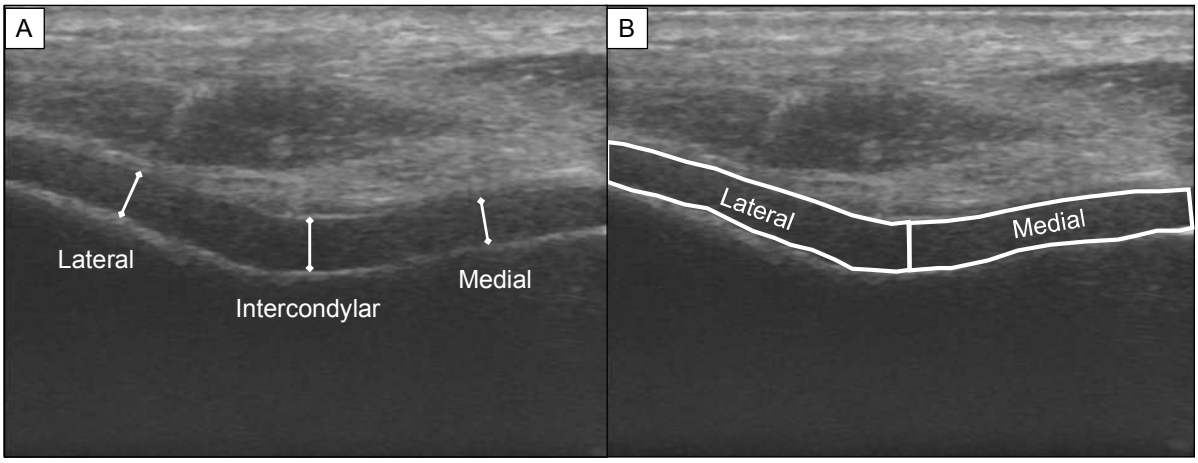
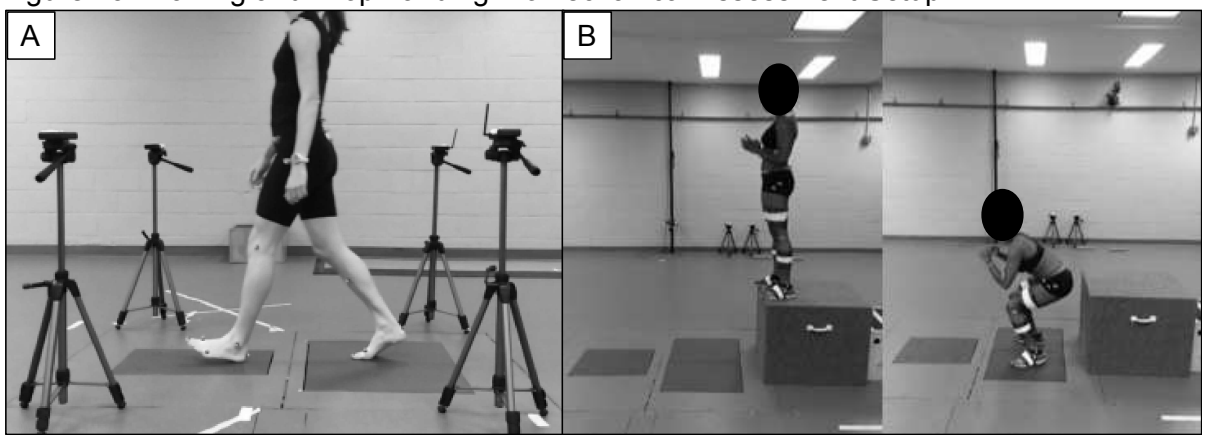


Figure 23. Walking and Drop-Landing Biomechanical Assessment Setup.



CHAPTER 6 - MANUSCRIPT 2

Acute Response of Cartilage Oligomeric Matrix Protein Concentration and its Association With Lower Extremity Loading during Walking and Drop-Landing

OVERVIEW

Context: An in depth understanding of the healthy physiologic cartilage response to activities of daily living and dynamic tasks is needed to better understand the complex relationship between cartilage health and loading that occurs during different movement tasks. Assessing serum cartilage oligomeric matrix protein (COMP) allows for the quantification of dynamic physiological processes related to cartilage metabolism. However, little is known regarding how biomechanical variables during different physical activities influence the acute COMP response following activity. **Objective:** To compare the acute serum COMP response between walking, drop-landing, and control conditions in healthy individuals. Secondarily, we sought to determine the association between the COMP response and lower extremity loading biomechanics during the walking and drop-landing conditions. **Design:** Repeated measures crossover study. **Setting:** Research laboratory. **Patients or Other Populations:** 40 healthy individuals without previous lower extremity injury. **Interventions:** A blood draw was performed in healthy individuals before and after a walking, drop-landing, and control condition to determine the acute serum COMP response following each condition. Additionally, we assessed walking and drop-landing biomechanics at the beginning of each of respective condition to evaluate relationships between lower extremity loading measures and the acute

COMP response. We utilized a repeated measures design in which each participant completed all conditions during independent data collection sessions separated by at least one week. **Main Outcome Measures:** Cartilage metabolism was quantified with the percent change of serum COMP from pre to post test in each condition. Peak magnitude and loading rate of the vertical ground reaction force, and internal knee extension, valgus, and varus moments were assessed during walking and drop-landing. Acute COMP response was compared between conditions with a one-way, repeated measures ANOVA. Associations between acute COMP response and lower extremity loading variables were assessed with Pearson product moment correlations or Spearman rank order correlations. **Results:** Healthy individuals presented with an increased COMP response to walking and drop-landing when compared to a control condition. Additionally, there was no difference in COMP response between the walking and drop-landing conditions. While greater internal knee valgus moment loading rate was associated with a lesser COMP response following walking, the remainder of our lower extremity loading biomechanics were not associated with COMP response for either loading condition. **Conclusions:** COMP increases following walking and drop-landing in health individuals, but these changes are not associated with lower extremity loading measures.

INTRODUCTION

Knee osteoarthritis (OA) is the leading musculoskeletal cause of total years lived with disability.[168] Knee OA is a disease that affects the entire joint, but declines in articular cartilage health are a hallmark sign of disease development.[2] Alterations in

cartilage loading are hypothesized to result in the initial breakdown of cartilage that leads to the development of OA.[6] A better understanding of the healthy physiologic cartilage response to activities of daily living (i.e. walking) and dynamic tasks (i.e. drop-landing) is needed to understand the complex relationship between cartilage health and loading that occurs during different movement tasks. Assessing various biochemical markers in serum allows for the quantification of dynamic physiological processes related to cartilage metabolism.[21]

Cartilage oligomeric matrix protein (COMP) is an essential structural and functional component of cartilage as it is involved in the organization of the cartilage extracellular matrix.[89] Previous reports have established the prognostic capability of COMP,[169] as elevated concentrations are observed in individuals with established OA[169] and following anterior cruciate ligament reconstruction (ACLR)[170] compared to healthy individuals. Additionally, COMP is described as mechano-sensitive[38] because it plays a role in the transduction of mechanical forces within the cartilage. Evidence of this mechano-sensitivity of COMP has been demonstrated as acute changes in COMP concentration in healthy individuals in response to common physical activity conditions (i.e. walking, running, drop-landing).[123-125,160] However, there is a dearth of information regarding how specific biomechanical variables during different physical activities are differentially associated with the acute COMP response following activity.

Knee cartilage is conditioned to the specific loading patterns that occur during gait.[116] The vertical ground reaction force (vGRF) represents a measure of general lower extremity loading that is associated with resting concentrations of a collagen metabolism biomarker in individuals at risk for OA development (i.e. ACLR).[138]

Additionally, the internal knee valgus moment, which is theorized to provide an estimate of medial knee compartment loading, is related to resting levels of enzymes responsible for cartilage breakdown in individuals following ACLR.[139] These studies,[138,139] however, do not provide evidence of how lower extremity biomechanics may be associated with acute biomarker responses. A preliminary study[160] in healthy individuals determined that an acute COMP response to walking at various speeds was associated with a combination of gait variables that included moments and angles at the hip, knee, and ankle. Due to the viscoelastic properties of cartilage,[29] cartilage is vulnerable to varying rates of loading as well as the overall peak magnitude of loading. Earlier animal studies,[32,112] as well as a recent gait study,[118] indicate that greater cartilage degradation is related to greater loading rates applied to the cartilage. Therefore, determining how the magnitude and rate of lower extremity loading from multiple planes of motion are associated with an acute biochemical cartilage response will be important in understanding the impact of activity on cartilage metabolism.

Therefore, the primary purpose of this study was to compare the acute serum COMP response between a walking, drop-landing, and control condition in healthy individuals. Secondarily, we sought to determine the association between the COMP response and lower extremity loading measures during the walking and drop-landing conditions. We hypothesized that we would see a greater acute COMP response following the walking and drop-landing conditions compared to the control condition, as well as a greater COMP response in the drop-landing compared to walking. Additionally, we hypothesized that biomechanics indicative of greater lower extremity loading would be associated with a greater COMP response following walking and drop-landing.

METHODS

Design

Ante-cubital blood draws were performed in healthy individuals before and after walking, drop-landing, and control conditions to determine the acute cartilage metabolic response (i.e. COMP change) to each condition (Figure 24). Additionally, we assessed walking and drop-landing biomechanics at the beginning of each respective condition to determine how lower extremity loading measures are related to the acute metabolic cartilage response. We utilized a repeated measures design in which each participant completed each condition during three independent data collection sessions separated by at least one week (11.7±9.0 days between sessions) at the same time of day (0.29±0.75 hours difference in time of day) to control for diurnal variation in serum COMP.[134] The order of the conditions was counterbalanced. Participants were instructed to limit their physical activity on the days that data collection occurred.

On the day of each data collection session, we began by collecting a urine sample to test urine specific gravity via refractometry to confirm that each participant was not dehydrated (i.e. urine specific gravity < 1.025) prior to testing.[140] Following hydration testing, participants were seated on a padded plinth with their back against a wall in a long-sit position with their knees in full extension[133] for one hour to unload the femoral articular cartilage and minimize the effect any preceding activity on the cartilage. Next, participants were positioned supine for the baseline blood sample collection. The participants were then immediately transferred across the laboratory with a wheel chair to begin setup for the biomechanical assessment during the walking/drop-

landing condition or remained on the padded plinth for the control condition. Next, the participants completed the activity condition. The participants wore the same pair of their own personal athletic footwear for all three sessions. Immediately following cessation of the activity condition, the participants were transferred back to the padded plinth and posttest blood sample collection were obtained within five minutes following the end of the condition.

Participants

We recruited a convenient sample of healthy individuals between the ages of 18 and 35 who self-reported participating in at least 30 minutes of physical activity at least three times per week. Additionally, we excluded individuals with a history of ligamentous or cartilage injury to the knee or hip, cartilage injury to the ankle, congenital or degenerative joint condition, orthopedic implant, lower extremity fracture, or upper extremity fracture. Additionally, those with current joint pain (quantified as greater than 2 on a 10cm visual analog scale) were excluded from participation. This study was part of a larger investigation that was powered to see a change in ultrasonography assessed cartilage thickness in healthy individuals. We conducted an *a priori* power analysis using data from medial femoral compartment thickness changes following 30 minutes of walking (pre avg= 2.23mm, post avg=2.09mm, pooled SD=0.42mm, effect size: d=0.33) published in a previous study.[132] We estimated that we would need 33 participants to determine statistical differences, with 80% power and an α level of 0.05, if the smallest effect we found in the current study across the three loading conditions and five total time points was similar to previously published research (d=0.33).[133] As larger effects

are observed in COMP change following various physical activities,[123,124] we decided to power the study on changes in cartilage thickness. Due to the time commitment of three separate three-hour data collection sessions, we over-sampled by 30% to ensure that we would achieve adequate statistical power in our final analyses if 30% of the initial sample were to drop-out of the study.

Screening Session

Prior to testing, participants were required to come to the laboratory for an initial session to determine their habitual walking speed and determine their corresponding comfortable step frequency, which was used to standardize the amount of time used for each of the activity conditions. Habitual over-ground walking speed was initially determined in our motion capture laboratory utilizing two sets of infrared timing gates (TF100, TracTronix, Lenexa, KS, USA). Participants were instructed to walk at a self-selected speed described as “comfortably walking on the sidewalk” through the 6-meter capture area.[137-139] After completing five familiarization trials, we recorded the time of the next five walking trials to determine their average habitual walking speed. Next, the speed on the treadmill (4Front, WOODWAY, Waukesha, WI, USA) was increased to the habitual walking speed of each participant and 60 seconds of walking was continued for the purpose of treadmill familiarization. After treadmill familiarization, study personnel manually counted the steps of each participant for one minute in order to determine the time each participant would need to reach 5000 steps (46.23±4.26 minutes).[122] This calculated time for each participant was used for all three activity conditions.

Quantifying Cartilage Metabolism

Blood Sample Collection

For both the baseline and posttest time points, the participants were positioned supine on the padded plinth, and 5mL of blood were collected from the antecubital vein in a serum separator tube vacutainer. Blood samples were placed on ice until centrifuged at 4°C for 15minutes at 4000rpm. Serum was pipetted equally into two cryovials and stored in an -80°C freezer until batch analysis after all participants were collected.

Analysis of Serum Cartilage Oligomeric Matrix Protein

Serum was assessed for cartilage oligomeric matrix protein (COMP) using commercially available enzyme-linked immunosorbent assays (Human COMP PicoKine ELISA; Boster Biological Technology; Pleasanton, CA, USA). Blood samples were analyzed in triplicate. The COMP assay detection sensitivity was <10pg/mL, and the intra-assay variability was 2.35%. Samples for a single individual were analyzed on a single plate to in order to control for inter-assay variation within participants. For data analysis, a percent change score was created to determine the COMP response from baseline to posttest time point following each condition (Equation 1).

Equation 1: COMP Response (%Δ) = $(\frac{\mathrm{COMP_{post}} - \mathrm{COMP_{pre}}}{\mathrm{COMP_{pre}}})*100$

Biomechanical Assessment of Lower Extremity Loading

Participant Preparation for Biomechanical Assessment

A modified retroreflective marker cluster/bony landmark setup was used for data collection. Marker clusters were secured bilaterally at the middle 1/3rd of the anterior lateral thigh, middle 1/3rd of the anterior lateral shank, middle of the dorsum of the foot, and over the sacrum. Additional bony landmark markers will be affixed with double sided tape at the L4/L5, manubrium, and bilaterally over the anterior superior iliac spine, greater trochanter, medial/lateral femoral epicondyle, medial/lateral malleoli, and acromion processes. Marker positions were collected using a 10-camera three-dimensional motion capture system with a sampling frequency of 120Hz with Vicon Nexus v1.4.1 motion capture software (Vicon Motion Systems, Centennial, CO) and lowpass filtered at 10Hz.[139] The cameras were interfaced with three total Bertec force plates (40cm x 60cm, FP406010, Bertec Corporation, Columbus, OH) collecting at 1200Hz and lowpass filtered at 75Hz.[139] Two of the force plates were positioned side by side and used to collect drop-landing data (Figure 25a), while two of the force plates were staggered to allow for bilateral limb collection during a single walking trial (Figure 25b).[139]

Walking Biomechanical Assessment

The participants walked shod through the capture area at their habitual walking speed (Figure 25a). Five practice trials were performed to familiarize the participants with the walking task. Five test trials were recorded in which: 1) both limbs individually landed on a single force plate, 2) maintained forward eye contact and were not “aiming”

for the force plates, 3) and maintained a consistent gait speed (±5% of the habitual walking speed calculated in the screening session).[137,138] Immediately following the collection of the last test trial, the participants were returned to the wheelchair and were transferred across the laboratory to begin the walking condition.

Drop-Landing Biomechanical Assessment

A 62cm platform was positioned behind the side-by-side force plates to allow for simultaneous collection of both limbs during a single drop-landing trial (Figure 25b). A separate step was positioned behind the 62cm platform and was utilized by the participants to ascend onto the platform. For each drop-landing trial, the participants ascended onto the platform and were instructed to drop down from the platform and perform a comfortable landing with each limb on a separate force plate. No specific instructions were provided to the participants on how to perform the double-legged landing. After each drop-landing, participants would walk around the box and ascend up the step in order to prepare for the next drop-landing trial. Drop-landings trials 5 through 10 were recorded and analyzed for the biomechanical assessment.

Analysis of Lower Extremity Loading

Individual trials from the walking and drop-landing biomechanical assessments were labeled to identify all of the retro-reflective markers within the Vicon Nexus motion capture software (version 1.8.5, Vicon Motion Systems, Oxford, UK). Once labeled, the marker trajectory data, synchronized with the ground reaction force, was exported from Vicon Nexus and further processed using The Motion Monitor software (version 8.0,

Innovative Sports Training, Chicago, IL, USA). A custom LabVIEW program (National Instruments Corp., San Antonio, TX) was used to measure our biomechanics variables during the early loading phase of walking and drop-landing. For the walking trials, the loading phase was defined as the first 50% of stance phase; defined as the interval from heel strike (vGRF > 20N) to toe-off (vGRF < 20N). The loading phase of drop-landing was defined as the first 100ms following ground contact (vGRF > 20N). Peak vGRF was determined during the loading phase of the walking and drop-landing conditions. Peak internal knee extension (EXT) moment, peak internal knee valgus (VAL) moment, and internal knee varus (VAR) moment moments during the loading phase were calculated using inverse dynamics as previously performed in our laboratory.[139] Greater EXT moments were depicted as negative values, while more positive VAL and VAR moments are indicative of greater moment.[160] Instantaneous loading rate of the vGRF (vGRF-LR), EXT, VAL, and VAR moments were calculated as the peak of first derivative of the force-time and moment-time curves.[139] Peak vGRF (xBW) and vGRF-LR (xBW/s) were normalized to participants' body weight.[138] Peak moments (xBW*Ht) and moment loading rates (xBW*Ht/s) were normalized to the product of participants' height and weight.[139]

Walking, Drop-Landing, and Control Conditions

Walking Condition

The participants were positioned on the treadmill and the speed was increased to the habitual walking speed determined during the screening session. This speed was

maintained for the time calculated during the screening session to reach 5,000 steps (46.23±4.31 minutes).

Drop-Landing Condition

The drop-landing biomechanical assessment occurred concurrently with the drop-landings used for the drop-landing condition. For the drop-landing condition, the participants continued performing drop-landings until they completed 120 total drop-landings. We selected the amount of drop-landings from the 62cm platform in order to match the high magnitude loading condition utilized in a previous study utilizing a similar drop-landing protocol.[123] The 120 drop-landing trials were evenly distributed over the same period of time utilized in the other conditions (i.e. 46.23±4.31 minutes).

Control Condition

During the control condition, participants remained on the treatment table following the baseline blood sample collection in a long-sit position for the same period of time utilized in the other conditions (i.e. 46.23±4.31 minutes).

Statistical Analysis

Comparison of Cartilage Oligomeric Matrix Protein Response between Conditions

A one-way repeated measures analysis of variance (RM-ANOVA) was used to determine if the baseline COMP concentration was similar between the walking, drop-landing, and control conditions. A separate one-way RM-ANOVA was used to compare acute COMP response between each condition. Outliers were defined as > two

standard deviations away from the mean of COMP response in any of the conditions. After outlier removal, a Shapiro-Wilk test was used to confirm normal distribution for COMP response. If there were significant differences in COMP response, we utilized paired samples t-tests and a Bonferroni correction ($p = 0.05/3 = 0.017$) to determine specific differences in COMP response between conditions.

Association between Cartilage Oligomeric Matrix Protein Response and Lower Extremity Loading during Walking and Drop-Landing Conditions

Separate Pearson product moment correlations were used to determine the association between COMP response and each measure of lower extremity loading during the walking and drop-landing condition. For the correlational analysis between each COMP response and lower extremity loading variable, outliers > two standard deviations away from the mean for either measure were removed for that individual analysis. Following outlier removal, if the data were still found to be non-normal with the Shapiro-Wilk test, a Spearman Rank-Order correlation was used to determine the association between COMP response and each lower extremity loading measure. Associations were classified as negligible (0.0 – 0.30), low (0.31 – 0.50), moderate (0.51 – 0.70), high (0.71 – 0.90), and very high (0.90 – 1.00).[148] Additionally, we included the 95% confidence intervals around each Pearson r and Spearman ρ to ensure that the intervals of each association did not cross zero.[149]

Post-Hoc: Comparison of Increased and Decreased COMP Responders following Walking and Drop-Landing

During our initial analysis comparing COMP response between conditions, we identified a heterogeneous COMP response to both the walking and drop-landing conditions, as some participants demonstrated an increased (i.e. posttest increased above baseline) while other displayed a decreased (i.e. posttest decreased below baseline) COMP response.

Our first *post hoc* test sought to determine if participants demonstrated a similar COMP response between the walking and drop-landing sessions. We created a binary COMP response outcome measure to indicate whether a participant's post-activity COMP concentration increased or decreased compared to the baseline COMP concentration for walking and drop-landing. We used a chi square analysis to determine if the frequency of being an increased COMP responder in the walking condition was similar to the frequency of being an increased COMP responder in the drop-landing condition. This analysis was used to determine if being an increased COMP responder (i.e. post COMP > baseline COMP) in the walking condition is associated with being an increased COMP responder in the drop-landing condition.

With our second *post hoc* test, we used a Pearson product moment correlation to determine whether the magnitude of COMP response following walking was associated with the magnitude of COMP response following drop-landing.

Lastly, we utilized separate independent t-tests to determine if lower extremity loading measures were different between the increased and decreased COMP responders in both the walking and drop-landing conditions.

All statistical analyses were performed using SPSS (version 21.0; IBM Corporation) with an *a priori* α level of $P < 0.05$.

RESULTS

Participants

Forty total participants were included in this study (Table 5); however, due to participant dropout between data collection sessions, not every participant completed each data collection session (walking n = 39, drop-landing n = 39, and control n = 40). The dropout in the walking condition was due to an unrelated injury between their 2nd and 3rd session, and the dropout in the drop-landing condition was due to the participant unwilling to complete the drop-landing protocol. Thirty-eight participants completed all conditions, and after outlier removal (outliers: walking n = 2; control n = 3), thirty-three participants were included in the ANOVA analysis. The correlational analyses included the maximum amount of participants for the walking and drop-landing condition and each lower extremity loading measure analyzed.

Comparison of COMP Response between Walking, Drop-Landing, and Control Conditions

Baseline COMP concentration was not different between the walking, drop-landing, or control conditions ($F_{2,64}=1.71$, $p=0.189$, Table 6.). There was a significant difference in COMP response between the conditions ($F_{2,64}=14.58$, $p<0.001$). COMP response was greater in the walking ($t_{32}=-4.291$, $p<0.001$) and drop-landing ($t_{32}=4.331$,

p<0.001) conditions compared to the control condition. COMP response was not different between the walking and drop-landing conditions (t_{32}=-0.535, p=0.596).

Association between COMP Response and Lower Extremity Loading during the Walking and Drop-landing Conditions

Greater walking valgus moment loading rate was associated with a lesser COMP response (r=-0.48, p=0.005, n=33; Table 6). However, no other walking lower extremity loading measure was significantly associated with the COMP response following walking (r/ρ range = -0.24 – 0.30). There were no significant associations between drop-landing lower extremity loading measures and COMP response (r/ ρ range = -0.30 – 0.26).

Post-Hoc: Comparison of Increased and Decreased COMP Responders following Walking and Drop-Landing

For this analysis, we only excluded individuals with outliers in the walking or drop-landing COMP response (walking, n=2); thus, 36 individuals were included in the following analyses. 12 and 10 participants presented with a decreased COMP response, while 24 and 26 participants presented with an increased COMP response following the walking and drop-landing conditions, respectively (Figure 26).

Being an individual with increased COMP response in the walking condition does mean that you will be an individual with an increased COMP response in the drop-landing condition (χ_1 = 0.277, p = 0.599). However, the magnitude of COMP response following walking was moderately associated with the magnitude of COMP response

following drop-landing (r = 0.55, p = 0.001). There were no significant differences in our lower extremity loading measures between the increased COMP responders and decreased COMP responders.

DISCUSSION

Previous studies assessing the COMP response following various physical activities in healthy individuals have utilized small samples sizes (i.e. n≤10[124,125,171] and n≤20[121,123,160]). This study represents the largest sample size utilized to determine the healthy COMP response following walking and drop-landing. We observed significantly increased COMP responses following both the walking and drop-landing condition when compared to the control condition. However, there was no difference in COMP response between the walking and drop-landing conditions. While the majority of our associations between COMP response and lower extremity loading biomechanics were not significant, we did observe that lesser valgus moment rate was associated with a greater COMP response. Furthermore, when separating our participants into groups based on an increased or decreased COMP response to walking and drop-landing, there were no differences between groups for any of our lower extremity loading variables for either condition.

Due to the mechano-sensitivity of COMP,[38] it is theorized that assessing the acute serum COMP response following various activities may effectively increase the sensitivity of COMP to be used as an indicator of acute cartilage turnover in response to mechanical cartilage loading.[12] Our protocol of 5,000 steps at habitual walking speed resulted in an average +4.1% increase in COMP, which is of similar magnitude

observed in previous studies following walking in healthy individuals (COMP response = +5%[160], +5.6%[126]). Earlier studies have observed a dose-dependent relationship between the magnitude of load during activity and the magnitude of COMP response,[124,160] which is why we hypothesized that we would observe a larger magnitude COMP response following drop-landing compared to walking. However, the COMP response was similar between our walking (+4.1%) and drop-landing (+4.8%) conditions. Even though the magnitude and rate of loading for a single drop-landing was much greater than that of each step, the increased frequency in steps (i.e. 5,000) compared to drop-landings (i.e.120) may explain the lack of difference in the magnitude of COMP response. Previous studies investigating the COMP response following different physical activities observed similar findings with no difference in COMP response between running and drop-landing[123] or between running and cycling.[172] Additionally, it is possible that our young and physically active patient population was able to withstand the greater magnitude loading of the drop-landing condition without experiencing an increased COMP response. Future studies should aim to investigate if individuals at risk for declining cartilage health (i.e. ACLR) would experience differences in COMP responses between a low and high magnitude loading condition.

The majority of magnitude and rate of loading variables that we assessed during the early loading phase of walking and drop-landing were not significantly associated with an acute COMP response. Our only significant association indicates that lesser valgus moment rate (i.e. medial compartment loading rate) is associated with an increased COMP response in individuals following walking. Potentially, due to the viscoelastic nature of cartilage,[29] acute changes in cartilage metabolism may be more

responsive to slower rates of loading. For our study, we included joint moments that were specific to loading mechanics at the knee. However, including lower extremity loading variables from multiple joints may provide a better understanding of the serum COMP response following physical activity. Additionally, we did not include any kinematic variables in our analysis because our focus was to determine how the magnitude of loading would alter the COMP response. Yet, previous work has indicated that knee kinematics affect the location in which loading occurs on the cartilage,[167] which may influence the specific COMP response. A recent investigation predicted 61% of the variance in the serum COMP response following an ambulation protocol using multiple kinetics and kinematics from several lower extremity joints.[160] Since the serum COMP response provides a global measure of cartilage stress within the body, creating a more holistic view of the all of biomechanics occurring within the lower extremity may allow us to better determine how biomechanics are related to the acute cartilage response following different activities.

Our initial analysis comparing the COMP response between the walking and drop-landing conditions provided evidence of a heterogeneous COMP response in which some individuals responded to walking and drop-landing with either an increased or decreased COMP response. We determined that individuals with an increased COMP response in the walking condition were not the same individuals experiencing an increased COMP response in the drop-landing condition. However, the overall magnitude of COMP response was associated between the walking and drop-landing conditions. These results indicate that even though the overall magnitude of COMP response is moderately associated between the walking and drop-landing conditions,

the direction of the COMP response is not necessarily the same in healthy individuals responding to two conditions with differing magnitude and frequency of loading. These findings of heterogeneous COMP responses following physical activity have been observed in other studies,[124,126,173] but this is the first investigation that attempts to provide a biomechanical explanation for these heterogeneous COMP responses. However, there were no differences in our lower extremity loading biomechanics between the increased and decreased COMP responders in both the walking and drop-landing condition. Future work is needed to best determine why individuals display heterogeneous COMP responses to walking and drop-landing, and why specific individuals respond differently to tasks of varying magnitude and frequency of loading.

While this study provides information regarding the healthy COMP response to walking and drop-landing, and how this response is associated with lower extremity biomechanics, there are some limitations that should be addressed. We assessed the serum COMP response, which provides a global COMP response and we are not able to confirm that the alterations in COMP are specifically due to changes in cartilage metabolism of the knee joint tested. However, the majority of COMP is released from articular cartilage and we included healthy individuals without any joint pathologies that may increase the COMP response.[174] Lack of association between our COMP response and knee specific kinetics may be due to our assessment of the global COMP response, as the COMP response is likely due to biomechanics occurring at all lower extremity joints. Knee moments have demonstrated to predict joint contact force;[157] however, joint moments do not provide direct quantification of compression force that occurs at the joint surface. Future studies should determine how more specific measures of cartilage

contact forces are associated with the acute COMP response. Our biomarker investigation was limited to the COMP response because this is the most commonly assessed cartilage biomarker acutely following acute physical activity.[123,124,126,160] However, there are other biochemical markers related to cartilage metabolism, as well as metabolism of other joint tissues that may provide insight into other aspects of the acute knee joint response to physical activity.[21] Even though we observed statistically significant increases in the COMP response following the walking and drop-landing conditions, the magnitude of COMP response following our drop-landing condition was lower than previously reported.[123] The individuals included in our study were much younger than participants in previous studies,[125,126] and potentially the conditions used in this study were of insufficient intensity to elicit a greater COMP response in our cohort of young, active individuals. Further research is needed to determine the effect of age on the COMP response to various conditions.

In conclusion, healthy individuals present with an increased COMP response to walking and drop-landing when compared to a control condition. Additionally, there is no difference in COMP response between the walking and drop-landing conditions. While greater knee valgus moment loading rate was associated with a decreased COMP response following walking, the remainder of our lower extremity loading biomechanics were not associated with COMP response following walking and drop-landing. Lower extremity loading biomechanics were not different between groups that were separated based on an increased or decreased COMP response following walking and drop-landing.

Table 5. Demographics

	Mean	SD
n	40 (20 male, 20 female)	
Age (years)	21.60	3.15
Height (meters)	1.72	0.09
Mass (kilograms)	68.32	11.39
Tegner	5.87	1.24
Walking Biomechanics Descriptive Data		
Walking Speed (meters/sec)	1.24	0.18
Walking Distance (kilometers)	3.44	0.35
Peak vGRF (xBW)	1.13	0.08
vGRF Loading Rate (xBW/s)	20.50	3.34
Peak VAL Moment (xBW*Ht)	0.029	0.007
VAL Moment Loading Rate (xBW*Ht/s)	1.142	0.614
Peak VAR Moment (xBW*Ht)	0.0039	0.0026
VAR Moment Loading Rate (xBW*Ht/s)	0.490	0.126
Peak EXT Moment (xBW*Ht)	-0.033	0.016
EXT Moment Loading Rate (xBW*Ht/s)	-1.777	0.651
Drop-Landing Biomechanics Descriptive Data		
Peak vGRF (xBW)	2.75	0.55
vGRF Loading Rate (xBW/s)	178.22	41.55
Peak VAL Moment (xBW*Ht)	0.115	0.107
VAL Moment Loading Rate (xBW*Ht/s)	11.58	8.62
Peak VAR Moment (xBW*Ht)	0.021	0.017
VAR Moment Loading Rate (xBW*Ht/s)	9.52	6.78
Peak EXT Moment (xBW*Ht)	-0.230	0.63
EXT Moment Loading Rate (xBW*Ht/s)	-16.38	3.31

vGRF = vertical ground reaction force, VAL = valgus, VAR = varus, EXT = extension
BW = body weight, HT = height, s = seconds

Table 6. Baseline and COMP Response Following Activity Conditions

Condition	Baseline (ng/mL)		Post %Δ		Post Absolute (ng/mL)	
	mean	sd	mean	sd	mean	sd
Walking	145.72	32.80	+4.10*	8.57	150.36	28.43
Drop-landing	144.10	27.11	+4.78*	7.74	149.98	24.19
Control	139.71	29.41	-2.92	5.51	135.2	27.1

*significantly different than control ($p<0.05$), %Δ = percent change, sd = standard deviation, ng = nanograms, mL = milliliters

Table 7. Association between COMP Percent Change and Lower Extremity Loading during Physical Activity Conditions

		Walking								Drop-Landing							
		vGRF	vGRF LR	KEM	KEM LR	VAL	VAL LR	VAR	VAR LR	vGRF	vGRF LR	KEM	KEM LR	VAL	VAL LR	VAR	VAR LR
COMP Change	r/ρ	**0.06**	**-0.24**	**-0.15**	**0.30**	**-0.04**	**-0.48***	*0.03*	**-0.17**	**0.11**	**0.23**	*-0.30*	**0.01**	*0.23*	*0.26*	*0.24*	*0.23*
	p	**0.72**	**0.17**	**0.38**	**0.085**	**0.820**	**0.005**	*0.86*	**0.32**	**0.53**	**0.17**	*0.08*	**0.96**	*0.19*	*0.13*	*0.15*	*0.17*
	n	**37**	**36**	**36**	**34**	**35**	**33**	*34*	**35**	**37**	**37**	*37*	**33**	*35*	*37*	*38*	*37*

Bolded = Pearson r; *Italicized = Spearman Rho ρ*; * = statistically significant, vGRF = vertical ground reaction force, LR = loading rate; EXT = internal knee extension moment, VAL = internal knee valgus moment, VAR = internal knee varus moment, r/ρ = Pearson r or Spearman rho association, 95% CI = lower, upper 95% confidence interval of the association, p = p-value, n = sample size

Table 8. Differences in Lower Extremity Loading Between Increased and Decreased COMP Responders

Outcome Measure	Responder	Walking				Drop-Landing			
		mean	sd	t	p	mean	sd	t	p
Peak vGRF	Decreased	1.11	0.06	-0.636	0.529	2.92	0.89	0.038	0.97
	Increased	1.13	0.09			2.91	0.73		
vGRF Loading Rate	Decreased	20.36	3.33	-0.343	0.734	196.07	87.33	0.099	0.922
	Increased	20.81	3.92			193.48	63.50		
Peak EXT Moment	Decreased	-0.029	0.013	0.752	0.458	-0.21	0.06	1.339	0.19
	Increased	-0.034	0.017			-0.25	0.08		
EXT Moment Loading Rate	Decreased	-2.29	0.93	-1.748	0.09	-17.80	5.81	0.318	0.752
	Increased	-1.77	0.77			-18.56	6.30		
Peak VAL Moment	Decreased	0.029	0.008	-0.384	0.704	0.085	0.106	-1.347	0.187
	Increased	0.030	0.008			0.163	0.163		
VAL Moment Loading Rate	Decreased	1.44	0.46	1.385	0.175	8.69	8.12	-1.247	0.221
	Increased	1.12	0.69			14.15	12.16		
Peak VAR Moment	Decreased	0.0044	0.0027	0.22	0.827	0.020	0.018	-0.236	0.815
	Increased	0.0042	0.0032			0.021	0.018		
VAR Moment Loading Rate	Decreased	0.53	0.14	0.642	0.526	7.43	6.00	-1.208	0.236
	Increased	0.50	0.15			10.92	7.87		

vGRF = vertical ground reaction force, EXT = extension, VAL = valgus, VAR = varus, sd = standard deviation, t = t-statistic, p = p-value, COMP = cartilage oligomeric matrix protein

Figure 24. Study design. Blood sample collection pre and post each activity condition.

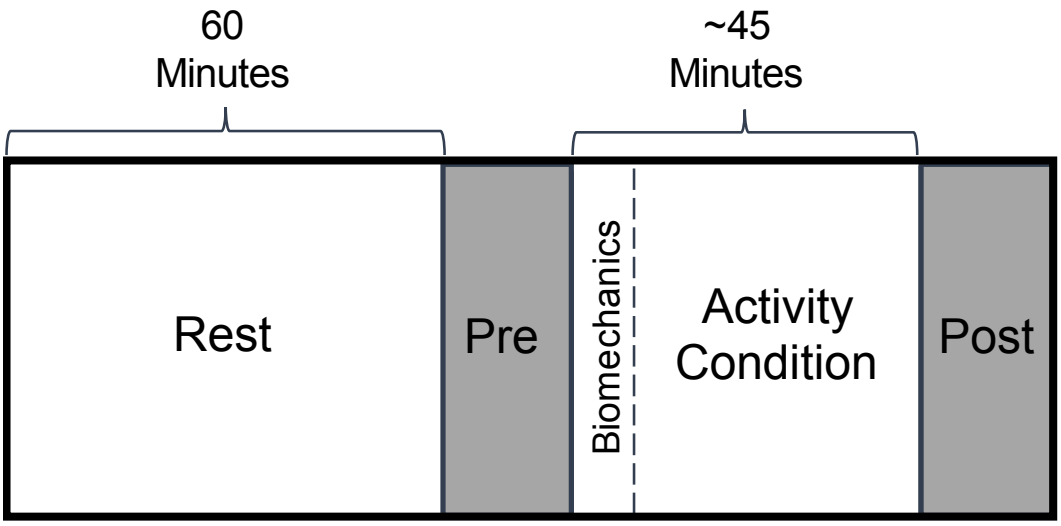


Figure 25. Walking and Drop-Landing Biomechanical Assessment Setup.

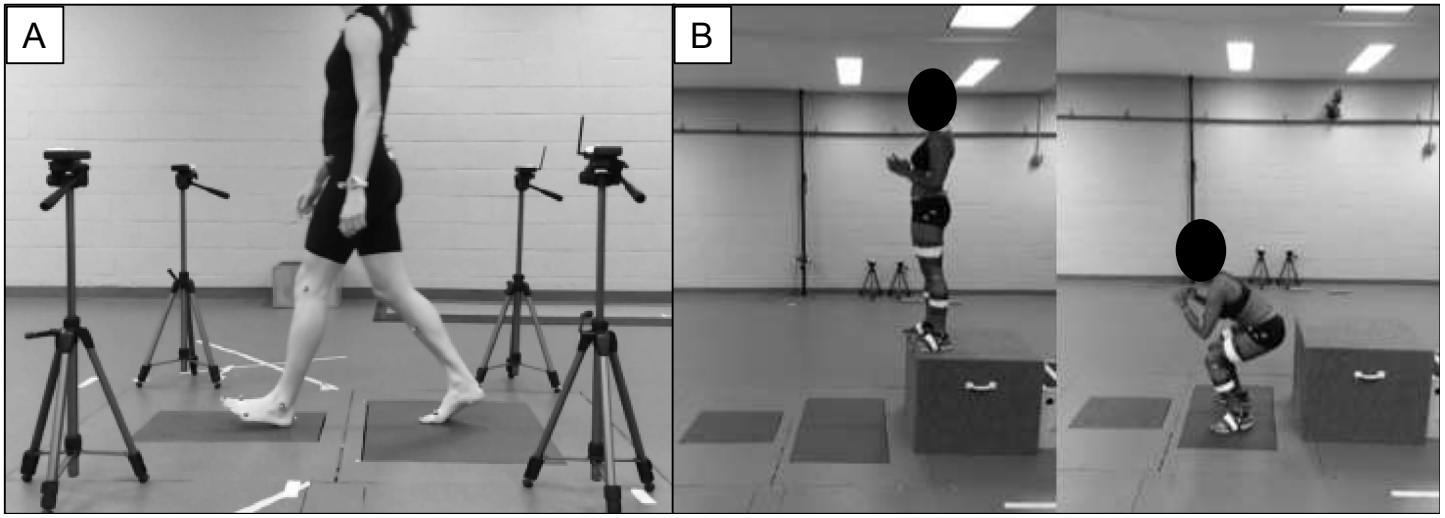
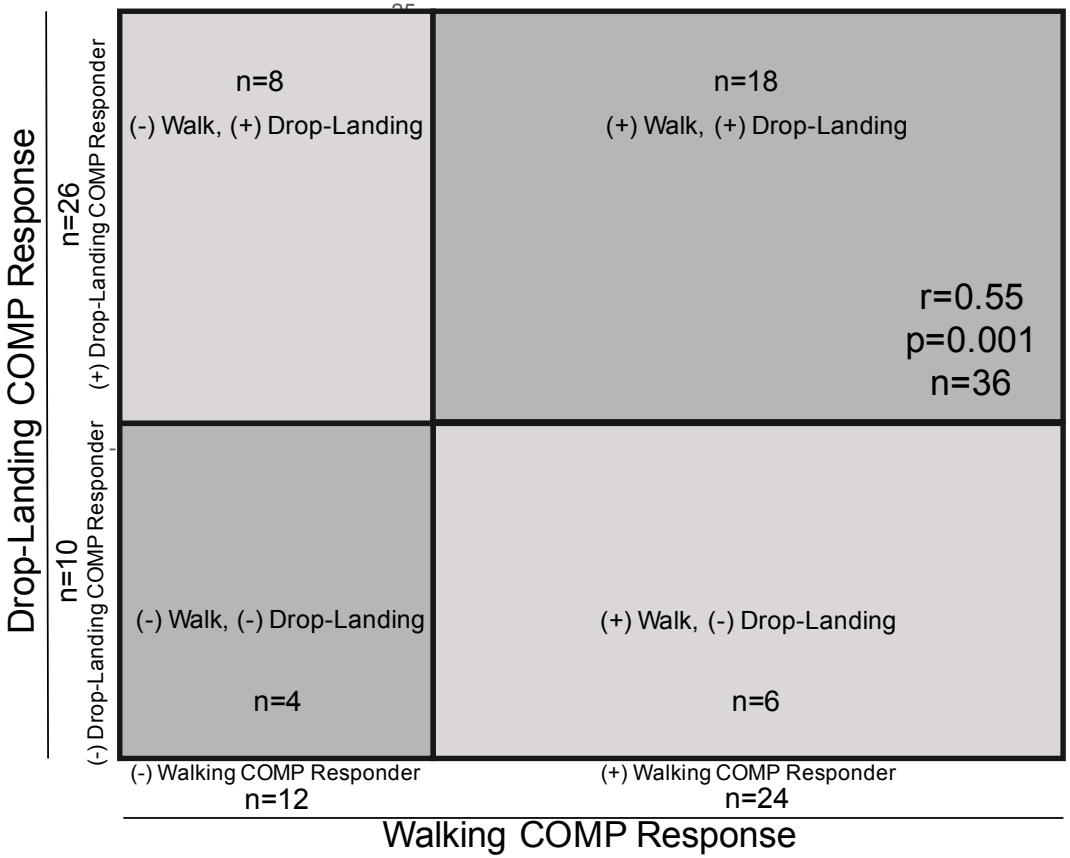


Figure 26. Associations between Walking COMP Response and Drop-Landing COMP Response. This figure combines a scatter plot of the association between the magnitude of the walking COMP response and the drop-landing COMP response with a 2x2 contingency table indicating the amount of increased and decreased COMP responders for the walking and drop-landing conditions.



COMP = cartilage oligomeric matrix protein, r = Pearson association, p = p-value, n = sample size

CHAPTER 7 - MANUSCRIPT 3

Associations between Femoral Cartilage Deformation and Serum Cartilage Oligomeric Matrix Protein Response Following Walking and Drop-Landing in Healthy Individuals

OVERVIEW

Context: A systems-based approach to assess cartilage health uses an acute bout of physical activity to create acute changes in cartilage structure and metabolism. Utilizing this systems-based approach is said to elevate the sensitivity of both the cartilage structure and metabolic outcome measures as a better prognostic indicator of cartilage health, but little is known how measures of cartilage structure and metabolism are associated. **Objective:** To determine the association between baseline US measures of cartilage health and baseline serum COMP in healthy individuals. The second purpose of this study was to determine the association between the change in US measures of cartilage structure and the serum COMP response following walking and drop-landing. **Design:** Repeated measures crossover study. **Setting:** Research laboratory. **Patients or Other Populations:** 40 healthy individuals with no previous history of lower extremity injury. **Interventions:** A femoral cartilage US assessment and an ante-cubital blood draw were performed in healthy individuals before and after walking and drop-landing conditions to determine the acute structural and metabolic cartilage response to each

condition. We utilized a repeated measures design in which each participant completed both conditions during independent data collection sessions separated by at least one week. **Main Outcome Measures:** Femoral articular cartilage was assessed with US to determine the thickness, area, and echo-intensity. Cartilage metabolism was quantified with serum COMP. Percent change scores from pre to post activity were calculated for each outcome measure. Associations between baseline and acute changes in cartilage metabolism and structure measures were assessed with Pearson product moment correlations. **Results:** Within the entire cohort, baseline US measures of femoral cartilage were not associated with baseline COMP concentration. Specifically in females, greater baseline cartilage thickness were associated with lower baseline COMP, but in males, lesser baseline cartilage thickness were associated with lower baseline COMP. In the entire cohort, greater medial femoral compartment deformation was associated with lesser COMP response following walking. Yet there were no other significant associations between US measures and COMP response in walking or drop-landing. **Conclusions:** While the majority of cartilage structure and metabolism markers were not associated within the entire cohort, sex may influence the association between these measures.

INTRODUCTION

Knee osteoarthritis (OA) leads to significant impairments in mobility and decreased quality of life,[175] and is the sixth leading cause of years living with disability worldwide.[3] A decline in cartilage health is a hallmark sign of OA; therefore, being able to effectively monitor subtle alterations in cartilage health is needed to successfully

detect early OA development.[46] The lack of preventative strategies is in part due to the maintenance of cartilage health being influenced by complex processes that affect the three primary components:[12] joint mechanics or loading, metabolism, and structure. Previous, well-designed research has evaluated alterations of each of these components in isolation.[6-8] However, utilizing a novel multi-faceted approach that seeks to understand the interaction between this broad range of individual cartilage components may provide new insight to *in vivo* cartilage function.[11] A systems-based approach[11,12] has been established to determine the interaction between components of cartilage health by assessing cartilage structure and metabolism prior to and following an acute bout of physical activity (i.e. joint loading). Utilizing this systems-based approach is said to elevate the sensitivity of both the cartilage structure[159] and metabolic[31] outcome measures as a better prognostic indicator of cartilage health, but little is known about the association between cartilage structure and metabolism..

Assessing resting cartilage volume and thickness utilizing magnetic resonance imaging[176] (MRI) or ultrasonography[17] (US) provides a reliable estimate of cartilage structure. A decrease in cartilage thickness is a hallmark sign of OA, but this may not occur until later stages of disease development.[20] Earlier stages of OA development are characterized by alterations in the cartilage composition,[20] without overt declines in cartilage thickness that alter the cartilage's ability to distribute loads and minimize joint stress during physical activity.[177] Previous investigators have quantified acute cartilage deformation following walking[133,155] and drop-landing[123] as a surrogate measure of cartilage composition, because cartilage deformation is theorized to be governed by composition of the tissue.[153] Thus, assessing cartilage deformation to determine the

functional response of cartilage following activity has been speculated as a more sensitive measure of cartilage health when compared to traditional cartilage structure measures.[120]

The cartilage thickness and cartilage composition alterations associated with the development of OA are often considered a result of a chronic slow-progressing continuum that begins with subtle alterations in cartilage metabolism.[21] Cartilage oligomeric matrix protein (COMP) is an essential structural and functional component of cartilage that is often assessed in resting serum samples as an indicator of cartilage metabolism.[89] Greater resting concentrations of serum COMP are interpreted as indicative of greater cartilage degradation in individuals with OA,[178] as well as in individuals at risk for OA development.[179] However, assessing the change in COMP concentration following activities of daily living (i.e. walking[125,160]) and more dynamic activities (i.e. running[121,123,124] and drop-landing[123]) have been attributed to normal cartilage turnover that occurs as a response to mechanical cartilage loading during activity rather than cartilage degradation. Previous reports have theorized that activities with greater magnitude of loading will result in a greater cartilage deformation, which will lead to greater acute COMP responses.[121,124,125] While this dose-dependent relationship in COMP response is primarily thought to be due to the overall magnitude of load on the cartilage, there is evidence that the frequency of loading may alter the relationship between COMP response and magnitude of cartilage deformation.[123] Therefore, determining the differential COMP response to walking and drop-landing may help to uncover the role of magnitude and frequency of loading on the metabolic cartilage response to loading.

Determining this association between cartilage structure and metabolism will be important to determine if the deformation that occurs during the mechanical loading associated with activity is related to the metabolic cartilage response. Uncovering this relationship between cartilage structure and metabolism will be important for understanding an abnormal association between cartilage structure and metabolism in individuals at risk for the development of OA. Therefore, the first purpose of this study was to determine the association between baseline US measures of cartilage health (i.e. thickness, area, and echo-intensity) and baseline serum COMP. The second purpose of this study was to determine the association between the change in US measures of cartilage health and the serum COMP response following walking and drop-landing in healthy individuals. We hypothesized that there would be a significant association between baseline US measures of cartilage health and baseline COMP concentrations, and that a greater change in US measures of cartilage health would be associated with a greater serum COMP response following walking and drop-landing.

METHODS

Design

In this study, a femoral cartilage US assessment and an ante-cubital blood draw were performed in healthy individuals before and after a walking and drop-landing condition to determine the acute structural and metabolic cartilage response to the each condition (Figure 27). We utilized a repeated measures design in which each participant completed both conditions during independent data collection sessions separated by at least one week (11.7±9.0 days between sessions) at the same time of day (0.28±0.74

hours difference in time of day) to control for diurnal variation in serum COMP[134] and femoral cartilage thickness.[135,136] The order of the walking and drop-landing conditions was counterbalanced. Participants were instructed to limit their physical activity on the days that data collection occurred.

On the day of each data collection session, we began by collecting a urine sample to test urine specific gravity via refractometry to confirm that each participant was not dehydrated (i.e. urine specific gravity < 1.025) prior to testing.[140] Following hydration testing, participants were seated on a padded plinth with their back against a wall in a long-sit position with their knees in full extension[133] for one hour to unload the femoral articular cartilage, permit fluid rebound, and minimize effect of the preceding activity on the cartilage. Next, the US cartilage assessment occurred immediately prior to the blood sample collection at both time points in each session. The participants were then immediately transferred across the laboratory with a wheel chair to begin the activity condition. The participants wore the same pair of their own personal athletic footwear for both sessions. Immediately following cessation of the condition, the participants were transferred back to the padded plinth with a wheel chair to begin the posttest US assessment followed by the posttest blood sample collection. Due to the proximity of the walking and drop-landing conditions and the accessibility of US, posttest US images and blood sample collection were obtained within five minutes following each condition.[133]

Participants

We recruited a convenient sample of healthy individuals between the ages of 18 and 35 who self-reported participating in at least 30 minutes of physical activity at least three times per week. Additionally, we excluded individuals with a history of ligamentous or cartilage injury to the knee or hip, cartilage injury to the ankle, congenital or degenerative joint condition, orthopedic implant, lower extremity fracture, or upper extremity fracture. Additionally, those with current joint pain (quantified as greater than 2 on a 10cm visual analog scale) were excluded from participation. This study was part of a larger investigation that was powered to see a change in ultrasonography assessed cartilage thickness in healthy individuals. We conducted an *a priori* power analysis using data from medial femoral compartment thickness changes following 30 minutes of walking (pre avg= 2.23mm, post avg=2.09mm, pooled SD=0.42mm, effect size: *d*=0.33) published in a previous study.[132] We estimated that we would need 33 participants to determine statistical differences, with 80% power and an α level of 0.05, if the smallest effect we found in the current study across the three loading conditions and five total time points was similar to previously published research *(d*=0.33).[133] As larger effects are observed in COMP change following various activities,[123,124] we decided to power the study on changes in cartilage thickness. Due to the time commitment of three separate three-hour data collection sessions, we over-sampled by 30% to ensure that we would achieve adequate statistical power in our final analyses if 30% of the initial sample were to drop-out of the study.

Screening Session

Prior to testing, participants were required to come to the laboratory for an initial session to determine their habitual walking speed and their corresponding comfortable step frequency, which was used to standardize the amount of time used for the walking and drop-landing conditions. Habitual over-ground walking speed was initially determined in our motion capture laboratory utilizing two sets of infrared timing gates (TF100, TracTronix, Lenexa, KS, USA). Participants were instructed to walk at a self-selected speed described as “comfortably walking on the sidewalk” through the 6-meter capture area.[137-139] After completing five familiarization trials, we recorded the time of the next five walking trials to determine their average habitual walking speed. Next, the speed on the treadmill (4Front, WOODWAY, Waukesha, WI, USA) was increased to the habitual walking speed of each participant and 60 seconds of walking was continued for the purpose of treadmill familiarization. After treadmill familiarization, study personnel manually counted the steps of each participant for one minute in order to determine the time each participant would need to reach 5000 steps (46.23±4.26 minutes).[122] The calculated time for each participant was used for both the walking and drop-landing conditions.

Ultrasonographic Assessment of the Femoral Articular Cartilage

Ultrasonographic Image Acquisition

US images were obtained in the dominant limb, which was defined as the self-reported limb that the participant preferred to use for kicking a ball.[132] Participants were positioned with their back against a wall and the knee of the dominant limb was

positioned at 140° of flexion using a manual goniometer (Figure 28).[133] A tape measure was secured to the treatment table and used to record the distance between the wall and the posterior calcaneus in order to standardize positioning for each participant during the posttest and throughout both data collection sessions. A single investigator performed all femoral cartilage US imaging using a LOGIQe US system (General Electric Co., Fairfield, CT, USA) with a 12MHz linear probe. The probe was placed transversely in line with the medial and lateral femoral condyles above the superior edge of the patella (Figure 28) and rotated to maximize the reflection of the articular cartilage surface, as previously reported.[17,141,142] A transparency grid was placed over the US screen to aid in reproducibility of the US image.[133] Once the intercondylar notch was centered on the grid, the locations of the lateral and medial femoral condyles at the edges of the screen were recorded. This probe positioning was replicated during subsequent US assessments to ensure similar probe placement between assessments. Three images were recorded, with the US probe being removed and repositioned on the knee between each recorded image, at baseline and immediately after the walking and drop-landing condition.

Ultrasonographic Imaging Processing

A single unblended investigator manually segmented the US images using ImageJ software (National Institutes of Health, Bethesda, MD, USA). All three of the femoral cartilage US images from each time point were processed and averaged for the following outcome measures:

Cartilage Thickness

Femoral cartilage thickness was assessed at the midpoints of the medial femoral condyle, lateral femoral condyle, and intercondylar notch as the straight-line distance in millimeters (mm) between the cartilage-bone interface to the synovial space-cartilage interface (Figure 29a).[17,133,141,142] Strong intra-session reliability for the cartilage thickness assessment has previously been established within our laboratory ($ICC_{2,k}$ = 0.966).[133]

Cartilage Area and Echo-intensity

The femoral cartilage was then segmented by individually outlining the cartilage of the medial and lateral femoral condyles to obtain the size (i.e. cartilage area [mm^2]) and the gray-scale value (i.e. cartilage echo-intensity) of the cartilage (Figure 29b). The medial and lateral areas were separated based on the location of the intercondylar thickness measure. Echo-intensity evaluates the average gray scale brightness of each pixel segmented on a scale from 0 (i.e. black; more water content) to 255 (i.e. white; lesser water content). US echo-intensity (i.e. grey-scale brightness) has primarily been used as a measure of "muscle quality",[28,143] with the echo-intensity representing the relative water content of muscle. Since cartilage is approximately 60-80% fluid and acute cartilage deformation is in part due to fluid exudation,[29,144] we may be able to use US echo-intensity to monitor acute changes in cartilage water content that occur with loading during activity.

Additionally, a percent change score from baseline to posttest was calculated to determine the acute cartilage response for each US measure following both conditions (Equation 1). A greater negative percent change of thickness and area indicates greater cartilage deformation. A greater negative percent change in echo-intensity is theorized to indicate an increase in cartilage water content.

Equation 1: Percent change (%Δ) = $(\frac{\text{mean}_{\text{post}} - \text{mean}_{\text{pre}}}{\text{mean}_{\text{pre}}})$*100

Quantifying Cartilage Metabolism

Blood Sample Collection

For both the baseline and posttest time points, the participants were positioned supine on the padded plinth, and five milliliters of blood were collected from the antecubital vein in a serum separator tube vacutainer. Blood samples were placed on ice until centrifuged at 4°C for 15minutes at 4000rpm. Serum was pipetted equally into two cryovials and stored at -80°C until a batch analysis that occurred after all participants were collected.

Analysis of Serum Cartilage Oligomeric Matrix Protein

Serum was assessed for cartilage oligomeric matrix protein (COMP) using commercially available enzyme-linked immunosorbent assays (Human COMP PicoKine ELISA; Boster Biological Technology; Pleasanton, CA, USA). Blood samples were analyzed in triplicate. The COMP assay detection sensitivity was <10pg/mL, and the intra-assay variability was 2.35%. Serum samples for each participant were analyzed on

a single plate to control for differences caused by inter-assay variation. For data analysis, we utilized resting concentration as well as calculating a percent change score to determine the COMP response from baseline to posttest for each condition (Equation 1).

Walking and Drop-Landing Activity Conditions

Walking Condition

The current study was part of a larger project that evaluated walking biomechanics. Therefore, following the baseline blood draw, participants were setup for the biomechanical assessment and performed 5 walking trials over a standard 6m walkway and were then transferred with a wheelchair across the laboratory to minimize any further non-standardized joint loading prior to beginning the treadmill walking condition. Participants were then positioned on the treadmill and increased their speed to their predetermined habitual walking speed from the screening session, and maintained this pace for the time calculated during the screening session to reach 5,000 steps (46.23±4.26 minutes).

Drop-Landing Condition

For the drop-landing condition, the participants ascended a set of two steps to position themselves on a 62cm platform. Participants were instructed to drop down from the platform and perform a comfortable double-legged landing (Figure 30). After each landing they would walk around the platform and back up the step in order to prepare for the next drop-landing trial. No specific instructions were provided to the participants

on how to perform the double-legged landing. Participants performed 120 drop-landing trials that were evenly distributed over the same period of time utilized in the walking condition (46.23±4.26 minutes).

Statistical Analysis

Association between Ultrasonographic Measures of Femoral Cartilage and Cartilage Oligomeric Matrix Protein

Separate Pearson product moment correlations were used to determine the association between baseline US measures and baseline COMP concentration, as well as determining the association between the percent change in each US measure and the COMP response following the walking and drop-landing conditions. For each correlational analysis between COMP response and each US measure, outliers > two standard deviations away from the mean for either measure were removed for that individual analysis. Following outlier removal, if the data were still found to be non-normal with the Shapiro-Wilk test, a Spearman Rank-Order correlation was used to determine the association between COMP and each US outcome measure. Associations were classified as negligible (0.0 – 0.30), low (0.31 – 0.50), moderate (0.51 – 0.70), high (0.71 – 0.90), and very high (0.90 – 1.00).[148] Additionally, we included the 95% confidence intervals around each Pearson r and Spearman ρ to ensure that statistically significant associations did not cross zero.[149]

Post-hoc Test: Sex-Specific Associations between Ultrasonographic Measures of Femoral Cartilage and Cartilage Oligomeric Matrix Protein

As previous reports have indicated sex differences in cartilage volume[150] and resting concentrations of serum COMP,[151] we performed separate *post hoc* associations individually for males and females to determine the association between cartilage structure and metabolism for each sex individually. All statistical analyses were performed using SPSS (version 21.0; IBM Corporation) with an *a priori* α level of $P < 0.05$.

RESULTS

Participants

Forty total participants were included in this study (Table 9); however, due to participant dropout between data collection sessions, not every participant completed each data collection session (walking n=39; drop-landing=39). The drop-out in the walking condition was due to an unrelated injury between the 2nd and 3rd session, and the drop-out in the drop-landing condition was due to the participant unwilling to complete the drop-landing protocol. The correlational analyses included the maximum amount of participants for the COMP response and US measure being analyzed.

Association between Ultrasonographic Measures of Femoral Cartilage and Cartilage Oligomeric Matrix Protein

Descriptive statistics for the baseline, posttest, and percent change of COMP and each US measure following walking and drop-landing can be found in Table 10.

Within the entire cohort, there were no significant associations between baseline COMP and any US measure (r range= -0.23 – 0.22; Table 11).

For the walking condition, greater medial femoral compartment area deformation was associated with a decreased COMP response (r=0.36, p=0.036; Table 12). However, no other US measure percent change was significantly associated with the acute COMP response following walking (Table 12). For the drop-landing condition, all associations between each US measure percent change and the acute COMP response were negligible and non-significant (r/ρ range = -0.15 – 0.22).

Post-hoc Test: Sex-Specific Associations between Ultrasonographic Measures of Femoral Cartilage and Cartilage Oligomeric Matrix Protein

In males, greater baseline COMP concentration was associated with lesser medial cartilage echo-intensity (i.e. more water content; r = -0.52, p = 0.023; Table 11). In females, greater baseline COMP was associated with lesser lateral cartilage area (r = -0.57, p = 0.014). While not all associations are statistically significant, the directions for all associations between cartilage structure measures and COMP for the males were positive (r range = 0.05 – 0.39), while all these same associations for females were negative (r range = -0.39 - -0.57). Additionally, the directions for all associations between cartilage echo-intensity measures and COMP for the males were negative (r range = -0.21 - -0.52), while all these same associations for the females were positive (r range = 0.29 – 0.36).

In males, increased COMP response was significantly associated with lesser medial cartilage area deformation ($r = 0.48$, $p = 0.036$; Table 12). In females, increased COMP response in females was significantly associated with lesser medial cartilage thickness deformation ($r = 0.46$, $p = 0.46$). However, no other significant associations were observed for females and no significant associations were reported in males.

DISCUSSION

This is the first study to examine associations between baseline cartilage US outcomes and baseline cartilage metabolism in healthy individuals, as well as determining the individual associations between the percent change in US measures and COMP response following walking and drop-landing. Within the entire cohort, we did not observe any significant associations between baseline COMP concentration and any of our US measures. However, when separating our cohort by sex, we observed that the directions of the associations between baseline COMP and each US measure were different for males compared to females. For the entire cohort, greater medial femoral cartilage deformation during walking was associated with a decreased COMP response; however, no other US measure was associated with COMP response in either walking or drop-landing. Separating the cohort by sex did not change the majority of the associations between the percent change in US measure and COMP response. While the overall analyses did not generally support associations between US measures and cartilage metabolism, the *post hoc* analyses potentially indicate that sex may influence the association between cartilage structure and metabolism.

While this is the first time that resting cartilage structure and COMP concentrations have been assessed in a healthy population. A previous cross-sectional investigation in individuals with OA observed that greater resting COMP concentration was associated with lesser medial cartilage volume.[180] These authors suggested that greater resting COMP concentrations are indicative of greater cartilage degradation, and that the association with lesser cartilage volume is due to long term alterations in cartilage metabolism leading to decreased cartilage structure.[180] When separating our cohort by sex, we observed a similar cross-sectional association in our female participants, as greater resting COMP concentrations were associated with lesser lateral cartilage area ($r=-0.57$, $p=0.014$). Additionally, while not all of the associations were statistically significant, it is interesting to note that the direction of association between every US structure measure and COMP concentration was positive for males and negative for females, while the direction of association between the US echo-intensity measures and COMP concentration were negative for males and positive for females. We cannot definitively state why these differences in association directions occur between males and females, but previous investigations have reported that on average, females present with smaller cartilage volume, even after controlling for age, height, weight and bone volume.[150] Females also have lower resting COMP concentrations compared to males.[151] The direct result of these differences in cartilage structure and metabolism between males and females remains unclear. Continued research is needed to elucidate why cartilage imaging measures are differentially associated with cartilage metabolism between males and females. Future work

assessing cartilage imaging and cartilage metabolism should separately analyze males and females, as analyses combining the sexes may confound the results.

Porcine cartilage explant studies have observed that the magnitude of COMP response is proportionate to the dynamic magnitude of load applied to the cartilage.[181] Previous *in vivo* studies have also speculated that greater acute cartilage deformation would lead to a greater increase in COMP concentration following various activities.[121,124,125] However, in our entire cohort, we observed that greater deformation during walking was associated with a decrease in COMP concentration. A previous study observed that following a drop-landing condition greater cartilage deformation was associated with a a decrease in COMP concentration following a drop-landing condition, but within the same study, there was not a significant association between cartilage deformation and COMP response following running.[123] This finding is similar to that of the current study, as we found a significant association between change in cartilage structure and metabolism following walking, but a non-significant association in drop-landing condition. Both of these studies observed a discrepancy between the association between cartilage deformation and cartilage metabolism after tasks of differing frequency and magnitude of loading (i.e. walking/running = high frequency/low magnitude, drop-landing = low frequency/high magnitude).[123] Thus, the association between cartilage deformation and COMP response may be dependent on the frequency of loading that occurs during a specific activity. Further work is needed to clarify the specific mechanisms that are driving this difference in direction of association in cartilage deformation and cartilage metabolism between activities of different frequencies.

While this study provides information regarding the association between cartilage imaging outcomes and cartilage metabolism, there are some limitations that will inform future research. The cartilage imaging outcomes assessed with US in this study are limited to the anterior femoral cartilage, but serum concentrations of COMP provide a global measure of cartilage metabolism. Thus, the lack of association between the majority of the US measures and COMP response may be due to the COMP response indicating a whole-body cumulative measure of acute cartilage turnover that is not representative of our specific alterations in US measures. An unblinded reader conducted US image analysis, and future studies should consider utilizing blinded readers unaware of the loading condition. Within our study design, we always conducted our US analysis prior to the blood sample collection. There is a small likelihood that this discrepancy in timing between the measures could affect their associations. However, as we were able to collect all post measurements within five minutes, we believe that our US and COMP measures are indicating the same time course following loading.

In conclusion, baseline US measures of femoral cartilage were not associated with cartilage metabolism within the entire cohort. However, separating the group by sex indicated that US cartilage measures and cartilage metabolism are differentially associated between males and females. Greater medial femoral compartment deformation was significantly associated with decreased COMP response following walking. Yet there were no other significant associations between US measures and COMP response in walking or drop-landing.

Table 9. Demographics

	Mean	SD
n	40 (20 male, 20 female)	
Age (years)	21.60	3.15
Height (meters)	1.72	0.09
Mass (kilograms)	68.32	11.39
Tegner	5.87	1.24
Walking Descriptive Data		
Walking Speed (meters/sec)	1.24	0.18
Walking Distance (kilometers)	3.44	0.35

n = samples size, SD = standard deviation

Table 10. Baseline, Post, and Percent Change of COMP and US Measures

Outcome	Compartment	Condition	Baseline Absolute		Post %Δ		Post Absolute	
			mean	sd	mean	sd	mean	sd
Thickness (mm)	Medial	Walking	2.20	0.4	-7.33	3.2	2.05	0.4
		Drop-landing	2.22	0.4	-9.94	3.8	2.00	0.4
	Intercondylar	Walking	2.25	0.5	-2.01	6.7	2.21	0.5
		Drop-landing	2.22	0.5	-3.16	7.5	2.14	0.4
	Lateral	Walking	2.14	0.3	-5.67	3.7	2.02	0.4
		Drop-landing	2.09	0.3	-7.21	4.2	1.94	0.3
Area (mm^2)	Medial	Walking	41.56	7.4	-6.97	4.1	38.69	7.2
		Drop-landing	42.55	7.0	-8.95	4.4	38.74	6.5
	Lateral	Walking	43.97	7.4	-5.31	4.5	41.64	7.3
		Drop-landing	44.18	7.5	-5.98	5.9	41.46	7.1
Echo-intensity	Medial	Walking	64.77	6.1	-1.22	4.7	63.85	5.4
		Drop-landing	64.67	5.9	-1.70	4.4	63.55	6.1
	Lateral	Walking	58.67	4.6	-0.40	4.3	58.34	4.0
		Drop-landing	59.58	5.3	-2.12	4.8	58.22	4.8
Cartilage Oligomeric Matrix Protein		Walking	146.9	38.8	4.06	9.9	150.6	31.2
		Drop-landing	147.3	31.1	4.41	7.5	152.7	27.9

COMP = cartilage oligomeric matrix protein, US = ultrasonography, %Δ = percent change, sd = standard deviation

Table 11. Associations between Baseline COMP and Baseline US Measures

			Medial			Lateral			Intercondylar Thickness
			Thickness	Area	Echo-Intensity	Thickness	Area	Echo-Intensity	
Baseline COMP	Combined	r	**0.09**	**0.22**	**-0.23**	**0.08**	**0.13**	**-0.17**	**0.04**
		95% CI	**-0.24, 0.40**	**-0.11, 0.51**	**-0.52, 0.10**	**-0.25, 0.39**	**-0.20, 0.44**	**-0.47, 0.16**	**-0.29, 0.36**
		p	**0.596**	**0.182**	**0.176**	**0.650**	**0.458**	**0.312**	**0.819**
		n	**37**	**37**	**37**	**37**	**37**	**37**	**37**
	Males	r	**0.26**	**0.27**	**-0.52***	**0.05**	**0.39**	**-0.21**	**0.06**
		95% CI	**-0.22, 0.64**	**-0.21, 0.65**	**-0.79, -0.09**	**-0.41, 0.49**	**-0.08, 0.72**	**-0.61, 0.27**	**-0.41, 0.50**
		p	**0.274**	**0.262**	**0.023**	**0.829**	**0.099**	**0.385**	**0.816**
		n	**19**	**19**	**19**	**19**	**19**	**19**	**19**
	Females	r	**-0.44**	**-0.42**	**0.36**	**-0.43**	**-0.57***	**0.29**	**-0.39**
		95% CI	**-0.75, 0.03**	**-0.74, 0.06**	**-0.13, 0.71**	**-0.75, 0.05**	**-0.82, -0.14**	**-0.20, 0.67**	**-0.72, 0.09**
		p	**0.065**	**0.083**	**0.138**	**0.079**	**0.014**	**0.239**	**0.112**
		n	**18**	**18**	**18**	**18**	**18**	**18**	**18**

* = statistically significant ($p<0.05$), p = p-value, n = sample size, COMP = cartilage oligomeric matrix protein

Table 12. Associations between COMP Percent Change and US Measure Percent Change

			Walking							Drop-Landing						
			Medial			Lateral				Medial			Lateral			
			Thickness	Area	Echo-Intensity	Thickness	Area	Echo-Intensity	Intercondylar Thickness	Thickness	Area	Echo-Intensity	Thickness	Area	Echo-Intensity	Intercondylar Thickness
COMP Change	Overall	r/ρ	**0.04**	**0.36***	**-0.15**	**0.24**	**0.07**	**-0.04**	**-0.21**	**0.22**	**-0.05**	**0.03**	**-0.05**	**-0.15**	**-0.02**	*0.03*
		95% CI	**-0.29, 0.36**	**0.03, 0.62**	**-0.46, 0.19**	**-0.10, 0.53**	**-0.26, 0.39**	**-0.37, 0.30**	**-0.51, 0.13**	**-0.11, 0.50**	**-0.36, 0.27**	**-0.29, 0.34**	**-0.36, 0.27**	**-0.45, 0.18**	**-0.34, 0.31**	*-0.29, 0.34*
		p	**0.831**	**0.036**	**0.405**	**0.162**	**0.695**	**0.830**	**0.238**	**0.176**	**0.786**	**0.836**	**0.749**	**0.389**	**0.918**	*0.858*
		n	**36**	**35**	**35**	**36**	**36**	**35**	**35**	**38**	**38**	**39**	**39**	**37**	**37**	*39*
	Males	r/ρ	**0.31**	**0.48***	*-0.02*	**-0.04**	**0.06**	**-0.19**	**-0.160**	**-0.19**	*0.02*	**0.17**	**-0.06**	*0.03*	**0.30**	*0.16*
		95% CI	**-0.17, 0.67**	**0.02, 0.77**	*-0.48, 0.45*	**-0.49, 0.42**	**-0.41, 0.50**	**-0.60, 0.30**	**-0.57, 0.32**	**-0.60, 0.30**	*-0.45, 0.48*	**-0.31, 0.58**	**-0.50, 0.41**	*-0.46, 0.50*	**-0.18, 0.66**	*-0.32, 0.57*
		p	**0.193**	**0.045**	*0.948*	**0.871**	**0.812**	**0.448**	**0.515**	**0.444**	*0.938*	**0.480**	**0.819**	*0.918*	**0.218**	*0.519*
		n	**19**	**18**	*18*	**19**	**19**	**18**	**19**	**18**	*18*	**19**	**19**	*17*	**19**	*19*
	Females	r/ρ	**-0.13**	**0.33**	**-0.26**	**0.44**	**0.11**	**0.10**	*-0.24*	**0.46***	**-0.07**	**-0.14**	**-0.01**	**-0.30**	**-0.23**	*-0.08*
		95% CI	**-0.57, 0.37**	**-0.18, 0.70**	**-0.66, 0.25**	**-0.05, 0.76**	**-0.39, 0.56**	**-0.40, 0.55**	*-0.657, 0.29*	**0.02, 0.75**	**-0.50, 0.38**	**-0.55, 0.32**	**-0.45, 0.43**	**-0.66, 0.16**	**-0.63, 0.27**	*-0.50, 0.38*
		p	**0.616**	**0.202**	**0.309**	**0.076**	**0.682**	**0.699**	*0.368*	**0.041**	**0.782**	**0.563**	**0.713**	**0.206**	**0.354**	*0.753*
		n	**17**	**17**	**17**	**17**	**17**	**17**	*16*	**20**	**20**	**20**	**20**	**20**	**18**	*20*

Bolded = Pearson r; *Italicized = Spearman Rho ρ*; * = statistically significant (p<0.05), 95% CI = upper, lower 95% confidence interval, p = p-value, n = sample size, COMP = cartilage oligomeric matrix protein

Figure 27. Study Design. A femoral cartilage ultrasonography assessment and blood sample collection was performed before and after each activity condition

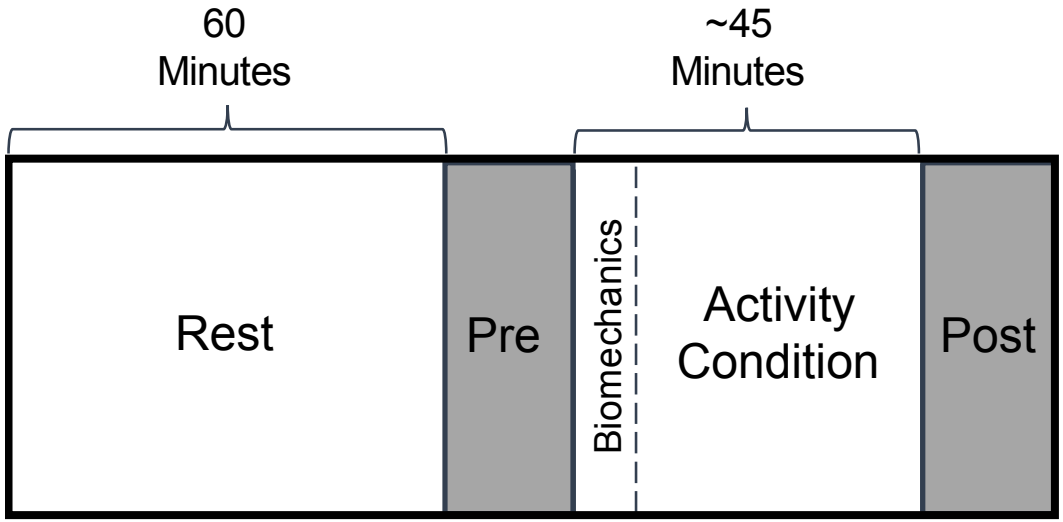


Figure 28. Femoral Cartilage Ultrasonography Setup and Participant Positioning.

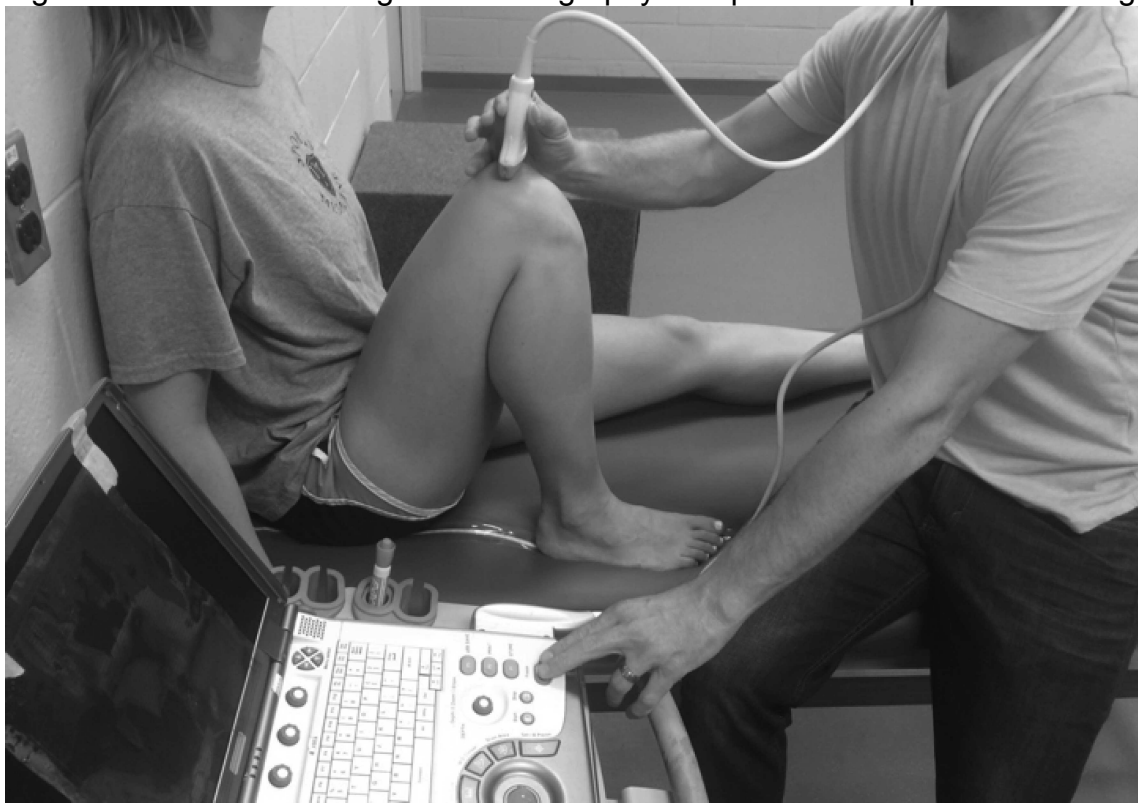


Figure 29. Femoral Cartilage Ultrasonography Outcome Measures. A) Cartilage Thickness; B) Cartilage Compartmental Area

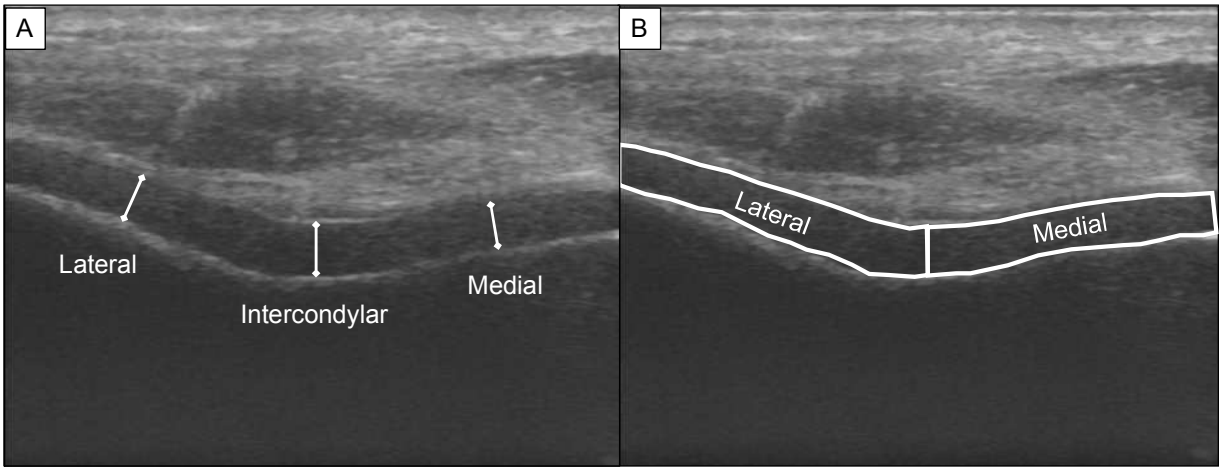
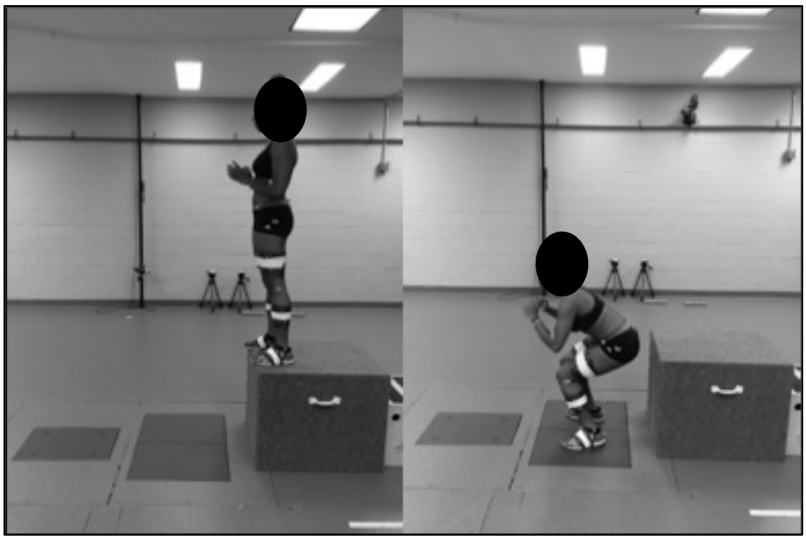


Figure 30. Drop-Landing Condition Setup.



REFERENCES

1. Glyn-Jones S, Palmer AJR, Agricola R, et al. Osteoarthritis. *The Lancet.* 2015;386(9991):376-387.

2. Hunter DJ. Osteoarthritis. *Best practice & research Clinical rheumatology.* 2011;25(6):801-814.

3. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. *Bulletin of the World Health Organization.* 2003;81(9):646-656.

4. Samson D, Grant M, Ratko T, Bonnell C, Ziegler J, Aronson N. Treatment of Primary and Secondary Osteoarthritis of the Knee. Evidence Report/Technology Assessment No. 157. *AHRQ Publication.* 2007;No. 07-E012.

5. Roos EM, Arden NK. Strategies for the prevention of knee osteoarthritis. *Nature reviews Rheumatology.* 2015.

6. Andriacchi TP, Mundermann A. The role of ambulatory mechanics in the initiation and progression of knee osteoarthritis. *Current Opinions Rheumatology.* 2006;18(5):514-518.

7. Madry H, Luyten FP, Facchini A. Biological aspects of early osteoarthritis. *Knee surgery, sports traumatology, arthroscopy.* 2012;20(3):407-422.

8. Roemer FW, Eckstein F, Hayashi D, Guermazi A. The role of imaging in osteoarthritis. *Best practice & research Clinical rheumatology.* 2014;28(1):31-60.

9. Felson DT. Osteoarthritis as a disease of mechanics. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2013;21(1):10-15.

10. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2013;21(1):16-21.

11. Chu CR, Andriacchi TP. Dance between biology, mechanics, and structure: A systems-based approach to developing osteoarthritis prevention strategies. *Journal of orthopaedic research.* 2015.

12. Andriacchi TP, Favre J, Erhart-Hledik JC, Chu CR. A systems view of risk factors for knee osteoarthritis reveals insights into the pathogenesis of the disease. *Annals of biomedical engineering.* 2015;43(2):376-387.

13. Guermazi A, Hayashi D, Roemer FW, Felson DT. Osteoarthritis: a review of strengths and weaknesses of different imaging options. *Rheumatic diseases clinics of North America.* 2013;39(3):567-591.

14. Bauer DC, Hunter DJ, Abramson SB, et al. Classification of osteoarthritis biomarkers: a proposed approach. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2006;14(8):723-727.

15. Hani A, Kumar D, Malik A, Ahmad R, Razak R, Kiflie A. Non-invasive and in vivo assessment of osteoarthritic articular cartilage: a review on MRI investigations. *Rheumatology International.* 2014;35(1):1-16.

16. Keen HI, Wakefield RJ, Conaghan PG. A systematic review of ultrasonography in osteoarthritis. *Annals of the rheumatic diseases.* 2009;68(5):611-619.

17. Naredo E, Acebes C, Möller I, et al. Ultrasound validity in the measurement of knee cartilage thickness. *Annals of the rheumatic diseases.* 2009;68(8):1322-1327.

18. Ostergaard M, Court-Payen M, Gideon P, et al. Ultrasonography in arthritis of the knee. A comparison with MR imaging. *Acta radiologica.* 1995;36(1):19-26.

19. Schmitz RJ, Wang H-MM, Polprasert DR, Kraft RA, Pietrosimone BG. Evaluation of knee cartilage thickness: A comparison between ultrasound and magnetic resonance imaging methods. *The Knee.* 2016; Epub ahead of print.

20. Guermazi A, Alizai H, Crema MD, Trattnig S, Regatte RR, Roemer FW. Compositional MRI techniques for evaluation of cartilage degeneration in osteoarthritis. *Osteoarthritis and cartilage.* 2015;23(10):1639-1653.

21. Lotz M, Martel-Pelletier J, Christiansen C, et al. Value of biomarkers in osteoarthritis: current status and perspectives. *Postgraduate medical journal.* 2014;90(1061):171-178.

22. Matzat SJ, van Tiel J, Gold GE, Oei EHG. Quantitative MRI techniques of cartilage composition. *Quantitative imaging in medicine and surgery.* 2013;3(3):162-174.

23. Tourville TW, Johnson RJ, Slauterbeck JR. Relationship between markers of type II collagen metabolism and tibiofemoral joint space width changes after ACL injury and reconstruction. *The American journal ….* 2013.

24. Lohmander SL, Roos H, Dahlberg L, Hoerrner LA, Lark MW. Temporal patterns of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee joint fluid after injury to the cruciate ligament or meniscus. *Journal of Orthopaedic Research.* 1994;12(1):21-28.

25. Petersson IF, Boegård T, Svensson B, Heinegård D, Saxne T. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. *British journal of rheumatology.* 1998;37(1):46-50.

26. Hosnijeh F, Runhaar J, van Meurs J, Bierma-Zeinstra SM. Biomarkers for osteoarthritis: Can they be used for risk assessment? A systematic review. *Maturitas.* 2015;82(1).

27. Jiao Q, Wei L, Chen C, et al. Cartilage oligomeric matrix protein and hyaluronic acid are sensitive serum biomarkers for early cartilage lesions in the knee joint. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals.* 2015:1-6.

28. Pillen S, Arts IMP, Zwarts MJ. Muscle ultrasound in neuromuscular disorders. *Muscle & nerve.* 2008;37(6):679-693.

29. Woo S, Buckwalter J. *Injury and Repair of the Musculoskeletal Soft Tissues.* Vol 405-26. Savannah, Georgia: American Academy of Orthopaedic Surgeons; 1988.

30. Neu CP. Functional imaging in OA: role of imaging in the evaluation of tissue biomechanics. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2014;22(10):1349-1359.

31. Erhart-Hledik JC, Favre J, Asay JL, et al. A relationship between mechanically-induced changes in serum cartilage oligomeric matrix protein (COMP) and changes in cartilage thickness after 5 years. *Osteoarthritis and cartilage.* 2012;20(11):1309-1315.

32. Radin EL, Martin RB, Burr DB. Effects of mechanical loading on the tissues of the rabbit knee. *Effects of mechanical loading on the tissues of the rabbit knee.* 1984.

33. Mündermann A, Dyrby CO. Secondary gait changes in patients with medial compartment knee osteoarthritis: increased load at the ankle, knee, and hip during walking. *Arthritis & ….* 2005.

34. Noehren B, Wilson H, Miller C, Lattermann C. Long-term gait deviations in anterior cruciate ligament-reconstructed females. *Medicine and science in sports and exercise.* 2013;45(7):1340-1347.

35. Miyazaki T, Wada M, Kawahara H, Sato M, Baba H, Shimada S. Dynamic load at baseline can predict radiographic disease progression in medial compartment knee osteoarthritis. *Annals of the rheumatic diseases.* 2002;61(7):617-622.

36. Bennell KL, Bowles K-A, Wang Y, Cicuttini F, Davies-Tuck M, Hinman RS. Higher dynamic medial knee load predicts greater cartilage loss over 12 months in medial knee osteoarthritis. *Annals of the rheumatic diseases.* 2011.

37. Andriacchi TP, Koo S, Scanlan SF. Gait mechanics influence healthy cartilage morphology and osteoarthritis of the knee. *The Journal of bone and joint surgery American volume.* 2009;91 Suppl 1:95-101.

38. Giannoni P, Siegrist M, Hunziker EB, Wong M. The mechanosensitivity of cartilage oligomeric matrix protein (COMP). *Biorheology.* 2003;40(1-3):101-109.

39. Maetzel A, Li LC, Pencharz J, Tomlinson G, Bombardier C. The economic burden associated with osteoarthritis, rheumatoid arthritis, and hypertension: a comparative study. *Ann Rheum Dis.* 2004;63(4):395-401.

40. Dillon CF, Rasch EK, Gu Q, Hirsch R. Prevalence of knee osteoarthritis in the United States: arthritis data from the Third National Health and Nutrition Examination Survey 1991-94. *J Rheumatol.* 2006;33(11):2271-2279.

41. Paradowski PT, Englund M, Roos EM, Lohmander LS. Similar mean scores, but large individual variations, in patient-relevant outcomes over 2 years in meniscectomized subjects with and without radiographic knee osteoarthritis. *Health Qual Life Outcomes.* 2004;2(1):38.

42. Hartwick M, Meeuwisse W, Vandertuin J, Maitland M. Knee pain in the ACL-deficient osteoarthritic knee and its relationship to quality of life. *Physiother Res Int.* 2003;8(2):83-92.

43. Holla JF, van der Leeden M, Knol DL, et al. The association of body-mass index and depressed mood with knee pain and activity limitations in knee osteoarthritis: results from the Amsterdam osteoarthritis cohort. *BMC musculoskeletal disorders.* 2013;14(1):296.

44. Singh G, Miller JD, Lee FH, Pettitt D, Russell MW. Prevalence of cardiovascular disease risk factors among US adults with self-reported osteoarthritis: data from the Third National Health and Nutrition Examination Survey. *The American journal of managed care.* 2002;8(15 Suppl):S383-391.

45. Reginster JY, Khaltaev NG. Introduction and WHO perspective on the global burden of musculoskeletal conditions. *Rheumatology (Oxford).* 2002;41 Supp 1:1-2.

46. Chu CR, Millis MB, Olson SA. Osteoarthritis: From Palliation to Prevention: AOA Critical Issues. *The Journal of bone and joint surgery.* 2014;96(15).

47. Brown TD, Johnston RC, Saltzman CL, Marsh JL, Buckwalter JA. Posttraumatic osteoarthritis: a first estimate of incidence, prevalence, and burden of disease. *Journal of orthopaedic trauma.* 2006;20(10):739-744.

48. Riordan EA, Little C, Hunter D. Pathogenesis of post-traumatic OA with a view to intervention. *Best Practice & Research Clinical Rheumatology.* 2014;28(1):1730.

49. Luc B, Gribble PA, Pietrosimone BG. Osteoarthritis Prevalence Following Anterior Cruciate Ligament Reconstruction: A Systematic Review and Numbers-Needed-to-Treat Analysis. *Journal of athletic training.* 2014;49(6):806-819.

50. Losina E, Weinstein AM, Reichmann WM, et al. Lifetime risk and age at diagnosis of symptomatic knee osteoarthritis in the US. *Arthritis care & research.* 2013;65(5):703-711.

51. Parkkari J, Pasanen K, Mattila VM, Kannus P, Rimpelä A. The risk for a cruciate ligament injury of the knee in adolescents and young adults: a population-based cohort study of 46 500 people with a 9 year follow-up. *British journal of sports medicine.* 2008;42(6):422-426.

52. Griffin LY, Albohm MJ, Arendt EA, et al. Understanding and preventing noncontact anterior cruciate ligament injuries: a review of the Hunt Valley II meeting, January 2005. *The American journal of sports medicine.* 2006;34(9):1512-1532.

53. Shepherd DET, Seedhom BB. Thickness of human articular cartilage in joints of the lower limb. *Ann Rheum Dis.* 1999;58(1):27-34.

54. Getgood A, Bhullar T, Rushton N. Current Concepts in Articular Cartilage Repair. *Orthopaedics and Trauma.* 2009;23(3):189-200.

55. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Annals of the rheumatic diseases.* 1957.

56. Eckstein F, Guermazi A, Gold G, et al. Imaging of cartilage and bone: promises and pitfalls in clinical trials of osteoarthritis. *Osteoarthritis and Cartilage.* 2014;22(10):1516-1532.

57. Hunter D, Tu X, LaValley M, et al. The meniscus and cartilage loss. *Arthritis Rheum.* 2004;50(9):S141-S141.

58. Bauer DC, Hunter DJ, Abramson SB, et al. Classification of osteoarthritis biomarkers: a proposed approach. *Osteoarthritis Cartilage.* 2006;14(8):723-727.

59. Koster IM, Oei EHG, Hensen JHJ, et al. Predictive factors for new onset or progression of knee osteoarthritis one year after trauma: MRI follow-up in general practice. *Eur Radiol.* 2011;21(7):1509-1516.

60. Guermazi A, Roemer FW, Felson DT, Brandt KD. Motion for debate: osteoarthritis clinical trials have not identified efficacious therapies because traditional imaging outcome measures are inadequate. *Arthritis and rheumatism.* 2013.

61. Kijowski R, Roemer F, Englund M, Tiderius CJ, Swärd P, Frobell RB. Imaging following acute knee trauma. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2014;22(10):1429-1443.

62. Conaghan PG, Felson D, Gold G, Lohmander S. MRI and non-cartilaginous structures in knee osteoarthritis. *Osteoarthritis and Cartilage.* 2006.

63. Guermazi A, Roemer FW, Haugen IK. MRI-based semiquantitative scoring of joint pathology in osteoarthritis. *Nature Reviews Rheumatology.* 2013(9):236-251.

64. Roemer FW, Frobell R, Lohmander LS, Niu J. Anterior Cruciate Ligament OsteoArthritis Score (ACLOAS): Longitudinal MRI-based whole joint assessment of anterior cruciate ligament injury. *Osteoarthritis and Cartilage.* 2014.

65. Frobell RB. Change in cartilage thickness, posttraumatic bone marrow lesions, and joint fluid volumes after acute ACL disruption: a two-year prospective MRI study of sixty-one subjects. *The Journal of bone and joint surgery American volume.* 2011;93(12):1096-1103.

66. Li H, Chen S, Tao H, Chen S. Quantitative MRI T2 Relaxation Time Evaluation of Knee Cartilage: Comparison of Meniscus-Intact and -Injured Knees After Anterior Cruciate Ligament Reconstruction. *The American journal of sports medicine.* 2015.

67. Li H, Hosseini A, Li J-SS, Gill TJ, Li G. Quantitative magnetic resonance imaging (MRI) morphological analysis of knee cartilage in healthy and anterior cruciate ligament-injured knees. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA.* 2012;20(8):1496-1502.

68. Theologis AA, Haughom B, Liang F, et al. Comparison of T1rho relaxation times between ACL-reconstructed knees and contralateral uninjured knees. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA.* 2014;22(2):298-307.

69. Gupta R, Virayavanich W, Kuo D, et al. MR T1ρ quantification of cartilage focal lesions in acutely injured knees: correlation with arthroscopic evaluation. *Magnetic resonance imaging.* 2014.

70. Hunter DJ, Zhang W, Conaghan PG, Hirko K. Systematic review of the concurrent and predictive validity of MRI biomarkers in OA. *Osteoarthritis and ….* 2011.

71. Hunter DJ, Zhang W, Conaghan PG, Hirko K. Responsiveness and reliability of MRI in knee osteoarthritis: a meta-analysis of published evidence. *Osteoarthritis and ….* 2011.

72. Conaghan PG, Hunter DJ, Maillefert JF. Summary and recommendations of the OARSI FDA osteoarthritis Assessment of Structural Change Working Group. *Osteoarthritis and ….* 2011.

73. Patel SA, Hageman J, Quatman CE, Wordeman SC, Hewett TE. Prevalence and Location of Bone Bruises Associated with Anterior Cruciate Ligament Injury and Implications for Mechanism of Injury: A Systematic Review. *Sports medicine (Auckland, NZ).* 2013.

74. Van Ginckel A, Verdonk P, Victor J, Witvrouw E. Cartilage status in relation to return to sports after anterior cruciate ligament reconstruction. *The American journal of sports medicine.* 2013;41(3):550-559.

75. Frobell RB, Le Graverand MPP, Buck R, et al. The acutely ACL injured knee assessed by MRI: changes in joint fluid, bone marrow lesions, and cartilage during the first year. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2009;17(2):161-167.

76. Frobell RB, Nevitt MC, Hudelmaier M, et al. Femorotibial subchondral bone area and regional cartilage thickness: a cross-sectional description in healthy reference cases and various radiographic stages of osteoarthritis in 1,003 knees from the Osteoarthritis Initiative. *Arthritis care & research.* 2010;62(11):1612-1623.

77. Buck R, Wyman B, Le Graverand M, Hudelmaier M, Wirth W, Eckstein F. Osteoarthritis may not be a one-way-road of cartilage loss – comparison of spatial patterns of cartilage change between osteoarthritic and healthy knees. *Osteoarthritis and Cartilage.* 2010;18(3):329-335.

78. Frobell RB. Change in cartilage thickness, posttraumatic bone marrow lesions, and joint fluid volumes after acute ACL disruption: a two-year prospective MRI study of sixty-one subjects. *The Journal of bone and joint surgery American volume.* 2011;93(12):1096-1103.

79. Andreisek G, White LM, Sussman MS, et al. Quantitative MR imaging evaluation of the cartilage thickness and subchondral bone area in patients with ACL-reconstructions 7 years after surgery. *Osteoarthritis and cartilage.* 2009;17(7):871-878.

80. Eckstein F, Wirth W, Lohmander LS, Hudelmaier MI, Frobell RB. Five-year follow-up of knee joint cartilage thickness changes after acute anterior cruciate ligament rupture. *Arthritis & rheumatology.* 2015;67(1):152-161.

81. Iagnocco A. Imaging the joint in osteoarthritis: a place for ultrasound? *Best practice & research Clinical rheumatology.* 2010;24(1):27-38.

82. Ostergaard M, Court-Payen M, Gideon P, et al. Ultrasonography in arthritis of the knee. A comparison with MR imaging. *Acta radiologica (Stockholm, Sweden : 1987).* 1994;36(1):19-26.

83. Bevers K, Zweers MC, van den Ende CH, et al. Ultrasonographic analysis in knee osteoarthritis: evaluation of inter-observer reliability. *Clinical and experimental rheumatology.* 2011;30(5):673-678.

84. Abraham AM, Goff I, Pearce MS, Francis RM, Birrell F. Reliability and validity of ultrasound imaging of features of knee osteoarthritis in the community. *BMC musculoskeletal disorders.* 2010;12:70.

85. Keen HI, Conaghan PG. Ultrasonography in osteoarthritis. *Radiologic Clinics of North America.* 2009.

86. Möller I, Bong D, Naredo E, et al. Ultrasound in the study and monitoring of osteoarthritis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2007;16 Suppl 3:7.

87. Kraus VB, Burnett B, Coindreau J, et al. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2011;19(5):515-542.

88. Bruyère O, Cooper C, Arden N, et al. Can We Identify Patients with High Risk of Osteoarthritis Progression Who Will Respond to Treatment? A Focus on Epidemiology and Phenotype of Osteoarthritis. *Drugs & aging.* 2015.

89. Tseng S, Reddi HA, Cesare PE. Cartilage Oligomeric Matrix Protein (COMP): A Biomarker of Arthritis. *Biomarker insights.* 2009;4:33.

90. Das BR, Roy A, Khan FR. Cartilage oligomeric matrix protein in monitoring and prognostication of osteoarthritis and its utility in drug development. *Perspectives in clinical research.* 2015;6(1):4-9.

91. Clark AG, Jordan JM, Vilim V, Renner JB. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity. *Arthritis ….* 1999.

92. Valdes AM, Meulenbelt I, Chassaing E. Large scale meta-analysis of urinary C-terminal telopeptide, serum cartilage oligomeric protein and matrix metalloprotease degraded type II collagen and their …. *Osteoarthritis and ….* 2014.

93. Blumenfeld O, Williams FMK, Hart DJ. Association between cartilage and bone biomarkers and incidence of radiographic knee osteoarthritis (RKOA) in UK females: a prospective study. *Osteoarthritis and ….* 2013.

94. Golightly YM, Marshall SW, Kraus VB, et al. Biomarkers of incident radiographic knee osteoarthritis: do they vary by chronic knee symptoms? *Arthritis and rheumatism.* 2011;63(8):2276-2283.

95. Kumm J, Tamm A, Lintrop M, Tamm A. The value of cartilage biomarkers in progressive knee osteoarthritis: cross-sectional and 6-year follow-up study in middle-aged subjects. *Rheumatology international.* 2013;33(4):903-911.

96. Sharif M, Saxne T, Shepstone L, et al. Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. *British journal of rheumatology.* 1995;34(4):306-310.

97. Lohmander LS, Ostenberg A, Englund M, Roos H. High prevalence of knee osteoarthritis, pain, and functional limitations in female soccer players twelve years after anterior cruciate ligament injury. *Arthritis and rheumatism.* 2004;50(10):3145-3152.

98. Lohmander LS, Saxne T, Heinegård DK. Release of cartilage oligomeric matrix protein (COMP) into joint fluid after knee injury and in osteoarthritis. *Annals of the rheumatic diseases.* 1994;53(1):8-13.

99. Harkey MS, Luc BA, Golightly YM, et al. Osteoarthritis-related biomarkers following anterior cruciate ligament injury and reconstruction: a systematic review. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2015;23(1):1-12.

100. Guermazi A, Roemer FW. Compositional MRI assessment of cartilage: what is it and what is its potential for sports medicine? *British journal of sports medicine.* 2015.

101. Wang L, Regatte RR. T1ρ MRI of human musculoskeletal system. *Journal of Magnetic Resonance Imaging.* 2015;41(3).

102. Regatte RR, Akella SVS, Lonner JH. T1ρ relaxation mapping in human osteoarthritis (OA) cartilage: comparison of T1ρ with T2. *… Resonance Imaging.* 2006.

103. Su F, Hilton JF, Nardo L, et al. Cartilage morphology and T1ρ and T2 quantification in ACL-reconstructed knees: a 2-year follow-up. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2013;21(8):1058-1067.

104. Theologis AA, Kuo D, Cheng J, et al. Evaluation of Bone Bruises and Associated Cartilage in Anterior Cruciate Ligament–Injured and –Reconstructed Knees Using Quantitative T1ρ Magnetic Resonance Imaging: 1-Year Cohort Study. *Arthroscopy: The Journal of Arthroscopic & Related Surgery.* 2011;27(1):65-76.

105. Maurits NM, Bollen AE, Windhausen A, De Jager AE, Van Der Hoeven JH. Muscle ultrasound analysis: normal values and differentiation between myopathies and neuropathies. *Ultrasound in medicine & biology.* 2003;29(2):215-225.

106. Reimers CD, Fleckenstein JL, Witt TN, Müller-Felber W, Pongratz DE. Muscular ultrasound in idiopathic inflammatory myopathies of adults. *Journal of the neurological sciences.* 1993;116(1):82-92.

107. Zheng H, Chen C. Body mass index and risk of knee osteoarthritis: systematic review and meta-analysis of prospective studies. *BMJ open.* 2015;5(12).

108. Runhaar J, van Middelkoop M, Reijman M, Vroegindeweij D, Oei EH, Bierma-Zeinstra SM. Malalignment: a possible target for prevention of incident knee

osteoarthritis in overweight and obese women. *Rheumatology (Oxford, England).* 2014;53(9):1618-1624.

109. Andriacchi TP, Mundermann A, Smith RL, Alexander EJ, Dyrby CO, Koo S. A framework for the in vivo pathomechanics of osteoarthritis at the knee. *Annals of biomedical engineering.* 2004;32(3):447-457.

110. Brandt KD, Myers SL, Burr D, Albrecht M. Osteoarthritic changes in canine articular cartilage, subchondral bone, and synovium fifty-four months after transection of the anterior cruciate ligament. *Arthritis and rheumatism.* 1991;34(12):1560-1570.

111. Liikavainio T, Bragge T, Hakkarainen M, Karjalainen PA, Arokoski JP. Gait and muscle activation changes in men with knee osteoarthritis. *The Knee.* 2010;17(1):69-76.

112. Ewers BJ, Weaver BT, Sevensma ET, Haut RC. Chronic changes in rabbit retro-patellar cartilage and subchondral bone after blunt impact loading of the patellofemoral joint. *Journal of orthopaedic research.* 2002;20(3):545-550.

113. Yang KH, Boyd RD, Schaffler MB, Burr DB, Radin EL. Differential Effect of Load Magnitude and Rate on the Initiation and Progression of Osteoarthritis. *Trans Orthop Res Soc.* 1989;14:148.

114. Mündermann A, Dyrby CO, Andriacchi TP. Secondary gait changes in patients with medial compartment knee osteoarthritis: increased load at the ankle, knee, and hip during walking. *Arthritis and rheumatism.* 2005;52(9):2835-2844.

115. Baliunas AJ, Hurwitz DE, Ryals AB, Karrar A. Increased knee joint loads during walking are present in subjects with knee osteoarthritis. *Osteoarthritis and ….* 2002.

116. Koo S, Andriacchi TP. A comparison of the influence of global functional loads vs. local contact anatomy on articular cartilage thickness at the knee. *Journal of biomechanics.* 2007;40(13):2961-2966.

117. Chehab EF, Favre J, Erhart-Hledik JC, Andriacchi TP. Baseline knee adduction and flexion moments during walking are both associated with 5 year cartilage changes in patients with medial knee osteoarthritis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2014;22(11):1833-1839.

118. Morgenroth DC, Medverd JR, Seyedali M, Czerniecki JM. The relationship between knee joint loading rate during walking and degenerative changes on magnetic resonance imaging. *Clinical biomechanics.* 2014;29(6):664-670.

119. Andriacchi TP. Osteoarthritis: Probing knee OA as a system responding to a stimulus. *Nature reviews Rheumatology.* 2012;8(7):371-372.

120. Eckstein F, Lemberger B, Gratzke C. In vivo cartilage deformation after different types of activity and its dependence on physical training status. *Ann Rheum Dis.* 2005;64(2):291-295.

121. Kersting UG, Stubendorff JJ, Schmidt MC. Changes in knee cartilage volume and serum COMP concentration after running exercise. *Osteoarthritis and Cartilage.* 2005;13(10):925-934.

122. Boocock M, McNair P, Cicuttini F, Stuart A. The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. *Osteoarthritis and Cartilage.* 2009;17:883-890.

123. Niehoff A, Müller M, Brüggemann L, et al. Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing. *Osteoarthritis and cartilage.* 2011;19(8):1003-1010.

124. Niehoff A, Kersting UG, Helling S, et al. Different mechanical loading protocols influence serum cartilage oligomeric matrix protein levels in young healthy humans. *European Journal of Applied Physiology.* 2010;110(3).

125. Mundermann A, Dyrby CO, Andriacchi TP, King KB. Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. *Osteoarthritis and Cartilage.* 2005;13(1):34-38.

126. Mündermann A, King KB, Smith RL, Andriacchi TP. Change in serum COMP concentration due to ambulatory load is not related to knee OA status. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society.* 2009;27(11):1408-1413.

127. Nishii T, Kuroda K, Matsuoka Y. Change in knee cartilage T2 in response to mechanical loading. *Journal of Magnetic Resonance Imaging.* 2008;28(1):175-180.

128. Mosher TJ, Liu Y, Torok CM. Functional cartilage MRI T2 mapping: evaluating the effect of age and training on knee cartilage response to running. *Osteoarthritis and Cartilage.* 2010;18(3):358-364.

129. Subburaj K, Kumar D, Souza RB, et al. The Acute Effect of Running on Knee Articular Cartilage and Meniscus Magnetic Resonance Relaxation Times in Young Healthy Adults. *The American journal of sports medicine.* 2012;40(9):2134-2141.

130. Souza RB, Fang C, Luke A, Wu S, Li X, Majumdar S. Relationship between knee kinetics during jumping tasks and knee articular cartilage MRI T1rho and T2 relaxation times. *Clinical biomechanics (Bristol, Avon).* 2012;27(4):403-408.

131. Kumar D, Kothari A, Souza RB, Wu S, Benjamin Ma C, Li X. Frontal plane knee mechanics and medial cartilage MR relaxation times in individuals with ACL reconstruction: A pilot study. *The Knee.* 2014.

132. Harkey MS, Blackburn JT, Davis H, Sierra-Arévalo L, Nissman D, Pietrosimone B. Ultrasonographic assessment of medial femoral cartilage deformation acutely following walking and running. *Osteoarthritis and cartilage.* 2016.

133. Harkey MS, Blackburn JT, Davis H, Sierra-Arévalo L, Nissman D, Pietrosimone B. Ultrasonographic Assessment of Medial Femoral Cartilage Deformation Acutely Following Walking and Running. *Osteoarthritis and cartilage.* 2016; Epub ahead of print.

134. Lottenburger T, Junker P, Hørslev-Petersen K. Diurnal variation of connective tissue metabolites in early and long-standing rheumatoid arthritis and in healthy individuals. *Scandinavian journal of rheumatology.* 2011;40(2):88-94.

135. Coleman JL, Widmyer MR, Leddy HA, et al. Diurnal variations in articular cartilage thickness and strain in the human knee. *Journal of biomechanics.* 2013;46(3):541-547.

136. Kilic G, Kilic E, Akgul O, Ozgocmen S. Ultrasonographic assessment of diurnal variation in the femoral condylar cartilage thickness in healthy young adults. *American journal of physical medicine & rehabilitation.* 2015;94(4):297-303.

137. Pietrosimone B, Blackburn JT, Harkey MS, et al. Walking speed as a potential indicator of cartilage breakdown following anterior cruciate ligament reconstruction. *Arthritis care & research.* 2016;68(6):793-800.

138. Pietrosimone B, Blackburn JT, Harkey MS, et al. Greater Mechanical Loading During Walking Is Associated With Less Collagen Turnover in Individuals With Anterior Cruciate Ligament Reconstruction. *The American journal of sports medicine.* 2016;44(2):425-432.

139. Pietrosimone B, Loeser RF, Blackburn JT, et al. Biochemical markers of cartilage metabolism are associated with walking biomechanics 6-months following anterior cruciate ligament reconstruction. *Journal of orthopaedic research.* 2017; Epub ahead of print.

140. Armstrong LE, Kavouras SA, Walsh NP, Roberts WO. Diagnosing dehydration? Blend evidence with clinical observations. *Current opinion in clinical nutrition and metabolic care.* 2016;19(6):434-438.

141. Özçakar L, Tunç H, Öken Ö, et al. Femoral cartilage thickness measurements in healthy individuals: learning, practicing and publishing with TURK-MUSCULUS. *Journal of back and musculoskeletal rehabilitation.* 2013;27(2):117-124.

142. Tuna S, Balcı N, Özçakar L. The relationship between femoral cartilage thickness and muscle strength in knee osteoarthritis. *Clinical rheumatology.* 2016;35(8):2073-2077.

143. Cartwright MS, Kwayisi G, Griffin LP, et al. Quantitative neuromuscular ultrasound in the intensive care unit. *Muscle & nerve.* 2013;47(2):255-259.

144. Eckstein F, Tieschky M, Faber S, Englmeier KH, Reiser M. Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise in vivo. *Anatomy and embryology.* 1999;200(4):419-424.

145. Bell AL, Pedersen DR, Brand RA. A comparison of the accuracy of several hip center location prediction methods. *Journal of biomechanics.* 1990;23(6):617-621.

146. Ferber R, Osternig LR, Woollacott MH, Wasielewski NJ, Lee J-H. Gait mechanics in chronic ACL deficiency and subsequent repair. *Clinical biomechanics (Bristol, Avon).* 2002;17(4):274-285.

147. Winter D. *Biomechanics and Motor Control of Human Movement.* 4 ed. Hoboken, NJ: John Wiley & Sons; 2009.

148. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi medical journal.* 2012;24(3):69-71.

149. Wright RW, Group M. Osteoarthritis Classification Scales: Interobserver Reliability and Arthroscopic Correlation. *The Journal of bone and joint surgery American volume.* 2014;96(14):1145-1151.

150. Cicuttini F, Forbes A, Morris K, Darling S, Bailey M, Stuckey S. Gender differences in knee cartilage volume as measured by magnetic resonance imaging. *Osteoarthritis and cartilage.* 1999;7(3):265-271.

151. Jordan JM, Luta G, Stabler T, et al. Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: the Johnston County Osteoarthritis Project. *Arthritis and rheumatism.* 2003;48(3):675-681.

152. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis & rheumatism.* 2012;64(6):1697-1707.

153. Eckstein F, Hudelmaier M, Putz R. The effects of exercise on human articular cartilage. *Journal of anatomy.* 2006;208(4):491-512.

154. Liu F, Kozanek M, Hosseini A, et al. In vivo tibiofemoral cartilage deformation during the stance phase of gait. *Journal of biomechanics.* 2010;43(4):658-665.

155. Lad NK, Liu B, Ganapathy PK, et al. Effect of normal gait on in vivo tibiofemoral cartilage strains. *Journal of biomechanics.* 2016;49(13):2870-2876.

156. Cotofana S, Eckstein F, Wirth W, et al. In vivo measures of cartilage deformation: patterns in healthy and osteoarthritic female knees using 3T MR imaging. *European Radiology.* 2011;21(6):1127-1135.

157. Wellsandt E, Khandha A, Manal K, Axe MJ, Buchanan TS, Snyder-Mackler L. Predictors of knee joint loading after anterior cruciate ligament reconstruction. *Journal of orthopaedic research.* 2017;35(3):651-656.

158. Manal K, Gardinier E, Buchanan TS, Snyder-Mackler L. A more informed evaluation of medial compartment loading: the combined use of the knee adduction and flexor moments. *Osteoarthritis and cartilage.* 2015;23(7):1107-1111.

159. Eckstein F, Reiser M, Englmeier KH, Putz R. In vivo morphometry and functional analysis of human articular cartilage with quantitative magnetic resonance imaging--from image to data, from data to theory. *Anatomy and embryology.* 2001;203(3):147-173.

160. Denning WM, Becker Pardo M, Winward JG, et al. Ambulation speed and corresponding mechanics are associated with changes in serum cartilage oligomeric matrix protein. *Gait & posture.* 2016;44:131-136.

161. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychological bulletin.* 1979;86(2):420.

162. Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. *The Journal of Strength & Conditioning Research.* 2005;19(1):231-240.

163. Kean CO, Birmingham TB, Garland SJ, Bryant DM, Giffin JR. Minimal detectable change in quadriceps strength and voluntary muscle activation in patients with knee osteoarthritis. *Archives of physical medicine and rehabilitation.* 2010;91(9):1447-1451.

164. Lüsse S, Claassen H, Gehrke T, et al. Evaluation of water content by spatially resolved transverse relaxation times of human articular cartilage. *Magnetic resonance imaging.* 2000;18(4):423-430.

165. Liess C, Lüsse S, Karger N, Heller M, Glüer CCC. Detection of changes in cartilage water content using MRI T2-mapping in vivo. *Osteoarthritis and cartilage.* 2002;10(12):907-913.

166. Mosher TJ, Smith HE, Collins C, et al. Change in knee cartilage T2 at MR imaging after running: a feasibility study. *Radiology.* 2004;234(1):245-249.

167. Koo S, Rylander JH, Andriacchi TP. Knee joint kinematics during walking influences the spatial cartilage thickness distribution in the knee. *Journal of biomechanics.* 2011;44(7):1405-1409.

168. Lopez AD, Murray CC. The global burden of disease, 1990-2020. *Nature Medicine.* 1998;4(11):1241-1243.

169. Hosnijeh F, Runhaar J, van Meurs J, Bierma-Zeinstra SM. Biomarkers for osteoarthritis: Can they be used for risk assessment? A systematic review. *Maturitas.* 2015;82(1):36-49.

170. Palmieri-Smith RM, Wojtys EM, Potter HG. Early Cartilage Changes After Anterior Cruciate Ligament Injury: Evaluation With Imaging and Serum Biomarkers-A Pilot Study. *Arthroscopy : the journal of arthroscopic & related surgery.* 2016;32(7):1309-1318.

171. Hyldahl RD, Evans A, Kwon S, et al. Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations: a pilot study. *European Journal of Applied Physiology.* 2016;116(11-12):2305-2314.

172. Roberts HM, Moore JP, Griffith-McGeever CL, Fortes MB, Thom JM. The effect of vigorous running and cycling on serum COMP, lubricin, and femoral cartilage thickness: a pilot study. *European journal of applied physiology.* 2016;116(8):1467-1477.

173. Cattano NM, Driban JB, Barbe MF, Tierney RT, Amin M, Sitler MR. Biochemical Response to a Moderate Running Bout in Participants With or Without a History of Acute Knee Injury. *Journal of athletic training.* 2016; Epub ahead of print.

174. Neidhart M, Hauser N, Paulsson M, DiCesare PE, Michel BA, Häuselmann HJ. Small fragments of cartilage oligomeric matrix protein in synovial fluid and serum as markers for cartilage degradation. *British journal of rheumatology.* 1997;36(11):1151-1160.

175. White DK, Zhang Y, Niu J, et al. Do worsening knee radiographs mean greater chances of severe functional limitation? *Arthritis Care & Research.* 2010;62(10):1433-1439.

176. Eckstein F, Cicuttini F, Raynauld JP, Waterton JC. Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. *Osteoarthritis and Cartilage.* 2006;14(Suppl A):A46-75.

177. Andriacchi TP, Favre J. The nature of in vivo mechanical signals that influence cartilage health and progression to knee osteoarthritis. *Current rheumatology reports.* 2014;16(11):463.

178. Clark AG, Jordan JM, Vilim V, Renner JB. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity. *Arthritis and rheumatism.* 1999.

179. Jiao Q, Wei L, Chen C, et al. Cartilage oligomeric matrix protein and hyaluronic acid are sensitive serum biomarkers for early cartilage lesions in the knee joint. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals.* 2016;21(2):146-151.

180. Berry PA, Maciewicz RA, Wluka AE, et al. Relationship of serum markers of cartilage metabolism to imaging and clinical outcome measures of knee joint structure. *Annals of the rheumatic diseases.* 2010;69(10):1816-1822.

181. Piscoya JL, Fermor B, Kraus VB, Stabler TV. The influence of mechanical compression on the induction of osteoarthritis-related biomarkers in articular cartilage explants. *Osteoarthritis and Cartilage.* 2005.