ACUTE EFFECT OF ALTERED MECHANICAL LOADING ON BIOMECHANICS AND BIOCHEMICAL MARKERS IN INDIVIDUALS WITH ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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ABSTRACT

Brittney A. Harkey: The Acute Effect of Altered Mechanical Loading in Individuals with Anterior Cruciate Ligament Reconstruction
(Under the direction of Brian Pietrosimone)

Context: The complex interaction between aberrant mechanical loading and altered cartilage metabolism is hypothesized to lead to the development of posttraumatic osteoarthritis (PTOA) following anterior cruciate ligament reconstruction (ACLR). There is a critical need for effective therapeutic strategies that are capable of manipulating mechanical loading for the purpose of maintaining homeostatic cartilage metabolism following ACLR. **Objective:** To utilize real-time biofeedback to acutely increase (i.e. overloading), acutely decrease (i.e. under-loading) and promote symmetrical loading between limbs during walking gait in individuals with ACLR to determine changes in lower extremity biomechanical outcomes and cartilage metabolism that occur following acute bouts of altered mechanical loading. **Participants:** 30 individuals with a primary, unilateral ACLR. **Interventions:** Participants completed four testing sessions. One of four loading conditions was completed during 20 minutes of treadmill walking, and included 1) control condition of normal walking, 2) a 5% increase (i.e. overloading) in peak vGRF, 3) a 5% decrease (i.e. under-loading) in peak vGRF, and 4) symmetrical peak vGRF between limbs. **Main Outcome Measures:** Root mean square error (RMSE) was calculated during the acquisition and recall periods. Lower biomechanical outcomes included peak vGRF, instantaneous vGRF loading rate, peak KEM, and knee flexion excursion. Catilage metabolism was quantified using serum oligomeric matrix protein (COMP). **Results:** Individuals with ACLR

demonstrate lesser RMSE during the acquisition of symmetrical loading as compared to the overloading and under-loading conditions. Peak vGRF was significantly greater during the overloading condition, and significantly lesser during the under-loading condition as compared to the control condition. Peak KEM and knee flexion excursion were significantly greater during the overloading condition as compared to the under-loading condition. Individuals with ACLR demonstrating an increase in COMP during the control condition demonstrated a significant decrease in COMP during the overloading condition as compared to the control. Individuals with ACLR demonstrating a lesser baseline peak vGRF also demonstrated a greater increase in COMP following 20 minutes of normal walking. **Conclusions:** Real-time biofeedback may be beneficial for altering mechanical loading during walking gait in individuals with ACLR. Acutely manipulating mechanical during walking gait may be able to influence cartilage metabolism in individuals with ACLR.

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- ACL - Anterior cruciate ligament
- ACLR - Anterior cruciate ligament reconstruction
- COMP - Cartilage oligomeric matrix protein
- CTX-II - C-terminal crosslinked telopeptide of type II collagen
- ECM - Extracellular matrix
- EMG - Electromyography
- GAGs - Glycosaminoglycans
- IL - Interleuikin
- KAM - External knee adduction moment
- KEM - Internal knee extension moment
- MMPs - Matrix metalloproteinases
- OA - Osteoarthritis
- PCM - Pericellular matrix
- PTOA - Posttraumatic osteoarthritis
- TIMPs - Tissue inhibitors or metallopeoteinases
- TNF-α - Tumor necoris factor alpha
- vGRF - Vertical ground reaction force

CHAPTER 1: INTRODUCTION

Osteoarthritis (OA) is the most prevalent chronic medical condition worldwide.[1] Knee OA specifically affects 12.1-16% of adults in the United States.[2,3] Individuals with knee OA experience pain, stiffness and a loss of function that results in a substantial adverse impact on a person's quality of life. Loss of function and increased disability likely lead to declines in physical activity,[4] and decreaed physical activity due to knee OA may be linked to secondary health complications (i.e diabetes, metabolic syndrome, coronary artery disease) and increase the risk of mortality.[5] As such, the presence of knee OA is a significant risk factor for the development of cardiovascular disease.[6]

A variety of factors increase the risk of knee OA development and include increased body mass,[7] female gender,[7] bony mal-alignment,[8] and synovitis.[7,9,10] Individuals with a history of a knee injury however are at a five times greater risk of developing posttraumatic OA (PTOA), a rapidly progressive form of OA, compared to individuals without a history of knee injury.[11] There are an estimated 900,000 cases of knee injuries annually in the United States, and PTOA accounts for 12% of all cases of OA.[12]

One third of all participants sustaining an anterior cruciate ligament (ACL) injury develop knee OA within the first decade following injury regardless of treatment,[13] with radiographic changes following ACL reconstruction (ACLR) reported to be greater than 70% more than 10 years post-surgery.[14,15] Individuals presenting with PTOA are at least a decade younger at the time of diagnosis as compared to individuals presenting with idiopathic OA, which increases the total number of years lived with disability due to PTOA.[12,16] As there is

currently no cure for knee OA, it is imperative to begin developing effective treatment strategies that reduce the risk of PTOA following ACLR.[16] In order to begin developing such strategies however, we must first determine specific therapeutic targets that may slow the progression towards PTOA.

The progression of PTOA in individuals with ACLR has been theorized to result from altered mechanical loading occurring about the knee[17] as well as metabolic alterations in joint homeostasis.[18] Joint injury initiates a robust inflammatory response that results in the production of both pro- and anti-inflammatory factors.[16] In turn, inflammatory cytokines up regulate matrix metalloproteinase (MMP) activity, enzymes responsible for cartilage catabolism.[16] Within the first week following ACLR synovial fluid concentrations of inflammatory biomarkers (Interleukin-6, Interleukin-1ra, and Interleukin-1α) are elevated compared to baseline.[19-21] Additionally, markers of cartilage turnover are also altered following ACLR. C-terminal crosslinked telopeptide of type II collagen (CTX-II), a marker of cartilage degradation, increases initially following ACLR and remains elevated up to one year following ACLR.[22] Persistent alterations in tissue metabolism may be also be influenced by aberrant mechanical loading that occurs following ACLR,[17,23-27] further complicating our understanding of PTOA development.

Despite ACLR offering beneficial clinical outcomes such as restored joint stability and high rates of return to physical activity,[28] individuals with an ACLR demonstrate alterations in mechanical loading for months[29,30] to years following surgery.[31,32] Individuals demonstrating asymmetrical tibiofemoral contact force during walking gait following ACLR are less likely to pass return to physical activity criteria[33] and demonstrate lesser self-reported function[29] as compared to those without inter-limb asymmetry during walking gait. Persistent asymmetrical

mechanical loading may also interact with altered tissue metabolism to promote cartilage degradation following ACLR.[26,27]

Maintaining appropriate mechanical loading of the articular cartilage is imperative for maintaining joint health,[34-36] therefore chronic alterations in mechanical loading following ACLR may result in changes in tissue metabolism that lead to cartilage degradation.[26,27] Animal models of osteoarthritis development demonstrate alterations in mechanical loading, both excessive[37,38] and insufficient,[36,39] lead to articular cartilage degradation. The application of a single blunt impact to the patellofemoral joint of rabbits results in greater number and depth of surface fissures in the articular cartilage when compared to patellofemoral joints not subject to a blunt impact.[38] Additionally, greater loading rate of a single blunt impact results in greater fissuring of the retropatellar cartilage in rabbits as compared to a lower loading rate.[37] Conversely, insufficient mechanical loading results in cartilage atrophy[40] and degradation of articular cartilage through an inflammatory response resulting in increased expression of MMPs.[41,42] Removal of sufficient mechanical loading results in a pro-inflammatory response that increases the expression of MMP-1,[41,42] and greater MMP concentration signals degradation of the articular cartilage.[43] Due to the repetitive nature of walking gait, subtle changes in mechanical loading applied over a long duration may lead to degradation of the articular cartilage following ACLR.[17,35]

At 6-months following ACLR individuals demonstrating a lesser peak external knee adduction moment during walking gait in the reconstructed limb also demonstrate greater concentrations of interleukin-6, a pro-inflammatory cytokine.[27] Additionally, lesser peak vertical ground reaction force (vGRF) instantaneous loading rate associates with greater plasma MMP-3 concentration in individuals with ACLR.[27] A separate study determined individuals developing

radiographic PTOA within 5 years of ACLR demonstrate lesser tibiofemoral contact force in the reconstructed limb during walking gait at the time of return to physical activity as compared to individuals who did not develop PTOA.[44] In a cohort of indiviudals who were 3 years post-ACLR, those who demonstrated lesser peak vGRF also demonstrated greater serum type-II collagen turnover.[26] While there is growing evidence that long-term alterations in mechanical loading may influence tissue metabolism following ACLR,[26,27,44] it remains unknown if acute alterations in mechanical loading also influence tissue metabolism in individuals with ACLR.

Acute bouts of mechanical loading induce a biochemical response as quantified via serum markers of cartilage metabolism in individuals without a history of knee injury.[45-47] Moreover, the biochemical response to an acute bout of mechanical loading has been associated with the development of knee OA; greater changes in serum cartilage oligomeric matrix protein (COMP) following 30 minutes of walking associate with cartilage thinning 5 years after ACLR.[47] A greater increase in COMP following acute increases or decreases in mechanical loading may indicate a greater metabolic response to alterations in mechanical loading.[48,49] Assessing the biochemical and biomechanical response to acute bouts of altered mechanical loading may inform the development of future interventions aiming to manipulate knee loading for the purposes of maintaining long-term joint health. However, it remains unknown if mechanical loading can be manipulated following ACLR, and if acute changes in mechanical loading promote beneficial changes in lower extremity biomechanics and biomarkers of cartilage turnover and inflammation.

Real-time biofeedback has been implemented to increase[50] and decrease[51-54] loading as well as promote symmetrical[55-58] loading between limbs during a variety of functional tasks in pathological and control populations. Real-time biofeedback offers the advantage of providing

instantaneous visual or auditory cues during movement allowing for immediate modifications in movement patterns.[59] Moreover, real-time biofeedback coupled with strength training improves recovery and lower extremity movement symmetry in individuals with knee[57,58] and hip arthroplasty.[55] Real-time biofeedback may be advantageous for manipulating knee joint loading during walking following ACLR. Manipulating knee joint loading following ACLR may reduce inflammatory and catabolic processes responsible for the initiation of cartilage breakdown.

The peak vGRF indicates the magnitude of loading applied to entire lower extremity during stance, and also contributes to other kinetic outcomes (i.e. peak KAM, internal knee extension moment) hypothesized to influence the development of PTOA.[60,61] Providing real-time biofeedback that aims to increase or decrease bilateral vGRF or create symmetrical peak vGRF between limbs during walking may be advantageous for manipulating loading following ACLR. While lesser peak vGRF during walking gait associates with greater type-II collagen turnover in individuals three years following ACLR, it remains unknown if acute changes in peak vGRF during walking gait result in simultaneous changes in lower extremity kinetics and kinematics and cartilage metabolism.

Therefore, the purpose of this study was to utilize real-time biofeedback to acutely increase peak vGRF (i.e. overloading), decrease peak vGRF (i.e. under-loading) and promote symmetrical peak vGRF between limbs in individuals with ACLR during walking gait, and to determine changes in lower extremity kinetics and kinematics and cartilage metabolism between overloading, under-loading, and symmetrical loading as compared to normal walking (i.e. control). The specific aims for this dissertation are as follows:

Specific Aims:

Specific Aim 1. Determine if individuals with an ACLR are able to acquire and recall average symmetrical loading, overloading loading and under-loading during walking gait using a real-time biofeedback intervention. Root mean square error (RMSE) was calculated to determine the absolute difference between peak vGRF in the reconstructed limb and the target value provided by the real-time biofeedback. We hypothesized individuals with ACLR would demonstrate greater RMSE during the acquisition period of the under-loading condition as compared to the overloading and symmetrical loading conditions. Additionally, we hypothesized individuals with ACLR would demonstrate greater RMSE during the recall period of the under-loading condition as compared to the overloading and symmetrical loading conditions.

Specific Aim 2. Determine the effect of symmetrical loading, overloading, and under-loading on lower extremity kinetics and kinematics in individuals with an ACLR. We determined vGRF loading rate, peak internal knee extension moment (KEM) and knee flexion excursion during the acquisition and recall periods of each loading condition (control, symmetrical loading, overloading, under-loading) as these variables have been demonstrated to influence PTOA pathogenesis.[62-64] We hypothesized that peak KEM and knee flexion excursion would significantly increase and decrease during the overloading and under-loading conditions, respectively, as compared to the control condition.

Specific Aim 3. Determine the effect of symmetrical loading, overloading and under-loading on cartilage metabolism in individuals with an ACLR. As COMP is the most commonly assessed marker of cartilage metabolism following acute loading protocols,[45,47] we

determined the effect of symmetrical loading, overloading, and under-loading on the change in serum COMP concentration immediately following completion of each loading condition.

Specific Aim 4. Determine the associations between baseline peak vGRF and the change in COMP following each loading condition in individual with an ACLR. We calculated the change in serum COMP immediately following each loading condition. We hypothesized that lesser baseline peak vGRF would associate with a greater change in serum COMP following 20 minutes of normal walking (i.e. control). We also hypothesized individuals with a lesser baseline peak vGRF would demonstrate a lesser change in serum COMP following the overloading condition, and individuals with a greater baseline peak vGRF would demonstrate a greater change in serum COMP following the under-loading condition.

CHAPTER 2: REVIEW OF LITERATURE

Epidemiology and Burden of Osteoarthritis

Osteoarthritis (OA) is one of the most prevalent chronic medical conditions globally, with 10-12% of the entire population in the United States afflicted with symptomatic OA of the knee.[3] Knee OA is a disease of the entire joint, and hallmark signs include cartilage loss, changes in subchondral bone, inflammation of the synovial fluid and degeneration of the meniscus.[65] As a result of the inflammatory and structural changes within the joint, individuals with knee OA experience an increase in functional disability.[1]

Major symptoms of OA include pain, stiffness and a loss of movement and function resulting in a substantial adverse impact on quality of life and a considerable economic burden. The risk of mobility disability (i.e. needing assistant walking or climbing stairs) attributed to knee OA alone is greater than any other medical condition in people older than 65.[66] On a global level, knee OA is the leading musculoskeletal cause of total years lived with disability[67] On average, a non-obese person with knee OA will lose 1.9 quality-adjusted life years due to knee OA specifically; obesity in conjunction with knee OA increases loss to 3.5 quality-adjusted life years.[68]

Declines in physical function subsequently result in declines in physical activity; physical activity is important not only for overall health, but also for improved physical function.[69] Current recommendations for physical activity include 150 minutes of moderate-vigorous physical activity per week[70] or 7,000 steps per day.[71] Less than half of OA participants have been reported to meet daily physical activity guidelines,[72,73] and more time spent in sedentary behavior

is associated with lower physical function.[4] Osteoarthritis is a likely risk factor for the development of cardiovascular disease;[6,74,75] knee OA is associated with several risk factors for the development of cardiovascular disease such as physical inactivity and hypertension.[76] Beyond physical disability, the chronic pain that accompanies knee OA also increases the risk for development of mood disorders such as depression.[77]

Despite the significant physical and global burden of knee OA, effective treatment strategies are lacking. Current diagnosis relies solely on radiographic changes of the joint, at which point irreversible cartilage damage has already occurred. In order to effectively manage OA it is imperative to begin developing strategies that slow the progression of OA before irreversible damage has occurred. In order to develop novel treatment strategies to slow the progression of knee OA, we must first understand the complex pathogenesis that leads to OA development.

The Physiology of Articular Cartilage

The Extracellular Matrix

The extracellular matrix (ECM) is critical for the functional properties of articular cartilage including stiffness, durability and load distribution. The ECM primarily consists of water, however the intricate network of macromolecules (i.e. proteoglycans, collagens) organizes and maintains water within the matrix. Articular cartilage is able to withstand compression because these macromolecules impede the free flow of water throughout the matrix. The ECM is composed primarily of proteoglycans, collagens, and chondrocytes.

Proteoglycans form the major macromolecule of articular cartilage ground substance and include glycosaminoglycans (GAGs), chondroitin sulfate and keratin sulfate. The structure of proteoglycans helps stabilize relationships with type II collagen, and helps control the flow of

water through the matrix.[78] Glycosaminoglycan chains contain a large number of negative ions that repel one another and provide a net negative charge to the matrix of the cartilage.[79] Interactions between the fixed charges of the GAGs provide cartilage the ability to withstand load. During compression, the negatively charged GAGs are forced closer together, which increases the resistive force between molecules, increasing the strength of the ECM and forcing water out of the articular cartilage.[78]

Type II collagen is the predominant collagen type found in articular cartilage. Due to the relationship between type II collagen and proteoglycans, type II collagen is important for maintaining a highly hydrated ECM.[79] The chondrocytes found within articular cartilage are responsible for maintenance of the ECM, which maintains the material properties of the cartilage and allows the articular cartilage to withstand loading.[79] Understanding how chondrocytes respond to mechanical stimuli may help explain what types of load are necessary for maintenance of normal cartilage.

The Pericellular Matrix

The pericellular matrix (PCM) is a small region of matrix that surrounds the chondrocytes and contains a higher concentration of proteoglycans and a fine arrangement of collagen fibers as compared to the ECM.[80] The PCM is composed of fibronectin, proteoglycans and collagen.[80] The PCM also contains type VI collagen, which has been hypothesized to anchor the chondrocyte to the PMC.[81] As the interaction between the ECM and chondrocytes is critical for the maintenance of articular cartilage, the PCM may play a key regulatory role in the maintenance of cartilage health.[80] During compression, the PCM affects the electrical, chemical and mechanical stimuli around a chondrocyte that in turn may affect chondrocyte activity.[80]

Articular Cartilage Structure

Cell shape and size, collagen fibril diameter, collagen fibril orientation relative to the articular surface, water content and proteoglycan content all vary based upon depth from the articular surface.[82] The superficial zone comprises the thinnest zone and contains elongated chondrocytes that lie parallel to the articular surface, allowing the superficial zone the ability to resist shearing forces across the surface. The transitional zone resides between the superficial and deep zones and contains collagen fibrils larger than those within the superficial zone. The deep zone is the thickest zone and contains the largest collagen fibrils, and contains less water as compared to the more superficial zones.

When subject to loading and deformation, articular cartilage exhibits a viscoelastic response, indicating that the response to loading varies even if the load is constant. This viscoelastic response depends upon the viscoelastic properties of the macromolecules that form the solid ECM and the frictional drag arising from the flow of interstitial fluid through the tissue.[78,83] Initially during compression water exudation occurs rapidly and the rate of deformation is rapid. The load applied to the surface of the articular cartilage is then balanced by the compressive stress within the ECM. As the compressive load remains constant cartilage deformation continues until the flow of interstitial fluid slows.[78] When fluid is then redistributed within the ECM the articular cartilage stress decreases over time.[78] A loss in proteoglycan content in articular cartilage has been demonstrated to influence the viscoelastic response of articular cartilage, hampering the ability to attenuate loading.[84]

Mechanisms of Osteoarthritis Development

Early Osteoarthritis Development

Mechanical injury, hereditary factors and ageing can influence the processes that lead to the development of OA. Initially during the hypertrophic repair phase of articular cartilage, GAG content is reduced which leads to increased water content and softening of the articular cartilage.[85] Cartilage mechanical properties are sensitive to composition and structure, therefore loss of GAG content leads to alterations in how the articular cartilage is able to resist compression.[86] Additionally, the release of type II collagen fragments from the cartilage initiates an inflammatory response that can promote cartilage degradation through increased expression of inflammatory mediators and cartilage-degrading proteinases.[87]

The Role of Inflammation in the Development of Osteoarthritis

Under normal conditions, chondrocytes have low metabolic activity and lack an ability to repair damaged cartilage.[18] As the cartilage begins to degrade however, the release of proteoglycan fragments into the synovium initiates a catabolic response. Inflammatory mediators such as cytokines and prostaglandins are released from synovial cells in response to the proteoglycan fragments which are considered foreign bodies within the synovium.[18] Inflammatory mediators then increase the production of matrix metalloproteinases (MMPs) from the chondrocytes, which in turn increase cartilage degradation.[18] Macrophages present in the synovial lining are also capable of expressing MMPs, or the macrophages can produce inflammatory mediators such as IL-1 and tumor necrocis factor α (TNF-α); IL-1 and TNF-α then induce MMP expression by chondrocytes, thus propagating cartilage destruction.[43]

Several MMPs have been suggested to be involved in the inflammatory pathway resulting in the development of OA, including MMP-13, MMP-2, and MMP-3. Specifically, MMP-2 is

capable of cleaving several collagens and can activate other MMPs produced by the chondrocytes of osteoarthritic cartilage.[88] In experimental models of knee OA MMP-3 expression is increased in the synovium and articular cartilage.[89] Greater plasma levels of MMP-3 correlate with greater joint space narrowing in individuals with knee OA.[90] In clinical and experimental data MMP-13 has been demonstrated to play a critical role in cartilage degradation in OA.[91,92] Tissue inhibitors of metalloproteinases (TIMPs) regulate MMP activity and may also be responsible for changes in MMP-mediated cartilage damage during OA. TIMP-1, TIMP-2 and TIMP-3 inhibit a wide array of MMPs; reductions in TIMPs activity lead to increased cartilage degradation through reduced inhibition of MMP activity.[93] Additionally, increased concentrations of inflammatory cytokines such as IL-6 and TNF-α are associated with less knee cartilage thickness.[94] Inflammation in OA also increases self-reported symptoms, as high-sensitivity C-reactive protein levels are associated with level of pain.[95,96]

The Role of Mechanical Loading in the Development of Osteoarthritis

Load-bearing joints such as the knee are subject to cyclic loading over time through daily activities such as walking. Under normal conditions the components of the ECM are under a constant state of turnover with a balance of catabolic and anabolic processes.[97] In a normal environment cyclical loading does not affect the articular cartilage. Altered joint loading however can lead to alterations in structure, composition, metabolism and mechanical properties of the articular cartilage.[97] High impact loading and static compression of articular cartilage lead to damage of articular cartilage, whereas cyclic and intermittent loading stimulate chondrocyte metabolism.[98] Specifically, high impact loading causes significant damage to the articular cartilage in addition to remodeling of subchondral bone.[98] Compression with high-rate loading causes damage to the collagen fibril network, in addition to increased proteoglycan release and

nitric oxide production.[99] The increase in proteoglycan release suggests mechanical stress alone can stimulate cell death as well as a range of biomechanical and biochemical alterations to the matrix.[100] Impact loads cause significant damage to the articular cartilage, including splitting of the ECM, increased cellular activity, increased tissue hydration and remodeling of the subchondral bone.[62] Mechanical compression of cartilage also induces a dose-dependent release of osteoarthritis biomarkers, including proteoglycan fragments and COMP.[101]

Mechanical loading during walking has been identified as a critical factor in the development and progression of OA. Most commonly, increased external knee adduction moments have been associated with medial tibiofemoral compartment OA due to increased mechanical load as compared to the lateral compartment. The knee adduction moment has been associated with cartilage changes in medial knee OA,[34,102] and is sensitive to disease progression.[103]

Although a majority of animal models and investigations in idiopathic knee OA demonstrate increased mechanical loading leads to the breakdown of articular cartilage, recent investigations in PTOA suggest a decrease in mechanical loading following ACLR may initiate cartilage breakdown. Several investigations[104-107] have reported individuals with an ACLR "off-load" their involved limb, or shift load to the contralateral limb during gait. Following ACLR, peak internal knee extension moments[104-107] and peak internal knee adduction moments[31,32,105] are reduced compared to contralateral limbs. A reduction in loading in the involved limb relative to the contralateral limb, or off-loading, may represent a movement strategy to protect the injured joint from excessive loading. Off-loading however, may be harmful to the injured joint as articular cartilage may become deconditioned over time. Deconditioning of the articular

cartilage, particularly during the early phases of rehabilitation, may increase the risk for cartilage breakdown once the individual returns to high level physical activity.[44]

In a cohort of 19 individuals with ACLR, a higher C2C:CPII ratio (type II collagen degradation relative to type II collagen synthesis) was moderately associated with lower peak vGRF during the first 50% of stance phase in the involved limb.[26] In knee OA patients, C2C:CPII ratios predict joint space narrowing or disease progression.[108] The association between the ratio of C2C:CPII and peak vGRF may indicate an adaptation in which individuals following ACLR accepting greater load on the injured limb up-regulated the synthesis of type II collagen in order to better attenuate joint loading.[26] As the participants with an ACLR were on average 40 months post reconstruction, a chronic reduction in peak vGRF over time may lead to increased cartilage degradation through a lack of appropriate loading stimulus which initiates cartilage synthesis.

Using an EMG-drivel model of the knee to estimate tibiofemoral contact forces, Wellsandt et al[44] further investigated the influence of reduced knee loading on OA development following ACLR. Individuals with an ACLR who developed OA within 5 years of reconstruction demonstrated lower peak knee adduction moments at baseline and 6 months following reconstruction as compared to individuals with an ACLR who did not develop knee OA. Individuals with an ACLR who developed OA demonstrated asymmetrical peak knee adduction moments (i.e reduced moment in involved limb) during walking at baseline and 6 months, yet symmetry was restored at 1 and 2 years following reconstruction. The group of individuals with an ACLR developing OA also demonstrated asymmetries in peak knee flexion moments at baseline and 6 months following reconstruction, with reductions in the involved limb as compared to the contralateral limb. At baseline, 6-month and 1-year time points following

reconstruction there were large differences in involved limb peak medial compartment contact force between individuals with an ACLR who did and did not develop knee OA. Peak medial compartment contact forces were lower at baseline and 6-month time-points in individuals who developed knee OA compared to those who did not develop knee OA.

As healthy cartilage increases thickness in response to high repetitive loading during walking,[34] the initial joint unloading commonly demonstrated in the involved limb following ACLR may represent a mechanism for the early cascade of events responsible for the progression of PTOA. Reductions in knee moments and contact forces early following reconstruction may lead to alterations in the structure of articular cartilage,[44] increasing the susceptibility to articular cartilage breakdown once joint loading increases in the involved limb in order to reach inter-limb symmetry one year following reconstruction.

The effect of mechanical loading on a joint depends upon the health status of the joint. Previous research has demonstrated in a healthy joint increased knee moments (i.e greater mechanical loading) demonstrate thicker articular cartilage.[109] Conversely, in individuals with knee OA, an increased knee adduction moment is associated with a decrease in cartilage thickness in the medial tibiofemoral compartment. While mechanical loading clearly plays a role in the development of knee osteoarthritis, it is likely the interaction between mechanical loading and the inflammatory response which progresses joint degeneration.

The Interaction between Mechanical Loading and Inflammation

Various types of aberrant loading also influence the inflammatory cascade of chondrocytes; pro-inflammatory mediators can be inhibited by increased hydrostatic pressure[110] whereas cyclic loading can produce an anti-inflammatory response.[111] Chondrocytes are exposed to a various array of biophysical signals that vary with the time and location of loading that

results in changes in the shape and volume of chondrocytes.[23] Chondrocytes sense physical signals through integrated action of ion channels in which the pericellular matrix serves as a transducer of the physical signals in the cell's environment.[80] At the cellular level the combination of biophysical factors (i.e. normal cyclical loading) and biochemical factors (i.e inflammatory agents) modulate the physiology of the chondrocytes and the health of the joint.[80]

Inflammatory mediators and cytokines appear to play an important role in altered loading models of osteoarthritis. Animal models demonstrate the importance of inflammatory pathways following abnormal mechanical loading. High impact loading causes only a small amount of chondrocyte death immediately following impact, yet chondrocyte death significantly increases over 48 hours which suggests inflammatory mediators released from the damaged cartilage continue to cause progressive cell death.[79]

Andriacchi et al[23] developed a model for overall joint homeostasis which depends on the interaction of a wide range of biomechanical signals ranging from full body mechanics to the mechanical environment of the cell. Alterations in commonly measured external loads applied to the knee joint, such as the peak external knee adduction and flexion moment influence internal knee joint contact forces that then influence the distribution of stress placed upon the cartilage. Alterations in cartilage stress then influence cell metabolism at the tissue level, and continue to propagate changes in external measures of joint loading. Due to the cyclical relationships between external loading and the mechanical environment at the cellular level, taking a multi-disciplinary approach which determines the interactions between categories of factors (i.e biology, structure and mechanics) known to increase the risk of OA development may be advantageous for developing treatment strategies to slow the progression of knee OA.[112]

There is imperative evidence that knee OA develops due to alterations in both mechanical and inflammatory processes. Developing treatments that target either abnormal process is difficult, as official diagnosis does not occur until after irreversible damage to the articular cartilage has occurred. Additionally, determining the early onset of disease remains a challenge. Posttraumatic knee osteoarthritis is a specific phenotype of OA that develops rapidly following traumatic knee injury. Using an injury-response model in order to determine key processes involved in the development of knee OA will allow for the development of early interventions that aim to slow the progression of OA. Injury to the ACL and subsequent reconstruction significantly increases the risk for the development of PTOA. Individuals with an ACLR demonstrate alterations in inflammatory processes as well as mechanical loading, both of which increase the progression of knee OA. Determining the interactions between mechanical loading and joint metabolism following ACLR may allow for the development of effective treatment strategies for knee OA.

Anterior Cruciate Ligament Injury

The anterior cruciate ligament (ACL) is a major ligamentous stabilizer of the knee, which restricts anterior tibial translation as well as rotational forces at the tibiofemoral joint. ACL rupture occurs in approximately 250,000 Americans each year[113,114] with one ACL rupture occurring every 1500 player-hours spent practicing or competing in sports such as football, skiing, basketball, and soccer.[115] ACL deficiency results in pain, increased instability and altered lower extremity function in a large proportion of patients.[116] In order to restore joint stability and increase function, ACLR is commonly performed. Total medical costs, encompassing diagnosis, surgical reconstruction, and post-operative rehabilitation of ACL injuries totals $3 billion in the United States annually.[117]

Anterior cruciate ligament reconstruction provides adequate short-term outcomes; ACLR is effective at regaining joint stability and up to 82% of patients return to some level of physical activity.[118] Unfortunately though, lingering deficits in lower extremity function persist for months[61] to years[119,120] following return to physical activity. A serious long-term consequence of ACL injury and reconstruction is the development of knee OA soon after trauma has occurred.[121] Specifically, one-third of all ACL injured patients develop OA within the first decade following injury regardless of treatment.[13] While mechanisms that contribute to the development of OA following ACL injury are not completely understood, current hypotheses have focused on influences from altered metabolic processes,[122] biomechanical alterations,[123] and deficits in neuromuscular function.[124,125]

Outcomes following ACLR and their Influence on the Development of PTOA

Neuromuscular Consequences of ACLR

Persistent quadriceps weakness has been well established as a consequence of ACLR. Specific alterations in underlying neural pathways responsible for muscle contraction are responsible for a reduction in voluntary quadriceps activation. Acutely following ACLR, reductions in spinal reflex excitability emerge while corticomotor excitability is up regulated in order to maintain voluntary control over the quadriceps.[126] Conversely, at the time of return to physical activity spinal reflex excitability returns while corticomotor excitability is reduced.[126] Moreover, at an average of 48 months following ACLR, individuals with an ACLR demonstrate a reduction in corticomotor excitability compared to individuals without a history of knee injury.[127]

Reduced quadriceps strength influences a variety of outcomes following ACLR. Quadriceps strength predicts 61% of the variance in self reported disability following ACLR.[128]

Additionally, Tourville et al[129] determined individuals with an ACLR demonstrating a reduction in quadriceps strength from initial injury to 4 years post-ACLR also demonstrated significant changes in tibiofemoral joint space width. Reduced quadriceps strength following ACLR directly influences lower extremity biomechanical patterns, as individuals with an ACLR with weak quadriceps display reductions in peak knee flexion angle and knee extension moments as compared to control participants. Individuals with an ACLR with strong quadriceps however did not display significant differences in peak internal knee extension moments during the first 50% of stance phase during walking as compared to control participants.[104] Additionally, quadriceps strength accounted for a significant proportion of the variance of peak internal knee extension moment.[104] Reduced knee flexion has been hypothesized to interfere with the normal ability of the knee to absorb shock during weight acceptance,[130] which may increase early degenerative changes within the joint. A reduction in knee flexion angle during the first half of stance during walking gait also associates with greater peak vGRF and greater vGRF loading rate.[130]

During the stance phase of gait, eccentric action of the quadriceps attenuates force through controlled flexion of the knee.[64,104] Therefore, reductions in quadriceps strength that result in reductions in knee extension moments during gait may lead to increased knee joint loading during walking. As the KEM is representative of the net quadriceps involvement during loading, a reduction in the KEM may lead to reduced energy absorption from the quadriceps, resulting in greater magnitude and loading rate of the articular cartilage.[63,64] Lower KEM during gait has been demonstrated in the ACLr limb compared to both the contralateral limb[29,32,60,106] and healthy control limbs.[104-107,131,132] Individuals with a smaller KEM during early stance demonstrate greater peak vGRF,[133] which has been associated with greater chondral damage[134] and depleted proteoglycan content[135] in canine ACL transection models. Additionally, rapidly

applied loads are also detrimental to joint health, as the application of rapidly applied loads diminishes the viscoelastic response of articular cartilage.[136]

Biomechanical Alterations Following ACLR

Cartilage Contact Patterns

Changes in kinematics during walking have been proposed to initiate a degenerative pathway for articular cartilage through shifting loading to areas of cartilage not conditioned to withstand chronic loading associated with the mechanics of walking.[34] Healthy cartilage adapts to loading during normal, cyclical activities such as walking through increasing cartilage thickness. Cartilage thickness is positively associated with physical activity[137] and cartilage volume is positively associated with muscle cross sectional area.[138] In healthy participants, the location of thickest femoral cartilage is significantly associated with knee flexion angle at heel strike.[139] The highest loads at the knee occur at heel strike, and typically the thickest regions of cartilage on the femur and tibia align when the knee is at full extension. Thus a shift in alignment at heel strike, such as alterations in knee flexion angle following ACLR, can shift normal load bearing to regions not previously conditioned to withstand the high loads that occur at heel strike.[35]

Scanlan *et al*[140] determined the involved limb of ACLR patients had significantly reduced peak knee extension at heel strike compared to the contralateral limb, yet the anterior-posterior location of the thickest femoral cartilage was not different between limbs. Alterations in knee extension at heel strike may cause non-weight bearing areas of the cartilage to withstand load it is not conditioned for. As mature cartilage has limited ability to adapt to increased demands[141] prolonged alterations in tibiofemoral kinematics are likely to damage articular cartilage over time.

Healthy cartilage adapts to the magnitude of loading via increased cartilage thickness and enhanced mechanical properties that are area specific.[35] A shift in loading patterns leads to an increase in fibrillation of the collagen network and subsequently an increase in friction within the joint. Increased friction at the joint surface increases the tangential force at the articular surface, resulting in tearing of collagen fibrils due to increased shear force.[35] Damage to collagen fibers in addition to increased shear force may then lead to an up-regulation of catabolic factors such as MMPs and interleukins.[35]

While ACLR is effective at restoring anterior-posterior joint stability, the restoration of rotational stability is not as definitive. Slight changes in rotational stability may also be responsible for cartilage deterioration through a reduction altering cartilage contact patterns.[35] A computational model of degradation has demonstrated that a 5° rotational shift may be enough to cause accelerated degradation of the cartilage.[142] ACLR and ACL deficient knees demonstrate alterations in tibial internal-external rotation relative to control participants during walking. Scanlan et al[143] determined an average external rotation offset of 2.3° during walking in ACLR limbs compared to healthy control limbs, with some ACLR limbs achieving a 5° offset.

Tibiofemoral Contact Force

Alterations in net moments acting about the knee (i.e knee adduction moment) have been proposed to influence the initiation and progression of knee OA.[144] The relationship between net moment and joint loading is not straightforward however, particularly when agonist/antagonists muscle groups are co-activated. For example a reduction in the net internal knee extension moment could result from a reduction in quadriceps activation, or an increase in flexor muscle activity.[145] Muscle forces are important contributors to total joint force, and therefore altered muscle activity may substantially influence loading of the articular cartilage.[146]

Electromyographic (EMG) modeling of muscle forces acting upon the knee can provide individual muscle activation strategies in order to provide an estimate of muscle forces acting about a joint.

Following ACL rupture patients display a reduction in both knee extensor and flexor muscle groups during walking compared to the contralateral limb.[147] An initial reduction in muscle force may be an attempt at offloading the injured joint following injury in order to prevent instability. Gardiner et al[148] further expanded upon these findings and demonstrated a significant reduction in overall tibiofemoral contact force in the injured compared to uninjured limb. Individually, peak medial and lateral tibiofemoral contact force was also significantly reduced in the injured limb compared to the uninjured limb.[148] A reduction in loading early following ACL injury may initiate alterations in the articular cartilage,[149] thus increasing the risk for deterioration following reconstruction and return to high levels of physical activity.

Following ACLR a proportion of patients continue to demonstrate alterations in tibiofemoral contact patterns at the time of return to physical activity.[33] Individuals with an ACLR who fail return to play criteria demonstrate significant tibiofemoral contact force asymmetries between limbs as compared to individuals with ACLR who pass return to play criteria.[33] Moreover, alterations in tibiofemoral contact force may influence the development of knee OA following ACLR. Individuals with an ACLR who develop knee OA within 5 years of reconstruction demonstrate greater asymmetries in medial tibiofemoral contact force 6 months following reconstruction compared to individuals with an ACLR who do not develop knee OA.[44] The involved limb of individuals with an ACLR developing knee OA demonstrated lower medial tibiofemoral contact force and internal knee abduction moments within the first 50% of stance during walking gait compared to the contralateral limb. Reductions in knee moments and contact

forces early following reconstruction may lead to alterations in the structure of articular cartilage,[44] increasing the susceptibility to breakdown once joint loading increases more than a year following reconstruction in the involved limb increases in order to reach symmetry.

Altered Biomechanical Movement Patterns

Sagittal Plane

Decreased quadriceps strength and lack of full extension range of motion are both common deficits following ACLR despite completion of rehabilitation and a full return to physical activity. Decreased quadriceps strength and decreased range of motion are associated with reduced internal knee extension moment[104] and increased knee flexion angle at heel strike[104,150] respectively. Adequate quadriceps strength can increase joint stability,[151] and plays a crucial role in modulating loading rate and impact forces during gait.[152]

The net internal quadriceps moment during the stance phase of gait counteracts the external knee flexion moment imposed by the vGRF.[153] Alterations in sagittal plane gait kinetics have been reported as both reductions in the internal knee extension moment[31,32,60,61,105,107,154] and reductions in the external knee flexion moment[132,140] in ACLR limbs compared to healthy knees. Both graft type[132] and graft orientation[140] have been demonstrated to alter the external knee flexion angle in ACLR patients. Scanlan *et al*[140] determined a more vertical coronal graft orientation was significantly associated with a lower peak external flexion moment. Vertical placement of the graft reduces the ability to resist anterior tibial translation produced by the internal quadriceps extension moment, therefore individuals with an ACLR adapted their gait pattern in order to reduce the demand placed upon the quadriceps and subsequently reduce joint instability by reducing the external knee flexion moment.[140] When comparing patients with a patellar tendon graft compared to a hamstring graft, Webster *et al*[132] determined patients with a

patellar tendon graft had a significantly lower external knee flexion moment during the weight acceptance phase, whereas the hamstring graft patients had significantly lower external knee flexion moments at terminal stance. The authors concluded the patients with a patellar tendon graft might experience donor site morbidity in which the patients decreased knee flexion during the weight acceptance phase in order to avoid pain. Additionally, the participants with a hamstring graft may have also limited knee extension in order to reduce the knee extension moment and the tension within the hamstring group.[132]

Greater knee flexion throughout the gait cycle has been demonstrated in individuals with an ACLR.[31,32,60] Loss of passive knee extension following ACLR occurs in 10-25% of knees,[155,156] which may be a primary reason for the loss of extension present at heel strike in individuals with an ACLR.[157] Patients with a loss of extensions are more than twice as likely to develop radiographic signs of OA within ten years following reconstruction,[155] and a primary cause may be a shift in cartilage contact patterns with the knee in less extension at heelstrike.[157] Tibiofemoral articular cartilage is conditioned such that the thickest areas of cartilage correspond with the knee extension angle at heel strike.[158] The loss of knee extension following ACLR alters the area of cartilage loaded during heel strike, thus increasing load on cartilage not accustomed to withstanding high compressive loads.[34]

Transverse Plane

Alterations in transverse plane rotation in excess of 5° have been suggested to be significant enough to cause acceleration of articular cartilage degeneration.[142] Webster *et al*[159] determined ACLR patients with both hamstring and patellar tendon grafts demonstrated less internal tibial rotation compared to a control cohort, with 42% of the ACLR limbs demonstrating a difference greater than 5°. Similarly, Scanlan *et al*[140] determined 85% of ACLR limbs

demonstrated greater tibial external rotation compared to contralateral limbs, with 54% demonstrating a difference in external rotation of 5° or greater. The femoral insertion of the ACL graft is in the lateral condyle, while the tibial insertion is centered in the medial-lateral direction. Over tensioning of the graft can lead to a more externally rotated tibia, which alters normal cartilage contact patterns.[160]

Frontal Plane

The external knee adduction moment (KAM) is commonly used as a surrogate for medial tibiofemoral joint loading during non-invasive biomechanical analysis of the lower extremity,[161] and has been associated with both OA severity and progression.[103] Greater KAM during gait predisposes the medial knee cartilage to greater loading and subsequent degeneration.[17,162] The KAM is a result of the vGRF passing medially to the center of the knee joint, causing an increase in the distance between the knee joint center and the resultant ground reaction force vector, and reflects the distribution and magnitude of the load transferred through the medial versus the lateral compartment of the tibiofemoral joint.[163] Loading applied to the medial compartment of the knee is approximately 2.5 times greater than the lateral compartment during normal gait,[164] and is most likely the reason the medial tibiofemoral compartment is most afflicted with OA. The amount of knee varus as well as the vGRF are associated with KAM,[165] indicating changes in kinetic or kinematic variables can influence overall medial tibiofemoral joint loading. The increase in medial tibiofemoral joint compartment loading following ACLR may be of tremendous concern as a 1% increase in the KAM is thought to increase the risk of knee OA by 6.5 times.[103]

As KAM has been proposed to associate with the progression of knee OA,[103] several recent research efforts have been reported in an attempt to determine if KAM is increased in

patients following ACLR as compared to healthy contralateral limbs[32,60,105,166,167] and compared to matched control participants.[31,105,159,166,168,169] Webster *et al*[165] demonstrated a reduced KAM in males undergoing unilateral ACLR with either a hamstring or patellar tendon graft when compared to a matched control group. While there were no significant differences in KAM between the two graft types, knee varus angle was reduced in the hamstring graft ACLR group compared to the matched controls whereas the vGRF was significantly reduced in the patellar tendon graft ACLR compared to the matched controls, potentially demonstrating unique biomechanical alterations to reduce knee joint load dependent upon graft type.[165] The authors speculated the medial hamstrings might apply a medially directed vector contributing to the net force exerted on the tibia relative to the femur during contraction that may contribute to some of the adductor force placed upon the knee.[165] As the medial hamstrings are harvested during ACLR in order to create the graft the medially directed force from the hamstrings may be reduced, thereby reducing knee varus. When assessing differences in the KAM following ACLR based upon sex, women were reported to have a 23% greater peak knee adduction moment compared to males.[167] When KAM was compared longitudinally from 12 month post-ACLR to greater than three years post-ACLR the KAM significantly increased in both limbs from the 12 month time point to the 3 year time point, and was significantly greater in the contralateral limb compared to the ACLR limb.[32] Increased KAM in the contralateral limb has also been reported to be greater than the ACLR limb of males at 1-year post-ACLR.[165] The external knee adduction moment has been demonstrated to be lower in the ACLR limb compared to the contralateral knee[105] as well as to healthy matched controls.[31] A reduced KAM may indicate an unloading biomechanical strategy to reduce pain and excessive loading. Additionally, the increased KAM applied to the contralateral limb may increase the demands on the cartilage propagate joint

breakdown in an otherwise uninjured joint. Varma *et al*[168] found no significant differences in KAM between ACLR and control groups. However, when separating the ACLR group based upon history of a concomitant meniscal tear, cartilage damage or MCL at the time of ACL injury, individuals with a history of concomitant injury demonstrated a significantly greater KAM compared to individuals with an isolated ACL injury.[168]

Kumar *et al*[60] further extrapolated the consequences of excessive KAM on cartilage changes following ACLR on cartilage changes found using T1rho MRI. Individuals with an ACLR demonstrating higher KAM also demonstrated greater T1rho relaxation times (Figure 9), indicating more proteoglycan loss within the joint.[60] Although there is not conclusive evidence that KAM is increased following ACLR, gender, time from injury and concomitant injury all appear to play a role in KAM.

Biochemical Alterations Following ACLR

The development of knee OA following ACLR is likely due to alterations in metabolic and inflammatory activity in addition to specific alterations in loading as previously described. The evaluation of biomarkers following acute injury or response to altered loading may provide insight into the early pathogenesis of disease before irreversible damage has occurred. Alterations in a variety of biomarker classifications have been detailed following ACL injury and reconstruction, from as early as days following injury to years following injury.

Inflammation

Following any injury, a standard pathway of inflammation, necrosis, revascularization and remodeling occurs in order for proper healing to occur.[19] Cellular changes that occur initially following injury are mediated through the release of a variety of inflammatory cytokines. In animal models of ACLR a disordered cytokine pattern of release, with an imbalance between

anabolic and catabolic mediators, leads to continued degradation rather than healing. Moreover, administration of an MMP inhibitor led to improved tissue healing,[170] further proving the influence of degradation enzymes in the acute phase following injury. Elevated cytokines following traumatic injury likely play a key role in abnormal tissue healing, particularly if there is an imbalance of catabolism relative to anabolism or if the time of inflammation is prolonged.

Patients with chronic ACL deficiency demonstrate a sustained inflammatory response, predisposing these patients to cartilage degradation and osteoarthritis development. Marks et al[171] assessed synovial fluid cytokine profiles as well as chondral damage following ACL injury. Concentrations of IL-1β and IL-1ra were significantly elevated compared to a reference group. Additionally, cytokine profiles differed in the ACL deficient group based upon severity of chondral injury. Those with an Outerbridge score of 0 (i.e least severe) demonstrated the greatest concentrations of IL-1ra, an antagonist of the pro-inflammatory cytokine IL-1.[171] In the group of ACL deficient patients demonstrating the most severe Outerbridge classification, concentrations of pro-inflammatory cytokines IL-1β and TNF-α were elevated compared to the other classifications. Pro-inflammatory cytokines IL-1β and TNF-α have been found to inhibit the synthesis of structural molecules that make up the cartilage matrix.[171] TNF-α may also increase the production of IL-1 and MMPs, thus propagating cartilage breakdown.

Following ACLR, alterations in inflammatory cytokine profiles have also been demonstrated. Within the first week following ACLR, patients present with increased synovial fluid concentrations of inflammatory cytokines such as IL-2, IL-1b, IL-6, and IL-1ra compared to a preoperative baseline concentration.[19] However, within 2 months following ACLR, there was no difference plasma concentration of IL-1a, IL-1b, IL-6, IL-1ra, TNF-α when compared to pre-operative concentrations in the same cohort of patients.[19] In support of the deleterious effect

of catabolic activity following ACLR, Kraus et al[172] administered IL-1ra and assessed the influence of IL-1ra on self-reported outcomes and pro-inflammatory profiles. Patients receiving IL-1ra treatment demonstrated greater improvement in self reported function quantified via the KOOS as compared to the control group. Additionally, the IL-1ra group demonstrated a decrease in IL-1α, a pro-inflammatory cytokine, as compared to the control group.

Alterations in inflammatory profiles following ACL injury and reconstruction are of concerns as increased concentrations of pro-inflammatory cytokines increase degradation of the cartilage matrix over time. Additionally, the addition of anti-inflammatory treatments has demonstrated the ability to improve outcomes initially following ACLR. Targeting alterations in inflammatory profiles following ACLR may be beneficial at slowing the progression of OA following traumatic injury.

Collagen and Proteoglycan Biomarkers

Articular cartilage is composed primarily of type-2 collagen, proteoglycans, and GAGs that allow the articular cartilage to resist compressive loads. Breakdown of the fundamental components of articular cartilage may be an early indicator of disease, which is detectable well before imaged evidence of disease.[173]

Within the first week following surgery, ACLR patients demonstrate increased synovial fluid concentrations of TIMP-1, a tissue inhibitor of MMPs which are known to degrade structural components of articular cartilage, as compared to concentrations before surgery suggesting an increase in.[174] Plasma concentrations of MMP-8 however were found to be no different than baseline concentrations before reconstruction.[174] A lack of change in MMP-8 following ACLR suggests a sustained inflammatory response, likely caused by the trauma of reconstruction itself.

C-telopeptide of type II collagen is a bi-product of articular cartilage degradation, and appears to have both diagnostic and prognostic capabilities for knee OA.[175] Within the first 2 months following reconstruction urinary concentrations of CTX-II have been demonstrated to be significantly greater than control participants.[176] Additionally, greater reductions in CTX-II following reconstruction associated with reductions in pain and increased self-reported function.[176] Within 1 year following reconstruction, ACLR patients demonstrate significantly greater urinary concentrations of CTX-II as compared to control participants.[22,176] ACLR participants also demonstrate greater synthesis to degradation ratios (CTX-II:CPII) when compared with control participants.[177] Furthermore, Tourville et al[177] determined greater CTX-II:CPII were significantly greater in ACLR participants with abnormal joint space width at 4 years following reconstruction compared to control participants. Individuals with an ACLR demonstrating abnormal joint space width also demonstrated worse pain and quality of life scores compared to ACLR patients with normal joint space width.[177]

The Interaction between Mechanical and Biochemical Alterations

System based approaches have been developed in order to characterize the multi-factorial development of knee OA. Alterations in structure (i.e. joint alignment, cartilage morphology), biology (i.e. cell metabolism, inflammation) and mechanics (i.e. ambulation) outside the homeostatic envelope of function create a pre-osteoarthritic state which increases the risk of disease.[112] As a majority of individuals undergoing ACLR are young and physically active, specific alterations in mechanics are likely outside the envelope of function as compared to biology or structural components.[112] Manipulating mechanics following ACLR is likely the most direct method to maintain joint homeostasis.[112] The previous sections detail the alterations in both mechanics and cartilage turnover following ACLR. As alterations in mechanical loading

and inflammation are likely a cyclical process, with one component directly influencing the other and vice versus,[178] it is imperative to further understand the interaction between mechanical loading and cartilage turnover following ACLR that may lead to OA progression.

Cartilage oligomeric matrix protein (COMP) is a glycoprotein found in articular cartilage the helps to stabilize and align type II collagen molecules. When articular cartilage is broken down, COMP is released into the circulation, making it a useful marker of cartilage degeneration.[179] Several studies[180-182] have reported higher COMP levels in OA patients than in healthy controls, suggesting increased turnover and damage to the cartilage matrix. The fact that COMP is mechano-sensitive and plays a role in transducing mechanical forces in the ECM[183] suggests the possibility a mechanical stimulus could be an approach to evaluate the sensitivity of COMP as a prognostic indicator of articular cartilage health.

Mechanical loading is essential for the development and maintenance of articular cartilage, with alterations in mechanical loading leading to degeneration. Cartilage oligomeric matrix protein interacts with collagen and is suggested to have a role in regulating fibril assembly as well as a structural role for maintaining the collagen network, therefore a loss of COMP likely influences the mechanical properties of articular cartilage.[184] COMP appears to be mechanosensitive as the time for concentrations of COMP to return to baseline increases as loading increases. Specifically, COMP has been demonstrated to return to baseline concentrations after 30 minutes following 60 minutes of walking,[48] within 24-48 hours following a marathon,[185] and within 6 days following an ultra-marathon.[186]

In a study assessing the diurnal variation in COMP, a putative half-life of COMP was calculated to be 7.4 hours, which COMP concentrations remaining stable throughout the day and the lowest concentrations occurring at night during bed rest.[187] Kersting et al[188] demonstrated a

moderate correlation between serum COMP concentration and knee joint cartilage volume following an hour of running. Following 30 minutes of running and jump-landing, serum COMP has been reported to increase by 30.7 and 32.2%, respectively.[45] When comparing cartilage deformation measured via ultrasound, there was significantly greater deformation during running as compared to jump landing.[45] Interestingly, during the running condition changes in COMP did not associate with changes in cartilage deformation yet during the drop landing condition changes in COMP did significantly associate with changes in cartilage deformation indicating lesser decrease in cartilage volume the greater the increase in serum COMP concentration.[45] It may be that the lower frequency of loading during the drop landing condition resulted in greater time for recovery of cartilage volume, and greater recovery of the articular cartilage led to greater COMP release. [45]

Helmark et al[189] exposed knee OA participants to a 30 minute moderate loading intervention consisting of unilateral knee extension exercises. Synovial fluid concentrations of COMP were significantly decreased as compared to baseline, yet there were no changes in IL-6 or CTX-II. Conversely, Andersson and colleagues[190] demonstrated an increase in serum COMP levels following 60 minutes of walking in participants with knee OA. Differences in COMP concentrations between these studies may be due to differences in mechanical loading during the intervention.

In a longitudinal analysis of COMP concentration immediately following and at 3.5 and 5.5 hours post 30 minutes of walking it was determined greater increases in COMP levels at 3.5 and 5.5 hours after 30 minutes of walking associated with thinner knee joint articular cartilage after 5 years.[47] The stimulus-response model of assessing changes in COMP following a loading protocol appears to induce meaningful short term changes that may not be detectable based upon

resting levels of COMP. Additionally using a stimulus-response model to determine relationships between changes in COMP and OA development allows reduced inter-subject variability as each participant is evaluated relative to their own baseline resting value.[47]

Utilizing COMP in order to determine the influence of altered mechanical loading in individuals with ACLR may provide insight as to the specific types of loading that are most beneficial for the articular cartilage.

The Use of Feedback to Alter Movement

Motor learning is the relatively permanent acquisition of motor skills, in which there are three stages of learning.[191] The cognitive stage of learning occurs first and is characterized by the learner's conscious attempt to determine the step-by-step process of how a task is to be completed. The associative stage then begins once the individual begins to acquire the basic movement pattern, and finally after extensive practice the individual reaches the autonomous stage in which movements are fluent and effortless.[191] During the autonomic stage the movement execution requires little or no attention.

Repetitive motions such as walking are governed by motor programs that coordinate both the timing and intensity of muscle contraction. Motor programs automate movement and therefore allow for attention to shift to higher level tasks rather than focusing on movement alone.[192] As motor programs are refined over many years, altering movement patterns following injury is difficult. A variety of variables that affect the learning of motor skills have been identified in order to allow researchers to implement strategies that enhance motor learning. The type and frequency of feedback administered, as well as the use of a model or physical guidance have been demonstrated to influence motor learning.[193]

Focus of Attention

Feedback directed towards an internal focus of attention cues a specific change in body movements, such as "Bend your knees when you land". Conversely, an external focus of attention occurs when feedback is directed towards the outcome of movement, such as "Keep the platform stable". An external focus of control is the preferred method of feedback as it accelerates the learning process and enhances efficient movement patterns.[194] Providing feedback that induces an external focus rather than an internal focus results in greater movement effectiveness and efficiency, and can also promote increased retention.[194]

Learning new or improving motor skills can be conducted with the use of instructions focusing on an internal focus of attention (focus on the movements themselves) or utilizing an external focus of attention in which the instructions direct the performer's attention away from their own body movements and to the effects that those movements have on the environment.[193] Typically, a novice learner is provided with specific instructions on how to correctly achieve the motor skill of interest, which refer to the coordination of the learner's body movements.[193] Directing the learner's attention to his or her own movements does not only disrupt the execution of automated skills but can also have degrading effects on learning.[193]

When learners focus on their body movements they are more likely to consciously intervene in order to control movement and disrupt the coordination of a number of relatively autonomic processes that normally control movement.[195] It has been suggested that an internal focus of attention couples agonist and antagonist muscle groups; increased co-contraction of agonists and antagonist muscle groups while utilizing an internal focus of attention limits the degrees of freedom of movements.[196] Increased co-contraction when utilizing an internal focus

of attention increases the recruitment of unnecessary motor units within muscles and creates noise within the motor system.[196]

Poor performance following feedback directing an internal focus of attention may in fact be due to a constrained motor system.[195] An internal focus of attention results in a more conscious type of control that may constrain the automatic control processing of the motor system.[197] Conversely, a external focus of attention facilitates motor learning as a focus on movement effect promotes the use of unconscious or automatic processes.[197] McNevin et al[195] demonstrated a lower root mean square error (RMSE) during a balance task when utilizing an external focus of control as compared to an internal focus of control. Participants were asked to balance on a sabilometer that contained a marker aligned with the toes, far inside the toes, or far outside the toes. When the external focus of control was placed further away from the body RMSE subsequently reduced, confirming the hypothesis of greater learning of motor skills as the feedback is driven away from the body movements themselves. Additionally, RMSE was reduced at the retention time point (3 days following) in the group of participants provided an external focus of control at the greatest distance from the body. The use of an external focus of attention is better suited for acquisition of complex motor skills as it enhances skill acquisition more efficiently, and increases the transfer of improved motor skills.[191]

Recent studies have demonstrated that instructions focusing on an external focus of attention result in greater knee flexion angles[198] and lower peak vGRF[199] during jump landing as compared to providing instructions focusing on an internal focus of attention. Gokeler et al[200] demonstrated individuals with an ACLR performed a single leg hopping task with greater knee flexion and greater time to peak knee flexion when provided an external focus of control compared to an internal focus of control.

Frequency of Feedback

While providing the correct type of feedback of important, the frequency of feedback delivery can also influence acquisition and retention. For example, providing knowledge of results after every 5 trials increases consistency and preciseness as compared to providing knowledge of results after every trial.[201] Providing feedback after each attempt at a task likely leads to constant corrections by the learner, making the learner unable to acquire a consistent motor pattern.[202] Lower frequency of feedback likely leads to a greater movement consistency as small adjustments can be made over several trials. Feedback provided after each time a task is performed may lead to overcorrection of an error which negatively impacts learning over time.[201]

A third hypothesis as to why higher frequency feedback is less effective for motor learning is that the learner becomes dependent upon receiving information in order to make adjustments. As the learner becomes dependent upon external information the capacity to detect and correct errors becomes inhibited, causing a block in the operations of memory recall and decreasing retention of the motor task.[203] Decreasing feedback frequency shifts the learner's dependence from external to internal cues in order to reinforce learning. Willy et al[204] has demonstrated the benefits of mirror gait retaining for the treatment of patellofemoral pain in female runners. The total amount of time feedback was provided was gradually reduced over the course of 8 training sessions. Following the intervention hip adduction and contralateral pelvic drop were significantly reduced when compared to baseline, and these changes persisted for three months.[204]

Assessment of Feedback

Acquisition of motor learning is assessed while the learner is provided with feedback, whereas retention of the task is assessed at any time following completion of feedback.

Researchers commonly utilize retention and transfer tasks that are performed after a certain time interval following training in order to negate the influence of any temporary performance-enhancing effects or performance degrading effects such as fatigue.[205] The root mean square error (RMSE) has been commonly used to assess motor skill acquisition and retention. [183,206] The RMSE represents the squared difference between the target and the actual value achieved by the individual.[195,206]

Real-Time Biofeedback

Real-time biofeedback allows learners to make immediate adjustments in movement patterns, and has a positive effect on task performance and influences motor memory. Real-time biofeedback has been implemented in a variety of methods in order to reduce biomechanical patterns known to increase the risk of sustaining an injury to the ACL.[59,191,205] During walking, real-time biofeedback on bilateral peak vGRF has been used to increase limb symmetry following total hip arthroplasty. White et al[55] implemented 8-weeks of real-time biofeedback while participants walked on a force-measuring treadmill at self- selected speed. Two bar graphs representing the peak force during the first half of stance were visually displayed on a step-by-step basis; participants attempted to walk with an equal loading distribution by matching the height, or force magnitude of the left and right bar graphs. Each participant completed three cycles of 5 minutes in which feedback was provided during the first 3 minutes and was removed for the last 2 minutes of each cycle. While the real-time biofeedback did not significantly change the peak force symmetry index, there was a significant improvement in loading rate symmetry when compared to baseline.

Biofeedback of peak force has also been utilized following total knee replacement in an attempt to increase movement symmetry. Zeni et al[57] determined real-time symmetry training of

peak vGRF during the leg press exercise in conjunction with standard rehabilitation following TKA was more effective than standard rehabilitation alone at improving clinical outcomes and inter-limb biomechanical symmetry during walking and completing a sit to stand task. Real-time biofeedback has also been implemented in OA patients in order to reduce medial compartment loading. By cueing a change in kinematic outcomes (i.e. toe out gait, contralateral trunk lean) the peak KAM of the involved limb subsequently reduced.[207,208]

CHAPTER 3: EXPERIMENTAL DESIGN AND METHODS

Overview: Aims 1-4

The purposes of Aims 1-4 are to determine how acutely altering joint loading influences lower extremity biomechanics and cartilage metabolism in individuals with an ACLR. Aim 1 determined if individuals with an ACLR demonstrate differences in RMSE during the acquisition and recall of symmetrical loading, overloading and under-loading during walking gait. Aim 2 determined how acutely increasing peak vGRF (i.e. overloading), decreasing peak vGRF (i.e. under-loading) and promoting vGRF symmetry between limbs influences lower extremity kinetics (i.e. vGRF loading rate, peak KEM) and kinematics (i.e. knee flexion excursion). Aim 3 determined how acutely increasing peak vGRF (i.e. overloading), decreasing peak vGRF (i.e. under-loading) and promoting vGRF symmetry between limbs influences cartilage metabolism (i.e. change in serum COMP). Aim 4 determined the association between baseline peak vGRF and the change in serum COMP following each loading condtition. This research seeks to provide a framework for future development of therapeutic interventions that proactively manipulate joint loading for the purpose of maintaining homeostatic cartilage metabolism following ACLR.

Participants: Aims 1-4

All participants were participating in unrestricted physical activity as allowed by their orthopaedic physician, which included at least 30 minutes of physical activity three times per week. We excluded individuals: 1) with a history of musculoskeletal injury to either leg (e.g.

ankle sprain, muscle strain) within 6 months prior to participation in the study, 2) a history of lower extremity surgery other than ACLR, 3) with a history of knee osteoarthritis or current symptoms related to knee osteoarthritis (e.g. pain, swelling, stiffness), 4) who were currently pregnant or planning to become pregnant while enrolled in the study, 5) with a history of cardiovascular restrictions that limited the participant's ability to participate in physical activity. Participants were asked to self-report age, sex, ACL graft type, and the date of ACL injury and ACLR. Height and weight were measured in the laboratory prior to testing.

Experimental Procedures: Aims 1-4

Experimental Design

This investigation utilized a single-blinded crossover study design in which each participant completed four separate testing sessions separated by a 1-week washout period. All participants provided informed consent prior to collection of any outcome measures, and the Institutional Review Board approved all study procedures. Each testing session had an identical testing protocol that included baseline assessments of each outcome measure, a 20-minute bout of loading, and serial post-loading condition assessments immediately, and 45 minutes following the loading condition (Figure 1). The 20-minute loading condition was the only component that differed during each testing session.

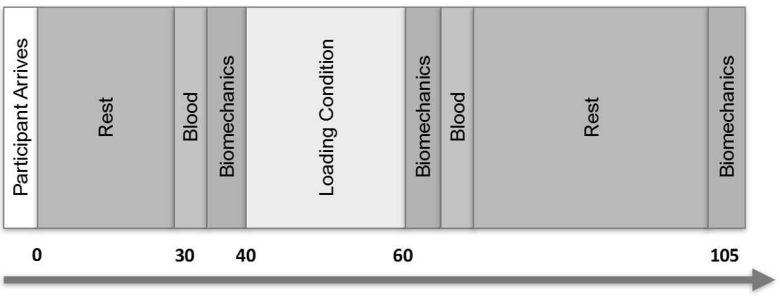


Figure 1: Experimental Protocol for Aims 1-4.

Experimental Procedures

Collection of Self-Selected Gait Speed

During the first control session, prior to treadmill walking, self-selected gait speed was determined using timing gates during 5 over-ground walking trials (Brower TC-Gate; Brower Timing Systems, Draper, Utah). This over-ground walking speed was used to set the speed of the treadmill, which remained consistent during all 4 testing sessions. Before beginning the first testing session (control session) participants walked on the treadmill for 5 minutes to allow for acclimation to treadmill walking.[50]

30-Minute Rest Period

Upon arrival to each testing session participants were seated in a padded treatment plinth with their knees in full extension for 30 minutes in order to allow for normalization of serum concentrations of COMP.[45-47]

Self-Reported Outcomes

During the resting portion of the initial testing session participants completed three self-reported outcome questionnaires administered via Qualtrics software (Provo, UT) through the University of North Carolina at Chapel Hill server. The following questionnaires were completed:

- **Knee Injury History Form**: The knee injury history form assessed general inclusion and exclusion criteria, determine previous history of lower extremity injury and surgery, and collect surgical information about the participant's ACLR.

- **The International Knee Documentation Committee (IKDC) Subjective Form:** The IKDC collects information relative to 1) symptoms, 2) sports and daily activities, and 3) current knee function and knee function prior to injury.[209]
- **Tegner Activity Scale**: The Tegner Scale assesses the highest level of physical activity the participant participated in before their ACLR, as well as current level of physical activity.[210]

Collection of Baseline Blood Samples

Five milliliters of antecubital venous blood were collected via a standard vacutainer serum separator tube with clot activator gel (BD Vacutainer® SST™, Becton Dickinson and Co., Franklin Lakes, New Jersey, USA) using a 21-gauge needle. Samples were placed on ice immediately until transported to the Applied Physiology Laboratory for long-term storage preparation following completion of the data collection session.

Collection of Baseline Lower Extremity Biomechanical Outcome Measures

Participant Set-Up

Individual passive markers were attached via double-sided tape to the trunk, pelvis, and bilateral thigh, shank and foot using double-sided tape (7th cervical vertebrae, anterior superior iliac spines, posterior superior iliac spines, sacrum, medial and lateral femoral epicondyles, medial and lateral malleoli, first metatarsal heads, fifth metatarsal heads, and posterior calcanei). Rigid clusters of 3 (right thigh, left shank) or 4 (left thigh, right shank) passive markers were secured to the thigh and shank using elastic wraps and secured with self-adhesive elastic wrap.

Instrumentation

Kinematic variables were collected at 100Hz using a 14 camera motion capture system, kinetic variables were collected at 1000Hz from two force plates imbedded within a split-belt treadmill (Bertec, Columbus, Ohio).[50] All biomechanical outcomes were acquired using Cortex motion capture tracking software (Motion Analysis Inc, Santa Rosa, CA).

Procedures

Once all passive markers were secured participants began walking at their preferred self-selected speed on the force-measuring treadmill. Biomechanical data were collected for one minute in order to determine baseline biomechanical outcomes (vGRF loading rate, peak KEM, and knee flexion excursion).

Real-Time Biofeedback Loading Conditions

During the baseline trial of the control session a custom MATLAB (Mathworks, Inc, Natick, MA) program processed and extracted left and right limb peak vGRF from the first 50% of the stance phase, which was used to determine the biofeedback targets for the three loading conditions (symmetrical loading, overloading, and under-loading) conducted in the subsequent sessions.

For the symmetrical loading, overloading, and under-loading sessions a projection screen directly in front of the treadmill displayed the real-time biofeedback. A second custom MATLAB script continuously computed the average of the previous four peak vGRF during the first 50% of stance phase, which was visually displayed as right and left blue bar graphs on the projection screen, with a red target line across the center. The target line for the symmetrical loading condition corresponded to the mean peak vGRF between the ACLR and contralateral limb collected during the baseline trial of the control session. The target line for the overloading

condition corresponded to a 5% increase in the baseline peak vGRF for the ACLR limb and the contralateral limb. The target line for the under-loading condition corresponded to a 5% decrease in baseline peak vGRF. The target line was always displayed in the center of the screen for each loading condition to maintain participant blinding to condition. Target values for the overloading (5% above baseline peak vGRF) and under-loading (5% below baseline peak vGRF) were determined individually for left and right leg based on the baseline value of each limb. Therefore, the overloading and under-loading conditions did not specifically cue inter-limb symmetry, rather a relative change in magnitude in each limb.

Before completing the real-time biofeedback loading condition a study investigator (BALH) conducted a brief presentation with each participant explaining the peak vGRF and how the biofeedback continuously displayed peak vGRF. Participants were instructed to match the height of each blue bar (i.e. peak vGRF) to the target line as close as possible during each loading condition and utilize any movement strategy possible to manipulate peak vGRF. During the initial presentation all participants were provided one strategy that focused on manipulating the vertical displacement of their center of mass (COM). Specifically, that increasing or decreasing the vertical displacement of their COM may result in a subsequent increase or decrease in peak vGRF, respectively. We provided one strategy to maximize the success of participants consistently reaching the target.

Assessment of Acquisition and Difficulty during the Loading Condition

One minute of biomechanical data was collected as previously described during the first minute Acquisition$_1$, and the final minute (i.e. minute 19) of the loading condition (Acquisition$_1$) to determine RMSE of each loading condition. Patients were asked to rate their perceived difficulty when attempting to reach the biofeedback target at Difficulty$_5$, Difficulty$_{10}$,

Difficulty$_{15}$, and Difficulty$_{20}$ via a 10cmVAS that ranged from "not at all difficult" (i.e. 0) to extremely difficult (i.e. 10).

Post-Loading Condition

Collection of Immediate Post-Loading Condition Lower Extremity Biomechanics

Immediately following completion of the 20-minute loading condition biomechanical data was collected for one minute as previously described in order to assess RMSE and lower extremity biomechanical outcomes (vGRF loading rate, peak internal knee extension moment, and knee flexion excursion) during immediate recall (Recall$_1$). For the collection of post-loading condition biomechanical outcomes participants were be instructed to "Walk as you had previously walked in order to match the force from each leg to the target."

Collection of Post Loading Condition Blood Sample

Five milliliters of whole blood samples were be collected as previously described at the following immediately following completion of Recall$_1$.

Collection of 45 Minute Post Loading Condition Lower Extremity Biomechanics

Following forty-five minutes of rest participants walked on the force-measuring treadmill and data will be collected for one minute as previously described in order to assess RMSE and lower extremity biomechanical outcomes at Recall$_{45}$. Participants were instructed to "Walk as you had previously walked in order to match the force from each leg to the target."

Data Processing and Reduction

During each session data was collected at four time points to be used for various analyses needed in order to address Specific Aims 1-4 (Figure 2). The blue rectangles indicate time

points where various outcome measures were assessed before, during and after each loading condition.

During the first collection time point (Baseline) biomechanical and biochemical outcomes were collected immediately before the loading condition (Aims 2-4). One minute of biomechanical outcomes was collected during the loading condition at 1) the second collection time point (Acq_1) which occurred during the first minute of the loading condition, and at 2) the third collection time point (Acq_{19}) which will occur during the final minute of the loading condition (Aim 1). The third collection time point occurred immediately following completion of the 20-minute loading condition (Rec_1) and consisted of collection of biomechanical and biochemical outcomes (Aims 2-4). The fourth collection time point occurred forty-five minutes following the 20-minute loading (Rec_{45}) and condited of collection of biomechanical outcomes (Aims 2-4).

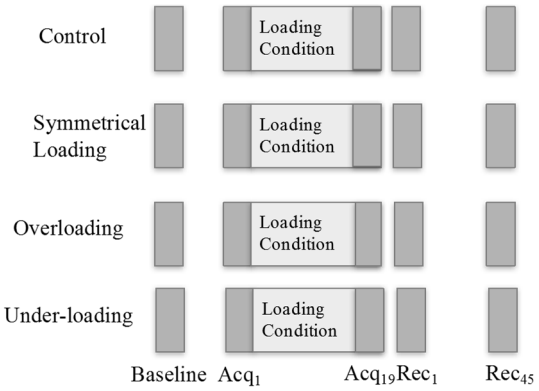


Figure 2. Analysis Time Points for Entire Study

Aim 1 – Acquisition, Recall and Difficulty

Root mean square error (RMSE) was calculated during each acquisition and recall trial using Equation 1 to determine the average difference in actual peak vGRF compared to the target value across each 60-second trial.

Equation 1:

$$RMSE = \sqrt{\frac{(Target\ vGRF - Actual\ vGRF)^2}{Total\ Steps}}$$

Patients were asked to rate their perceived difficulty when attempting to reach the biofeedback target at $Difficulty_5$, $Difficulty_{10}$, $Difficulty_{15}$, and $Difficulty_{20}$ via a 10cmVAS that ranged from "not at all difficult" (i.e. 0) to extremely difficult (i.e. 10). Perceived difficulty VAS we analyzed using a tape measure, with greater scores indicating more perceived difficulty.

Aim 2 – Lower Extremity Biomechanics

Kinematic data were filtered using a 4th order low-pass Butterworth filter with a cut-off frequency of 6Hz, and kinetic data were filtered using a 4th order low-pass Butterworth filter with a cut-off frequency of 100Hz.

The static calibration trial and functional hip joint centers were used to scale a seven segment, 18 degree-of-freedom model of the pelvis and left and right lower extremities.[211] The filtered marker and force data were used to estimate sagittal plane knee angle (flexion [+]) and internal sagittal plane knee moment (extension [-]) using previously described inverse dynamics calculations.[212] All outcomes were identified during the first 50% of the stance phase of gait, which we determined as the the interval from initial contact (vGRF ≥ 20N) to toe-off (vGRF ≤ 20N) and stride-averaged across the 60-second trial using a custom-built MATLAB program. The body mass of each participant was converted to Newtons (N) and used to normalize peak vGRF (xBW) and instantaneous vGRF loading rate (xBW/seconds). Instantaneous vGRF loading rate was determined by calculating the first derivative of the slope from the force-time

curve. Peak internal KEM was normalized to the product of bodyweight and height (xBW*meters). Knee flexion excursion was calculated from sagittal plane knee angle at initial contact to peak knee flexion angle. Four percent change scores were calculated for each biomechanical outcome during each loading condition.

The first percent change score determined the change in each biomechanical outcome from Baseline to Acq_1 using the following equation:

$$Percent\ Change = \frac{Acq_1 - Baseline}{Baseline} * 100$$

The second percent change score determined the change in each biomechanical outcome from Baseline to Acq_{19} using the following equation:

$$Percent\ Change = \frac{Acq_{19} - Baseline}{Baseline} * 100$$

The third percent change score determined the change in each biomechanical outcome from Baseline to Rec_1 using the following equation:

$$Percent\ Change = \frac{Rec_1 - Baseline}{Baseline} * 100$$

The fourth percent change score determined the change in each biomechanical outcome from Baseline to Rec_{45} using the following equation:

$$Percent\ Change = \frac{Rec_{45} - Baseline}{Baseline} * 100$$

Aim 3 – Biochemical Markers

Once transported to the Applied Physiology Laboratory, baseline and post-loading condition sera samples were centrifuged (IECCentra-8R Refrigerated Centrifuge, International Equipment Company, Needham Heights, Massachusetts, USA) at 3000 g for 10 minutes at 4° C.[26] Sera samples were pipetted into sterile 1.5 ml polypropylene long-term storage cryogenic

vials (Nalgene 1.5 ml Long-term Storage Cryogenic Vial, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples were stored at -80° C until all data was collected.

Biomarker Data Analysis: Enzyme Linked Immunosorbent Assays

Serum COMP concentration was assessed via a commercially available specific enzyme-linked immunosorbant assay (ELISA) (BosterBio, Pleasanton, CA) with an assay detection sensitivity of <10pg/ml. Unknown samples were diluted 33x. All assays were performed in triplicate determinations for standards and unknowns and demonstrated inter- and intra-assay variability <10%. Sample and standard microplate addresses were recorded in a spreadsheet for each assay. Well absorbance values were recorded in the corresponding spreadsheet cell addresses. The mean values for individual assay standards were calculated and the standard curve for each assay was determined and evaluated for optimal curve fitting.

First, a percent change scores was calculated for each of the four testing sessions for serum COMP from Baseline to Rec_1 using the following equation:

$$Percent\ Change = \frac{Rec_1 - Baseline}{Baseline} * 100$$

Sample Size Analysis

In order to determine the sample size needed to sufficiently power all four of the specific aims in the proposed study, we collected pilot data on 3 participants who were provided with real-time biofeedback which cued average symmetrical loading between limbs, as well as real-time biofeedback to increase and decrease symmetrical loading to varying magnitudes. We calculated Cohen's *d* effect sizes for biomechanical outcome measures of interest using mean differences and variability to. Cohen's *d* effect sizes for changes in COMP were estimated based

upon previously published researched assessing changes in COMP following loading.[46] Effect sizes for each outcome are depicted in Table 1.

Table 1. Effect Sizes for Sample Size Analysis

Outcome Measure	Cohen's *d* Effect Size	Conditions Assessed for Mean	Conditions Assessed for Variance
RMSE	1.21	Average Symmetrical Loading vs Symmetrical Underloading at 105% Body Weight	Pooled Average Symmetrical Loading and Symmetrical Underloading at 105% Body Weight
RMSE	0.07	Average Symmetrical Loading vs Symmetrical Overloading at 125% Body Weight	Pooled Average Symmetrical Loading and Symmetrical Overloading at 125% Body Weight
RMSE	1.21	Symmetrical Underloading at 105% Body Weight vs Symmetrical Overloading at 125% Body Weight	Pooled Symmetrical Underloading and 105% Body Weight vs Symmetrical Overloading at 125% Body Weight
Knee Flexion Excursion	1.35	Average Symmetrical Loading vs Symmetrical Underloading at 105% Body Weight	Pooled Average Symmetrical Loading and Symmetrical Underloading at 105% Body Weight
Knee Flexion Excursion	0.5	Average Symmetrical Loading vs Symmetrical Overloading at 125% Body Weight	Pooled Average Symmetrical Loading and Symmetrical Overloading at 125% Body Weight
Knee Flexion Excursion	2.07	Symmetrical Underloading at 105% Body Weight vs Symmetrical Overloading at 125% Body Weight	Pooled Symmetrical Underloading and 105% Body Weight vs Symmetrical Overloading at 125% Body Weight
Knee Flexion Excursion	0.95	Average Symmetrical Loading vs Symmetrical Underloading at 5% above Average Symmetrical Loading	Symmetrical Underloading at 105% Body Weight
Knee Flexion Excursion	0.5	Average Symmetrical Loading vs Symmetrical Overloading at 5% below Average Symmetrical Loading	Symmetrical Overloading at 125% Body Weight
Peak vGRF	0.39	Average Symmetrical Loading vs Symmetrical Underloading at 5% above Average Symmetrical Loading	Maximum variability to meet the target of 5% change from Average Symmetrical Loading
Peak vGRF	1.44	Average Symmetrical Loading vs Symmetrical Overloading at 5% below Average Symmetrical Loading	Maximum variability to meet the target of 5% change from Average Symmetrical Loading
COMP	1.44	Baseline Serum COMP vs Serum COMP following 30 minutes of Running	Pooled Baseline Serum COMP vs Pooled Post-Running Serum COMP

As effect sizes for each outcome ranged from weak to strong, we chose to power this study to be able to detect statistical significance if differences between conditions and over time were moderately sized effects (Cohen's $d = 0.5$). We chose not to power this study based off of the smallest effect size of 0.07 as this resulted in an estimated sample size of 332 participants needed to achieve statistical power. We used G*Power Statistical Power Analysis Software v3.1 to estimate the sample size needed in order to detect a moderate effect size of 0.5. As our largest ANOVA model for Specific Aims 1-3 is a 4 x 4 (condition x time) repeated measures ANOVA, we chose to estimate the total number of participants needed to detect a 0.5 effect size with $\alpha = 0.05$ and $1 - \beta = 0.8$ using a within condition and within factor repeated measures ANOVA with 4 conditions and 3 time points. We estimated that 29 participants would be needed to detect significant differences using the ANOVA models for Specific Aims 1-3. For Specific Aim 4, we

estimated 30 participants were needed in order to detect a correlation coefficient of 0.49 with $\alpha = 0.05$ and $1 - \beta = 0.8$, therefore we have chosen to include 30 participants for this study.

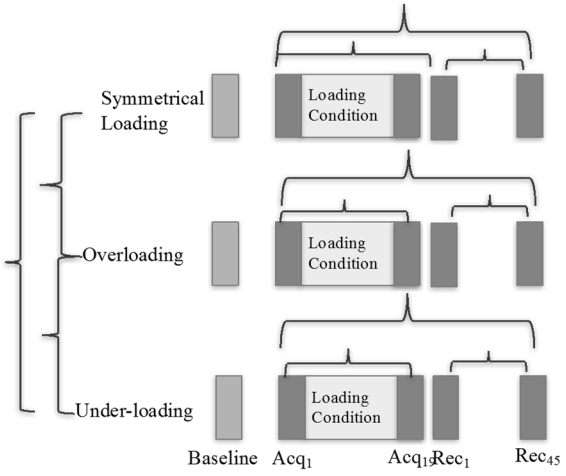
Statistical Analysis

Normality was be assessed for each outcome measure using the Shapiro-Wilk test and assessment of skewness and kurtosis values for each outcome. Outliers were determined via box plots as any data point greater than 3 standard deviations from the mean.

Aim 1 – Acquisition, Retention and Difficulty

We conducted separate 3x4 (condition x time) repeated measures ANOVAs to determine differences in RMSE and perceived difficulty between loading conditions (symmetrical, overloading, under loading). Bonferroni adjusted pairwise comparisons were used for *post hoc* analyses if significant main effects were determined ($P \leq 0.05$).

Figure 3. Analysis Time Points for Acquisition And Recall

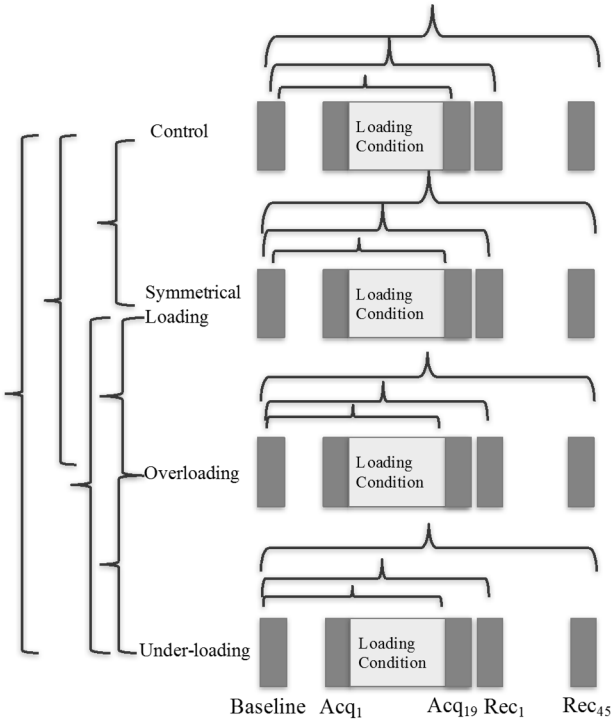


Dependent samples t-tests were used for *post hoc* analyses if a significant condition x time interaction was determined. We adjusted the P-value for multiple comparisons as we compared three loading conditions (P=0.05/3) at each time point (adjusted P = 0.017). We calculated three Cohen's *d* effect sizes with corresponding 95% confidence intervals (95% CI) at each time point to determine the magnitude of differences in percent change scores between each loading condition. Cohen's *d* effect sizes were classified as strong ≥ 0.80, moderate 0.79 – 0.50, and small ≥ 0.49.

Aim 2 – Lower Extremity Biomechanics

With all participants included, we conducted separate 4x4 (condition x time) repeated measures ANOVAs to determine differences in percent change from baseline between loading conditions (symmetrical, overloading, under-loading, control) at each time point ($Acquisition_1$, $Acquisition_{19}$, $Recall_1$, $Recall_{45}$). Bonferroni adjusted pairwise comparisons were used for *post hoc* analyses if significant main effects were determined ($P \leq 0.05$). Dependent samples t-tests were used for *post hoc* analyses if a significant condition x time interaction was determined. We adjusted the P-value for multiple comparisons as we compared four loading conditions (0.05/4) at each time point (adjusted P = 0.0125). As a *post hoc* analysis we determined the influence of outliers on our results. Outliers for each outcome measure were identified via box plots, and were defined as any data point >3 standard deviations away from the mean. Rather than remove each outlier identified at any single time point, participants were only removed from an analysis if their data point was classified as an outlier at two or more time points within each loading condition. Outliers were first removed from each outcome, and then we conducted the identical statistical analysis described above.

Figure 4. Analysis of Biomechanical Outcomes



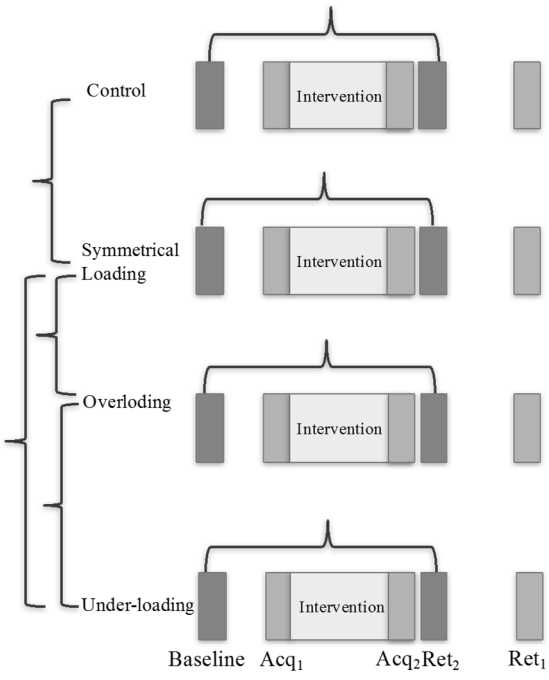
We calculated six Cohen's *d* effect sizes with corresponding 95% confidence intervals (95% CI) at each time point to determine the magnitude of differences in percent change scores between each loading condition. Cohen's *d* effect sizes were classified as strong ≤ 0.80, moderate 0.79 – 0.50, and small ≥ 0.49.

Aim 3 – Biochemical Markers

We conducted a 4x1 (condition x time) repeated measures ANOVA to determine differences in $COMP_{CHANGE}$ between loading conditions (symmetrical, overloading, under-loading, control). Bonferroni adjusted pairwise comparisons were used if significant a main effect was determined ($P \leq 0.05$).[213]

We calculated six Cohen's *d* effect sizes[214] with corresponding 95% confidence intervals (95% CI) to determine the magnitude of difference in $COMP_{CHANGE}$ between each loading condition. Cohen's *d* effect sizes were classified as strong ≤ 0.80, moderate 0.79 – 0.50, and small ≥ 0.49.[214] Effect sizes between conditions were calculated as follows:

Figure 5. Analysis of Biochemical Outcomes



$$Effect\ Size = \frac{Average\ Loading - Control}{Pooled\ SD}$$

$$Effect\ Size = \frac{High\ Loading - Control}{Pooled\ SD}$$

$$Effect\ Size = \frac{Low\ Loading - Control}{Pooled\ SD}$$

Aim 4 – Associations between Changes in Biomechanics and Biochemical Markers

For our primary analysis we conducted bivariate two-tailed Pearson Product-Moment correlations between baseline peak vGRF and $COMP_{CHANGE}$ within each loading condition. It is known that gait speed associates with peak vGRF,[215] and time since ACLR associates with serum biomarkers of cartilage degradation;[176] therefore, we secondarily conducted partial correlations between peak vGRF and $COMP_{CHANGE}$ while independently controlling for gait speed and time since ACLR. Correlation coefficients were interpreted as negligible (0.0 – 0.3), low (0.31 – 0.5), moderate (0.51 – 0.7), high (0.71 – 0.9) and very high (0.9-1.0).[216] Statistical significance was determined *a priori* as $P \leq 0.05$, and all statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Version 21, IBM Corp., Somers, NY).

CHAPTER 4: RESULTS

Specific Aim 1

Determine if individuals with an ACLR are able to acquire and recall average symmetrical loading, overloading loading and under-loading during walking gait using a real-time biofeedback intervention.

Root Mean Square Error

We determined a significant condition x time interaction for RMSE ($F_{6,57} = 3.094$, P=0.007). At $Acquisition_1$ participants demonstrated significantly lesser RMSE during the symmetrical loading condition as compared to the under-loading (t_{29}=-4.164, P<0.001) and overloading (t_{29}=-3.304, P=0.003) conditions. RMSE was not significantly different between the under-loading and overloading conditions at $Acquisition_1$ (t_{29}=-0.085, P=0.932). At $Acquisition_{19}$ participants demonstrated significantly greater RMSE during the under-loading condition as compared to the overloading condition (t_{29}=-2.993, P=0.006) and the symmetrical loading condition (t_{29}=-2.858, P=0.008). RMSE was not significantly different between the symmetrical loading condition and the overloading condition at $Acquisition_{19}$ (t_{29}=0.343, P=0.734). At $Recall_1$ participants demonstrated significantly lesser RMSE during the symmetrical loading condition as compared to the under-loading condition (t_{29}=-2.928, P=0.007). There were no RMSE differences between the under-loading condition (t_{29}=-0.745, P=0.462) and the symmetrical loading condition (t_{29}=-1.573, P=0.127) compared to the overloading condition at $Recall_1$. At $Recall_{45}$ participants demonstrated no significant differences in RMSE between the symmetrical,

overloading and under-loading conditions (P>0.017). There were strong effect size with conclusive 95% CIs that did not cross zero for RMSE between the symmetrical and under-loading condition at $Acquisition_1$. No other effect sizes evaluating between condition standardized differences was strong at any time point.

Perceived Difficulty

Perceived difficulty demonstrated significant main effects for time ($F_{3,27}$=15.924, P<0.001) and condition ($F_{2,28}$=8.026, P=0.001). Across all time points, participants reported significantly lesser difficulty during the symmetrical loading condition as compared to the under-loading condition (P=0.002). Perceived difficulty during the overloading condition was not significantly different from the symmetrical loading condition and the under-loading condition (P>0.017). Across all loading conditions, participants reported significantly greater difficulty at $Difficulty_5$ (VAS = 5.30±2.03cm) as compared to $Difficulty_{10}$ (VAS = 4.85±0.58cm; P=0.01), $Difficulty_{15}$ (VAS = 4.48±0.59cm; P=0.002) and $Difficulty_{20}$ (VAS = 4.17±0.60cm; P=0.001). Additionally, participants reported significantly greater difficulty at $Difficulty_{10}$ as compared $Difficulty_{20}$ (P=0.002). Participant perceived difficulty was not significantly different between $Difficulty_{15}$ and $Difficulty_{20}$ (P=0.023). There were no strong effect sizes between conditions at any time point.

Specific Aim 2

Determine the effect of symmetrical loading, overloading, and under-loading on lower extremity kinetics and kinematics in individuals with an ACLR.

Kinetic Outcomes

Peak Vertical Ground Reaction Force

We found a significant condition x time interaction for the percent change in peak vGRF ($F_{15,252}$=3.282, P=0.001). At $Acquisition_1$, participants demonstrated significantly greater change in peak vGRF during the overloading condition as compared to the symmetrical loading condition (t_{29}=-5.263, P<0.001), under-loading condition (t_{29}=4.419, P<0.001) and the control (t_{29}=-9.487, P<0.001). Participants demonstrated a significant decrease in peak vGRF during the under-loading condition as compared to the symmetrical loading condition (t_{29}=-10.908, P<0.001) and the control (t_{29}=4.47, P<0.001).

At $Acquisition_{19}$, participants demonstrated a greater change in peak vGRF during the overloading condition as compared to the symmetrical loading condition (t_{29}=-5.078, P<0.001), under-loading condition (t_{29}=-11.65, P<0.001) and the control (t_{29}=-5.584, P<0.001). Participants demonstrated a significant decrease in peak vGRF during the under-loading condition as compared to the symmetrical loading condition (t_{29}=5.246, P<0.001) and the control (t_{29}=5.5.645, P<0.001).

At $Recall_1$, participants demonstrated significantly greater peak vGRF during the overloading condition as compared to the symmetrical loading condition (t_{29}=-4.584, P<0.001), under-loading condition (t_{29}=-5.119, P<0.001) and the control (t_{29}=-4.965, P<0.001). Participants demonstrated significantly lesser peak vGRF during the under-loading condition as compared to the symmetrical loading condition (t_{29}=5.119, P<0.001) and the control (t_{29}=5.263, P<0.001).

At $Recall_{45}$, participants demonstrated significantly greater peak vGRF during the overloading condition as compared to the symmetrical loading condition (t_{29}=-3.488, P<0.001),

under-loading condition (t_{29} =-9.13, P<0.001) and the control (t_{29} =-4.17, P<0.001). Participants demonstrated significantly lesser peak vGRF during the under-loading condition as compared to the symmetrical loading condition (t_{29} =5.771, P<0.001) and the control (t_{29} =6.334, P<0.001). Peak vGRF was not significantly different between the symmetrical loading condition and the control condition at any time point (P>0.0125).

One outlier was identified for peak vGRF. Removal of this participant did not change our results as previously described. All between condition effect sizes were strong and demonstrated conclusive confidence intervals not crossing zero at each time point, except when the control condition was compared to the symmetrical condition.

Instantaneous Vertical Ground Reaction Force Loading Rate

Instantaneous vGRF loading rate demonstrated a significant main effect for condition ($F_{3,118}$ =10.282, P<0.001). Across all time points, participants demonstrated a significantly greater change in instantaneous vGRF during the overloading condition as compared to the symmetrical loading condition (P=0.007), the under-loading condition (P>0.017) and the control condition (P<0.001). The change in instantaneous vGRF loading rate was not significantly different between any other conditions (P>0.05).

No outliers were determined for instantaneous vGRF. Strong between condition effect sizes with conclusive confidence intervals were determined between the overloading and control conditions across all time points. No other between condition effect sizes were strong across all time points.

Peak Internal Knee Extension Moment

Peak KEM demonstrated significant main effects for time ($F_{3,118}$=7.409, P<0.001) and condition ($F_{3,28}$=13.247, P<0.001). Across all conditions, participants demonstrated a lesser

change in peak KEM at $Acquisition_1$ as compared to $Acquisition_1$ (P=0.01) and $Recall_1$ (P=0.006). Additionally, the change in peak KEM was greater at $Recall_1$ as compared to $Recall_{45}$ (P=0.012). The change in peak KEM was not significantly different between any other time points. Across all time points, the change in peak KEM was significantly greater in the overloading condition as compared to the symmetrical loading condition (P= 0.026), the under-loading condition (P< 0.001), and the control condition (P= 0.001). The change in peak KEM was not significantly different between any other conditions.

We determined that there were two outliers (>3 standard deviations from the mean during two or more time points) for peak KEM. Removal of these outliers did not change our results as compared to when all participants included in the analysis. Between condition effect sizes were strong and demonstrated conclusive confidence intervals at $Acquisition_1$, $Acquisition_{19}$ and $Recall_1$ when comparing 1) overloading and control, 2) overloading and symmetrical loading, and 3) overloading and under-loading. At $Recall_1$ between condition effect sizes were strong and demonstrated conclusive confidence intervals when comparing 1) overloading and control, and 2) overloading and under-loading. No other between condition effect sizes were strong across all time points.

Knee Kinematics

Knee Flexion Excursion

With all participants included, knee flexion excursion demonstrated a significant main effect for time ($F_{15,118}$ =13.157, P<0.001). Across conditions, the change in knee flexion excursion was significantly greater at $Acquisition_{19}$ as compared $Acquisition_1$ (P=0.007) and $Recall_{45}$ (P=0.001). Participants demonstrated greater knee flexion excursion at $Retention_1$ as compared to $Retention_{45}$ (P<0.001). Additionally, the change in knee flexion excursion was

significantly greater at $Recall_1$ as compared to $Acquisition_1$ (P=0.003) and compared to $Recall_{45}$ (P<0.001). Knee flexion excursion was not significantly greater at $Acquisition_{19}$ as compared to $Retention_1$ (P>0.0125).

We found four outliers (>3 stnadard deviations from the mean for two or more time points) for knee flexion excursion. Following removal of the outliers, there was a significant time x condition interaction for knee flexion excursion ($F_{15,114}$=2.228, P=0.021). At $Acquisition_1$ participants demonstrated significantly greater knee flexion excursion during the overloading condition as compared to the under-loading condition at (t_{25} = -2.844, P=0.009). Knee flexion excursion was not significantly different between any other conditions at $Acquisition_1$ (P>0.017).

At $Acquisition_{19}$, participants demonstrated significantly greater knee flexion excursion during the overloading condition as compared to the symmetrical loading condition ($t_{,25}$ = -7.332, P<0.001) and the under-loading condition (t_{25} = -2.549, P=0.017). The change in knee flexion excursion was not significantly different between any other conditions at $Acquisition_{19}$ (P>0.017).

At $Recall_1$, participants demonstrated significantly greater knee flexion excursion during the overloading condition as compared to the symmetrical loading condition (t_{25} = -5.361, P<0.001) and the under-loading condition (t_{25} = -3.577, P=0.001). The change in knee flexion excursion was not significantly different between any other conditions at $Recall_1$ (P>0.017).

At $Recall_{45}$, participants demonstrated significantly greater knee flexion excursion during the overloading condition as compared to the symmetrical loading condition (t_{25} = -3.803, P=0.001) and the under-loading condition (t_{25} = -3.580, P=0.001). The change in knee flexion excursion was not significantly different between any other conditions at $Recall_{45}$ (P>0.017).

Specific Aim 3

Determine the effect of symmetrical loading, overloading and under-loading on cartilage metabolism in individuals with an ACLR.

Within our entire cohort, $COMP_{CHANGE}$ was not significantly different between loading conditions ($F_{3,118} = 1.506$, P=0.219, Figure 1). Between condition effect sizes for $COMP_{CHANGE}$ were small and demonstrated inconclusive confidence intervals crossing zero when comparing all loading conditions.

Post-hoc Analysis

Twenty-four participants demonstrated an increase in $COMP_{CHANGE}$ during the control condition. In the sub-group of participants demonstrating an increase in $COMP_{CHANGE}$ during the control session we determined $COMP_{CHANGE}$ was significantly different between conditions ($F_{3,94} = 3.388$, P = 0.023). Participants demonstrated a significantly lesser $COMP_{CHANGE}$ during the overloading condition as compared to the control (P=0.020). The $COMP_{CHANGE}$ was not significantly different between any other loading conditions (P>0.05). There was a strong effect for $COMP_{CHANGE}$ between the overloading condition and the control with conclusive confidence intervals not crossing zero. There was a moderate effect for $COMP_{CHANGE}$ between the overloading condition and the control with conclusive confidence intervals not crossing zero. We determined a moderate between condition effect size indicating the $COMP_{CHANGE}$ during the overloading condition was lesser than the $COMP_{CHANGE}$ during the control condition. All other effect sizes were weak with inconclusive confidence intervals crossing zero.

We determined six participants demonstrated a decrease in $COMP_{CHANGE}$ during the control condition. In the cohort of participants demonstrating a decrease in $COMP_{CHANGE}$ during the control condition we determined $COMP_{CHANGE}$ significantly differed across loading

conditions ($F_{3,24}$ = 8.853, P = 0.031). Participants demonstrated a significantly greater $COMP_{CHANGE}$ during the under-loading condition as compared to the control condition (P=0.034). The $COMP_{CHANGE}$ was not significantly different between any other loading conditions (P>0.05). Between condition effect sizes for $COMP_{CHANGE}$ were strong and demonstrated conclusive confidence intervals not crossing zero when demonstrating that: 1) $COMP_{CHANGE}$ was increased during the symmetrical loading condition as compared to the control, 2) $COMP_{CHANGE}$ was increased during the overloading loading condition as compared to the control, 3) $COMP_{CHANGE}$ was increased during the under-loading condition as compared to the control, 4) symmetrical loading and overloading, and 5) symmetrical loading and under-loading.

Specific Aim 4

Determine the associations between baseline peak vGRF and the change in COMP following each loading condition in individual with an ACLR.

Complete $COMP_{PRE}$ and $COMP_{POST}$ data were available for 26 participants during each session, and complete vGRF data was obtained from all 30 participants. During the control condition, participants demonstrating a greater baseline peak vGRF also demonstrated a lesser $COMP_{CHANGE}$ (r = -0.437; P = 0.020). The association between baseline peak vGRF and $COMP_{CHANGE}$ during the control condition remained significant when controlling for gait speed (r = -0.467; P = 0.014) and time since reconstruction (r = -0.428; P = 0.026). Baseline peak vGRF was not significantly associated with $COMP_{CHANGE}$ during the symmetrical loading condition (r = 0.002; P = 0.993), during the overloading condition (r = 0.129; P = 0.504; Figure 4), or during the under-loading condition (r = 0.215; P = 0.272).

CHAPTER 5: MANUSCRIPT 1

Real-time Biofeedback During Walking Gait Promotes Symmetrical Loading in Individuals with Anterior Cruciate Ligament Reconstruction

OVERVIEW

Context: Individuals with anterior cruciate ligament reconstruction (ACLR) demonstrate asymmetrical loading during walking gait that may contribute to alterations in tissue metabolism. Providing real-time biofeedback that cues a change in peak vertical ground reaction force (vGRF) during walking gait may be beneficial for promoting symmetrical loading in individuals with ACLR. **Objective:** To determine differences in acquisition, recall, and perceived difficulty individuals with ACLR when provided real-time biofeedback during walking gait that cued a 5% increase in peak vGRF (i.e. overloading), a 5% decrease in peak vGRF (i.e under-loading), and symmetrical peak vGRF between limbs (i.e. symmetrical loading). **Design:** Single-blind, crossover study. **Setting:** Research laboratory. **Patients or Other Populations:** 30 individuals with ACLR. **Interventions:** Participants completed three testing sessions separated by at least 7 days. During each session, one of three loading conditions was completed during 20 minutes of treadmill walking. Loading conditions included 1) a 5% increase (i.e. overloading) in peak vGRF, 3) a 5% decrease (i.e. under-loading) in peak vGRF, and 4) symmetrical peak vGRF between limbs. Acquisition was assessed during the first ($Acquisition_1$) and final minute ($Acquisition_{19}$) real-time biofeedback was displayed. Recall was assessed during the first minute ($Recall_1$) following removal of the real-time biofeedback and again 45minutes following removal

(Recall$_{45}$). Perceived difficulty was assessed every five minutes real-time biofeedback was displayed. **Main Outcome Measures:** Root mean square error (RMSE) was calculated during acquisition and retention. Perceived difficulty was assessed using a 10cm visual analog scale that ranged from "not at all difficult" to "extremely difficult". **Results:** Data were analyzed using separate 3 x 4 (condition x time) ANOVAs. There was a significant condition x time interaction for RMSE. Post hoc analysis indicated lesser RMSE during the symmetrical loading condition compared to overloading and under-loading at Acquisition$_1$. At Acquisition$_{19}$ and Recall$_1$ RMSE was significantly lesser during the symmetrical loading condition than the under-loading condition. RMSE was not significantly different between loading conditions at Recall$_{45}$. Participants demonstrated significantly lesser perceived difficulty during the symmetrical loading condition as compared to the under-loading condition. Perceived difficulty decreased across time during all loading conditions. **Conclusions:** Real-time biofeedback may be advantageous for promoting symmetrical loading during walking gait in individuals with ACLR.

INTRODUCTION

Approximately 85% of individuals return to physical activity following anterior cruciate ligament reconstruction ACLR,[28] yet almost half of all of individuals with ACLR demonstrate radiographic evidence of post-traumatic knee osteoarthritis (PTOA) within two decades following reconstruction.[13,14,217] The etiology of PTOA is multifactorial,[18,24,25] and it is hypothesized that the development of aberrant mechanical loading following ACL injury and ACLR contribute to the development of PTOA.[23,34,178] Specifically, inter-limb asymmetries in mechanical loading during walking gait result in decreased self-reported function following ACLR,[29,33] and chronic increases[103,218] or decreases[27,44] in mechanical loading during walking

gait may lead to alterations in tissue metabolism.[24,112] Feedback is a commonly employed and effective intervention for cuing changes in aberrant movement patterns and reducing mechanical loading during functional tasks such as jump landing.[219] Utilizing feedback to cue a change in mechanical loading during walking gait may be beneficial for improving long-term outcomes realted to knee health following ACLR.

Providing simple, traditional feedback cues such as "bend your knees more" is effective at reducing loading in discrete, non-repetitive tasks such as jump-landing.[53,219] Cyclical movements, however, are governed by spinal-level central pattern generators that function to automate the timing and intensity of muscle contraction during movement.[220] The automaticity of walking gait likely renders traditional feedback cues ineffective at eliciting lasting changes in gait over time.[192] Whereas traditional feedback is typically provided following completion of a task,[53] real-time biofeedback offers the advantage of providing instantaneous cues to adjust movement during repetitive tasks.[59] Real-time biofeedback may be advantageous for eliciting changes in movement patterns during walking gait following ACLR.

Real-time biofeedback has been previously implemented to increase[50] and decrease[51-54] loading as well as promote symmetrical loading[55-58] between limbs during a variety of functional tasks in healthy participants and multiple patient populations (i.e. knee osteoarthritis, total hip and knee arthoplasty). Coupling real-time biofeedback with lower-extremity strength training improves lower extremity movement symmetry following total hip[55] and knee[57] arthroplasty. Individuals with total hip arthroplasty demonstrate significant improvements in inter-limb symmetry during walking gait when real-time biofeedback of peak vertical ground reaction force (vGRF) is administered during walking gait,[55] which is an estimate of the vertical force applied to the entire lower extremity during the stance phase of gait.[55,57] Providing real-time biofeedback

that elicits a change in peak vGRF during walking gait may be advantageous for restoring symmetrical mechanical loading during walking gait following ACLR.

Successful motor skill acquisition during the period of time that real-time biofeedback is provided, as well as successful recall of the motor skill following removal of real-time biofeedback are critical for understanding motor learning.[221] In order to begin developing interventions that effectively manipulate aberrant loading, it is imperative to understand if individuals with ACLR can manipulate peak vGRF during walking gait when provided a single session of real-time biofeedback. Therefore, the purpose of our study was to determine if individuals with ACLR acquire and recall movement patterns of overloading (i.e. increased peak vGRF), under-loading (i.e. decreased vGRF), and symmetrical loading (i.e. identical peak vGRF between limbs) during treadmill walking when provided a single session of real-time biofeedback. Secondarily, as task difficulty influences task acquisition and recall,[222] we sought to determine differences in perceived difficulty of using real-time biofeedback to cue overloading, symmetrical loading, and under-loading during treadmill walking in individuals with ACLR.

METHODS

Design

For this crossover study, each participant completed four testing sessions (control, symmetrical, overloading, under-loading) that were conducted at the same time of day (mean time difference between sessions = 0.29±0.48 hours) with at least a 7-day interval between each session (mean = 8.83±2.29 days). We blinded the participants to the symmetrical, overloading, under-loading loading condition implemented via real-time biofeedback. The biofeedback targets for the three loading conditions were determined during the control session, which was always

the first session conducted for each participant. No biofeedback was provided during the control session. The order of loading conditions elicited via real-time biofeedback during the remaining three testing sessions was block randomized prior to participant enrollment. All testing

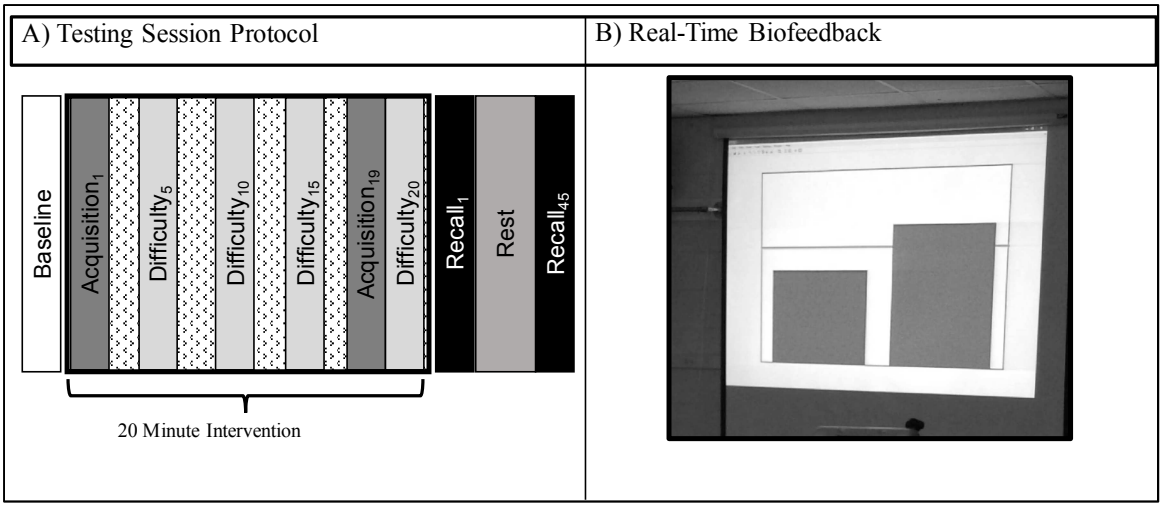


Figure 6A. Aim 1 Testing Session Protocol. All four testing sessions were conducted in the same order. Participants were instructed to walk in a normal gait pattern during the baseline trial. Immediately following the baseline trial real-time biofeedback was displayed to the participant during the 20-minute intervention. No biofeedback was provided during the control session. Acquisition of each loading condition (i.e. symmetrical loading, overloading, under loading) was assessed during the first minute of the intervention ($Acquisition_1$) and during the final minute (i.e. minute 19) of the intervention ($Acquisition_{19}$). Perceived difficulty was assessed following the 5^{th} ($Difficulty_5$), 10^{th}($Difficulty_{10}$), 15^{th} ($Difficulty_{15}$), and 20^{th} ($Difficulty_{20}$), minute of the intervention. Immediate recall was assessed during the first minute following removal of the real-time biofeedback ($Recall_1$) and following 45 minutes of rest ($Recall_{45}$). **Figure 1B.** The real-time biofeedback displayed a blue bar graph for each limb, which represented the magnitude of the first peak of the vGRF. A red target line was placed in the center of the screen, and participants were instructed to alter their movement in order to match each blue bar (i.e. peak vGRF) to the red line during each step.

procedures followed the same order for each session, which consisted of four 60-second walking trials (Figure 6A).

An additional baseline trial was collected during the control session to determine the target values for the following three loading conditions utilizing real-time biofeedback. Acquisition of each loading condition (i.e. symmetrical loading, overloading, under-loading) was assessed during the first minute ($Acquisition_1$) and during the final minute (i.e. minute 19) of the loading condition ($Acquisition_{19}$) when real-time biofeedback was provided. Immediate recall was assessed during the first minute following removal of the real-time biofeedback ($Recall_1$)

and following 45 minutes of rest ($Recall_{45}$). Perceived difficulty was assessed following the 5th ($Difficulty_5$), 10th($Difficulty_{10}$), 15th ($Difficulty_{15}$), and 20th ($Difficulty_{20}$) minute of the loading condition when real-time biofeedback was provided and quantified using a 10cm visual analog scale (VAS). The Institutional Review Board at the University of North Carolina at Chapel Hill approved all methods and all participants provided written informed consent prior to participation.

Participants

We recruited a convenient sample of individuals between 18-35 years of age who underwent a primary, unilateral ACLR using either a patellar tendon or hamstring autograft from the university community. All participants were participating in unrestricted physical activity as allowed by their orthopaedic physician, which included at least 30 minutes of physical activity three times per week. We excluded individuals: 1) with a history of musculoskeletal injury to either leg (e.g. ankle sprain, muscle strain) within 6 months prior to participation in the study, 2) a history of lower extremity surgery other than ACLR, 3) with a history of knee osteoarthritis or current symptoms related to knee osteoarthritis (e.g. pain, swelling, stiffness), 4) who were currently pregnant or planning to become pregnant while enrolled in the study, 5) with a history of cardiovascular restrictions that limited the participant's ability to participate in physical activity. Participants were asked to self-report age, sex, ACL graft type, and the date of ACL injury and ACLR. Height and weight were measured in the laboratory prior to testing. All participants completed the subjective portion of the International Knee Documentation Committee index to evaluate self-reported disability and the Tegner Activity Scale to measure level of physical activity. We estimated we would detect a moderate effect (Cohen's $d = 0.50$)

for RMSE between loading conditions, which we determined from our pilot data collected for this study in three healthy individuals. Therefore, in order to detect a moderate effect size of 0.50 with 80% power and an alpha level of 0.05 we would need to enroll 29 participants (G*Power Statistical Power Analysis Software v3.1[223])

Collection of Acquisition, Recall, and Perceived Difficulty

A dual-belt, force-sensing treadmill (Bertec, Columbus, OH) with two 70 x 40 inch force plates (Model S020008) was used to acquire all kinetic data (i.e. peak vGRF). Kinetic data were sampled at 1000Hz and filtered using a 4th order, low-pass Butterworth filter with a cut-off frequency of 100Hz. During the first control session, prior to treadmill walking, self-selected walking speed was determined using timing gates during 5 over-ground walking trials (Brower TC-Gate; Brower Timing Systems, Draper, Utah) and used to set the speed of the treadmill for the subsequent three testing sessions. Before beginning the first testing session (control session) participants walked on the treadmill for 5 minutes to allow for acclimation to treadmill walking.[50]

Peak vGRF was identified during the first 50% of the stance phase, which was defined as the interval from initial contact (vGRF $\geq$ 20N) to toe-off (vGRF $\leq$ 20N). Peak vGRF was extracted from each step during $Acquisition_1$, $Acquisition_{19}$, $Recall_1$, and $Recall_{45}$ using a second custom MATLAB script. Root mean square error (RMSE) was calculated during each acquisition and recall trial using Equation 1 to determine the average difference in actual peak vGRF compared to the target value across each 60-second trial.

Equation 1:

$$RMSE = \sqrt{\frac{(Target\ vGRF - Actual\ vGRF)^2}{Total\ Steps}}$$

Patients were asked to rate their perceived difficulty when attempting to reach the biofeedback target at $Difficulty_5$, $Difficulty_{10}$, $Difficulty_{15}$, and $Difficulty_{20}$ via a 10cmVAS that ranged from "not at all difficult" (i.e. 0) to extremely difficult (i.e. 10). Perceived difficulty was analyzed using a tape measure, with greater scores indicating more perceived difficulty.

Real-Time Biofeedback Conditions

During the baseline trial of the control session a custom MATLAB (Mathworks, Inc, Natick, MA) program processed and extracted left and right limb peak vGRF from the first 50% of the stance phase, which was used to determine the biofeedback targets for the three loading conditions (symmetrical loading, overloading, and under-loading) conducted in the subsequent sessions.

For the symmetrical loading, overloading, and under-loading sessions a 72 inch projection screen directly in front of the treadmill displayed the real-time biofeedback (Figure 6B). A second custom MATLAB script continuously computed the average of the previous four peak vGRF during the first 50% of stance phase, which was visually displayed as right and left blue bar graphs on the projection screen, with a red target line across the center. The target line for the symmetrical loading condition corresponded to the mean peak vGRF between the ACLR and contralateral limb collected during the baseline trial of the control session. The target line for the overloading condition corresponded to a 5% increase in the baseline peak vGRF for the

ACLR limb and the contralateral limb. The target line for the under-loading condition corresponded to a 5% decrease in baseline peak vGRF. The target line was always displayed in the center of the screen for each loading condition to maintain participant blinding to loading condition. Target values for the overloading (5% above baseline peak vGRF) and under-loading (5% below baseline peak vGRF) conditions were determined individually for left and right leg based on the baseline value of each limb. Therefore, the overloading and under-loading conditions did not specifically cue inter-limb symmetry, rather a relative change in magnitude in each limb.

Before completing the real-time biofeedback intervention a study investigator (BALH) conducted a brief presentation with each participant explaining the peak vGRF and how the biofeedback continuously displayed peak vGRF. Participants were instructed to match the height of each blue bar (i.e. peak vGRF) to the target line as close as possible during each loading condition and utilize any movement strategy possible to manipulate peak vGRF. During the initial presentation all participants were provided one strategy that focused on manipulating the vertical displacement of their center of mass (COM). Specifically, that increasing or decreasing the vertical displacement of their COM may result in a subsequent increase or decrease in peak vGRF, respectively. We provided one strategy to maximize the success of participants consistently reaching the target. Real-time biofeedback was not provided during the assessment of recall ($Recall_1$, $Recall_{45}$), and participants were instructed to "Walk in the same manner as when attempting to match each blue bar to the target line."

Statistical Analysis

First, normality was assessed using the Shapiro-Wilk test and skewness and kurtosis values, and outliers were identified via box plots as any data point greater than three standard deviations from the mean.We conducted separate 3x4 (condition x time) repeated measures ANOVAs to determine differences in RMSE and perceived difficulty between loading conditions (symmetrical, overloading, under-loading). Bonferroni adjusted pairwise comparisons were used for *post hoc* analyses if significant main effects were determined.[213] The alpha level for our main effect of time was adjusted to P=0.0125 as 4 conditions were collapsed across 4 separate time points (0.05/4). The alpha level for our main effects for condition were adjusted to P=0.0166 as we compared 3 separate conditions collapsed across all time points (0.05/3). Dependent samples t-tests were used for *post hoc* analyses if a significant condition x time interaction was determined. In the case of an interaction, our alpha level was adjusted to P = 0.004 (0.05/12) at each time point. We calculated three Cohen's *d* effect sizes with corresponding 95% confidence intervals (95% CI) at each time point to determine the magnitude of differences in percent change scores between each loading condition. Cohen's *d* effect sizes were classified as strong $\geq$ 0.80, moderate 0.79 – 0.50, and small $\geq$ 0.49.[214] All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Version 21, IBM Corp., Somers, NY).

RESULTS

Thirty individuals with ACLR were enrolled into this study (Table 2). All outcomes were normally distributed, and no outliers were determined for any outcome measure; therefore all participants were included within each analysis.

Root Mean Square Error

We determined a significant condition x time interaction for RMSE ($F_{6,174}$ = 3.094, P=0.007; Figure 7). At $Acquisition_1$ participants demonstrated significantly lesser RMSE during the symmetrical loading condition as compared to the under-loading (t_{29}=-4.164, P<0.001) and overloading (t_{29}=-3.304, P=0.003; Table 2) conditions. RMSE was not significantly different between the under-loading and overloading conditions at $Acquisition_1$ (t_{29}=-0.085, P=0.932). There was a strong effect size with conclusive confidence intervals at $Acquisition_1$ indicating lesser RMSE during the symmetrical loading condition as compared to the under-loading condition (Effect Size = -0.87[-1.40 to -0.34]; Table 3).

At $Acquisition_{19}$, while not statistically significant at our Bonferroni adjusted alpha level, participants demonstrated greater RMSE during the under-loading condition as compared to the overloading condition (t_{29}=-2.993, P=0.006) and the symmetrical loading condition (t_{29}=-2.858, P=0.008). There was a moderate effect size with conclusive confidence intervals not crossing zero at $Acquisition_{19}$ indicating greater RMSE during the under-loading condition as compared to the overloading condition (Effect Size = -0.71[-1.23 to -0.18]; Table 3). RMSE was not significantly different between the symmetrical loading condition and the overloading condition at $Acquisition_{19}$ (t_{29}=0.343, P=0.734). At $Recall_1$ participants demonstrated lesser RMSE during the symmetrical loading condition as compared to the under-loading condition, yet this was not statistically significant at our Bonferroni adjusted alpha level (t_{29}=-2.928, P=0.007). The effect size comparing RMSE during the under-loading condition and the overloading condition was moderate and demonstrated conclusive confidence intervals not crossing zero (Effect Size = -0.54 [-1.06 to -0.03]; Table 3). There were no RMSE differences between the under-loading condition (t_{29}=-0.745, P=0.462) and the symmetrical loading condition (t_{29}=-1.573, P=0.127)

compared to the overloading condition at $Recall_1$. At $Recall_{45}$ participants demonstrated no significant differences in RMSE between the symmetrical, overloading and under-loading conditions ($P>0.004$).

Perceived Difficulty

Perceived difficulty demonstrated significant main effects for time ($F_{3,58}=15.924$, $P<0.001$) and condition ($F_{2,58}=8.026$, $P=0.001$; Figure 8). Across all time points, participants reported significantly lesser difficulty during the symmetrical loading condition as compared to the under-loading condition ($P=0.002$; Table 3). Perceived difficulty during the overloading condition was not significantly different from the symmetrical loading condition and the under-loading condition ($P>0.0125$). Across all loading conditions, participants reported significantly greater difficulty at $Difficulty_5$ (VAS = 5.30±2.03cm) as compared to $Difficulty_{10}$ (VAS = 4.85±0.58cm; $P=0.01$), $Difficulty_{15}$ (VAS = 4.48±0.59cm; $P=0.002$) and $Difficulty_{20}$ (VAS = 4.17±0.60cm; $P=0.001$). Additionally, participants reported significantly greater difficulty at $Difficulty_{10}$ as compared $Difficulty_{20}$ ($P=0.002$). Participant perceived difficulty was not significantly different between $Difficulty_{15}$ and $Difficulty_{20}$ ($P=0.023$). There were no strong effect sizes between conditions at any time point (Table 3).

Table 2. Manuscript 1 Participant Demographics

Sex	21 Female (70%); 9 Male (30%)
Age (years)	20.43±2.91
Height (cm)	172.70±10.81
Mass (kg)	73.16±16.10
BMI	24.42±4.25
Time since Surgery (months)	47.83±26.97
Graft Type	HS = 16 (53%) ; PT = 14 (47%)
IKDC	86.49±9.51
Tegner	7.47±1.33

Data presented as mean ± standard deviation. HS = hamstring tendon autograft; PT = patellar tendon autograft

Table 3. Root Mean Square Error

	$Acquisition_1$	$Acquisition_{19}$	$Recall_1$	$Recall_{45}$	Condition Mean
Symmetrical Loading	26.68±10.49	31.50±17.06	31.73±16.32	34.80±8.28	31.18±12.99
Overloading	37.98±22.42*	30.32±9.81	37.52±14.13	41.68±18.04	36.88±10.99
Under-loading	38.41±15.58*	39.37±14.98	40.60±16.03*	36.93±13.12	38.28±12.67
Time Point Mean	34.36±11.76	33.73±10.42	36.62±10.40	37.81±11.08	

Data presented as mean ± standard deviation. * indicates significantly greater than symmetrical loading condition; # indicates significantly lesser than under-loading condition

Table 4. Root Mean Square Error and Perceived Difficulty Effect Sizes

Root Mean Square Error

	Acquisition 1		**Acquisition 19**		**Recall 1**		**Recall 45**	
Conditions Compared	Effect Size	95% CI	Effect Size	95% CI	Effect Size	95% CI	Effect Size	95% CI
Symmetrical vs Overloading	-0.64	-1.16 to -0.12	0.08	-0.42 to 0.59	-0.37	-0.88 to 0.14	-0.48	-1.00 to 0.03
Symmetrical vs Under-loading	-0.87	-1.40 to -0.34	-0.48	-1.00 to 0.03	-0.54	-1.06 to -0.03	-0.19	-0.70 to 0.32
Overloading vs Under-loading	-0.64	-1.15 to -0.12	-0.71	-1.23 to -0.18	-0.20	-0.71 to 0.31	0.30	-0.21 to 0.81

Perceived Difficulty

	Difficulty 5		**Difficulty 10**		**Difficulty 15**		**Difficulty 20**	
Conditions Compared	Effect Size	95% CI	Effect Size	95% CI	Effect Size	95% CI	Effect Size	95% CI
Symmetrical vs Overloading	-0.22	-0.72 to 0.29	-0.20	-0.71 to 0.31	-0.18	-0.68 to 0.33	-0.14	-0.64 to 0.37
Symmetrical vs Under-loading	-0.59	-1.11 to -0.07	-0.67	-1.19 to -0.05	-0.67	-1.19 to -0.15	-0.64	1.16 to -0.12
Overloading vs Under-loading	-0.43	-0.94 to 0.08	-0.53	-1.04 to -0.01	-0.52	-1.04 to -0.01	-0.54	-1.05 to -0.02

Table 5. Perceived Difficulty

	$Difficulty_5$	$Difficulty_{10}$	$Difficulty_{15}$	$Difficulty_{20}$	Condition Mean
Symmetrical	4.56±2.8	4.13±2.34	3.78±2.38	3.51±2.41	3.99±2.23
Overloading	5.12±2.30	4.57±2.01	4.19±2.23	3.83±2.18	4.43±2.03
Under-loading	6.23±2.79	5.84±2.71	5.46±2.57	5.17±2.71	5.67±2.59*
Time Point Mean	5.30±2.03	4.84±1.86$^{\#}$	4.47±1.88$^{\#}$	4.16±1.94$^{\#,\wedge}$	

Data presented as mean ± standard deviation in centimeters. * indicates significantly greater than symmetrical loading condition across all time points; $^{\#}$ indicates significantly greater than Difficulty5 across all conditions; ^ indicates significantly greater than $Difficulty_{10}$

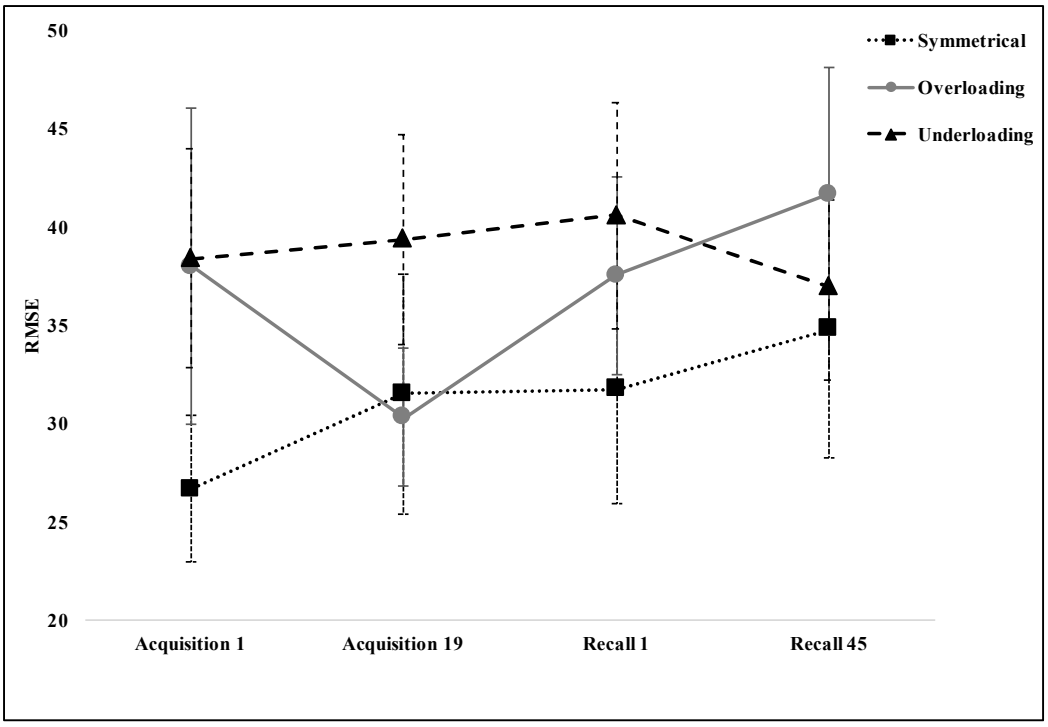


Figure 7. Root Mean Square Error. Data presented as mean RMSE (root mean square error) at each time point, with corresponding 95% confidence intervals.

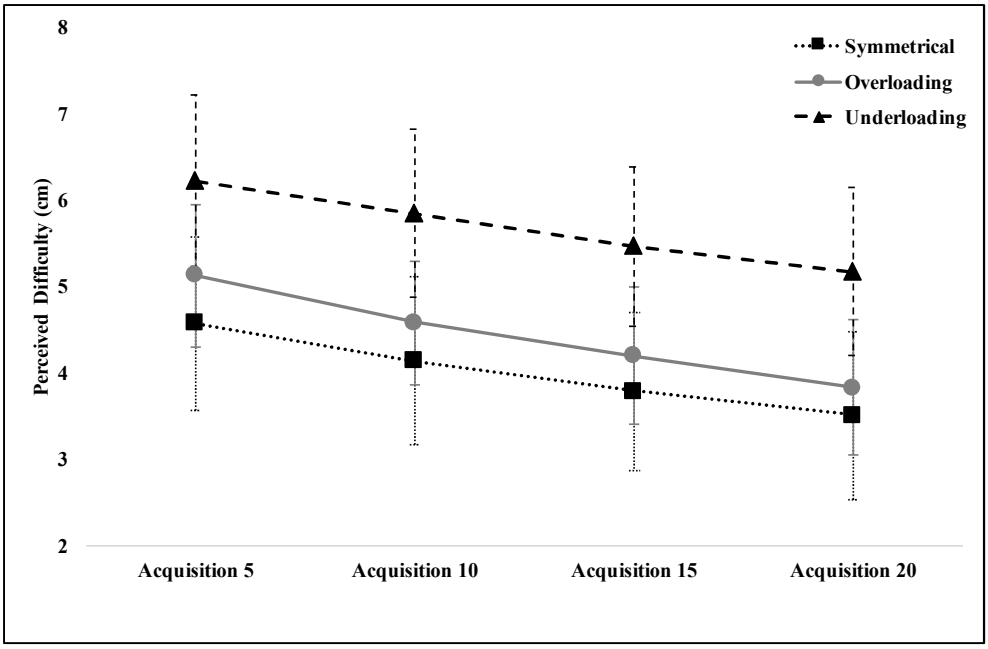


Figure 8. Perceived Difficulty. Data presented as mean perceived difficulty in centimeters (cm) at each time point, with corresponding 95% confidence intervals.

DISCUSSION

Overall, we determined individuals with ACLR demonstrate lesser RMSE during the first minute of acquisition of symmetrical loading when provided real-time biofeedback of peak vGRF as compared to the overloading and under-loading conditions. During the final minute of acquisition there was no significant difference in RMSE between the symmetrical loading and overloading conditions. Participants demonstrate the greatest RMSE during acquisition and the first minute following removal of the real-time biofeedback (i.e. $Recall_1$) during the under-loading condition. There was no significant difference in RMSE between the three loading conditions (symmetrical loading, overloading, under-loading) forty-five minutes following removal of the real-time biofeedback (i.e. $Recall_{45}$). Participants reported the greatest amount of perceived difficulty during the under-loading condition across all time points, yet perceived

difficulty significantly decreased throughout the real-time biofeedback intervention in each condition.

These data suggest real-time biofeedback that cues a change in peak vGRF may be most beneficial for promoting symmetrical loading during walking gait in individuals with ACLR. Perceived difficulty decreased over time during the symmetrical loading, overloading, and under-loading conditions. Therefore, real-time biofeedback may be a feasible intervention for cuing a change in peak vGRF in individuals with ACLR across a variety of loading conditions.

It is known that individuals with ACLR who demonstrate greater inter-limb asymmetries at 6 months following ACLR also demonstrate worse self-reported function[29] and are less likely to pass return to sport criteria.[29,33] Utilizing real-time biofeedback to reduce inter-limb asymmetry during walking gait, particularly early following ACLR when asymmetries are more distinct,[32] may be beneficial for improving long-term outcomes. In the current study, we found that individuals with ACLR demonstrate significantly lesser RMSE during the first minute of feedback aimed at improving symmetry of peak vGRF between limbs (RMSE mean = 26.68±10.49) as compared to increasing (Overloading RMSE mean = 37.98±22.42) or decreasing peak vGRF (Under-loading RMSE mean = 38.41±15.58). Task difficulty influences skill acquisition, with greater difficulty yielding poorer task acquisition.[222] Our overloading and under-loading conditions required a consistent 5% change in peak vGRF from baseline values, whereas the change elicited during the symmetrical loading condition was dependent upon the magnitude of inter-limb asymmetry in peak vGRF at baseline. The mean percent change from baseline peak vGRF during the symmetrical loading condition in our cohort was 0.84±1.53%. Producing a smaller magnitude of change in peak vGRF during the symmetrical loading condition likely resulted in lesser RMSE, and is reflected through lesser perceived difficulty

reported during the symmetrical loading condition (VAS=3.99±0.64cm) as compared to the overloading (VAS=4.43±0.61) and under-loading conditions (VAS=5.67±0.69).

We detrmined greater RMSE during the overloading condition as compared to the symmetrical loading condition at $Acquisition_{1;}$ yet there was no significant difference in RMSE between the overloading (RMSE = 30.32±9.81) and symmetrical loading (RMSE = 31.50±17.06) conditions at $Acquisition_{19}$. It is possible that more time is required to consistently walk with a 5% increase in peak vGRF during the overloading condition; yet individuals with an ACLR were able to reduce RMSE at $Acquisition_{19}$ as compared to $Acquisition_{1}$ during the overloading condition. Greater variability (i.e. lesser RMSE) in vGRF during the overloading condition may result from chronic deficits in proprioception following ACLR.[224] Evidence[224] suggests somatosensory deficits continue to persist for years following ACLR despite restoration of mechanical joint stability. Adaptations within the central nervous system following ACLR may be responsible for persistent alterations in neuromuscular control which may adversely affect the ability to make adjustments during motor tasks.[225] Peak vGRF is influenced by sagittal plane knee kinematics during walking gait.[226] A reduction in knee joint proprioception may reduce an individual's ability to replicate knee joint position consistently during each step, resulting in greater stride-by-stride variability in peak vGRF and an increase in RMSE.

During the under-loading condition, our participants demonstrated significantly greater RMSE during $Acquisition_{1}$ as compared to the symmetrical loading condition. At $Acquisition_{19}$ participants demonstrated notably greater RMSE during the under-loading condition (RMSE mean = 39.37±14.98) as compared to the overloading condition (mean = 30.32±9.81) and the symmetrical loading condition (mean = 31.50±17.06). However, following our Bonferroni adjustment (P=0.004), these results were not statistically significant. Individuals with lower

vGRF demonstrate lesser knee motion during the stance phase of walking gait as compared to those with greater peak vGRF following ACLR.[226] Greater difficulty in adapting movement strategies yielding a decrease in peak vGRF while maintaining a constant gait speed may have resulted in greater error during the acquisition period of the under-loading condition. While RMSE was significantly greater during the under-loading condition, there was no significant difference in RMSE forty-five minutes following removal of the biofeedback. Greater task difficultly promotes greater recall,[227] and may partially explain the greater RMSE demonstrated during the under-loading condition. During the task acquisition period the learner continuously evaluates movement outcomes and uses this information to modulate future responses.[228] Greater task difficulty, which may have been experience during the under-loading condition that demonstrated more RMSE during the acquisition period, may have forced participants to continuously utilize working memory[227] to modulate their movement during each subsequent step in an attempt to reach the target line. Participants may have continuously adjusted their movement pattern during each step to reach the target line, which may have better refined the movement pattern during the under-loading condition. Greater refinement of the movement pattern during the under-loading may have resulted in non-significant differences in RMSE between the three loading conditions (i.e. symmetrical loading, overloading, under-loading) at $Recall_{45}$.

Current rehabilitation guidelines emphasize a criterion-based progression that promotes inter-limb symmetry for key clinical outcomes (i.e. range of motion, strength, functional tasks).[229] As regaining quadriceps strength is imperative for improving self-reported function following ACLR,[230] quadriceps strength has a clearly defined return-to-sport criterion of at least 90% symmetry.[229] Asymmetrical loading during walking gait associates with lower self-reported

function[33] and biomarkers of tissue metabolism,[27] yet the requirements for achieving symmetrical loading during walking gait are not clearly defined. We determined individuals with ACLR demonstrate the least amount of error when acquiring a symmetrical loading pattern during real-time feedback. Further refinement of real-time biofeedback for implementation into clinical practice may be beneficial for restoring symmetrical loading patterns following ACLR.

Limitations

While the current study provides insight into the ability of individuals with ACLR to acquire and recall changes in peak vGRF during a single session of real-time biofeedback, there are limitations that that can inform the development of future research. In this study we utilized a crossover design in which all participants received all three loading conditions, rather than a fully randomized controlled trial. In order to minimize the potential for a learning effect, the order of the loading conditioned was randomized and a one-week washout was provided between each testing session. Motor learning theory suggests utilizing blocked and random practice promotes retention of motor skills,[228] whereas our participants were provided with 20 minutes of continuous real-time biofeedback during each session. As our primary aim was to determine if individuals with ACLR can acquire real-time biofeedback curing a change in peak vGRF, and not to assess long-term motor learning, we chose to provide continuous feedback to maximize skill acquisition. Future studies are necessary to determine the optimal feedback frequency in order to promote retention of changes in peak vGRF following ACLR. Following removal of the real-time biofeedback we specifically cued participants to continue walking as they did when they were attempting to reach the target time, therefore we assessed recall rather than retention. As this was a single session of real-time biofeedback, retention of the movement was unlikely.

Future research is needed to determine the retention of altered peak vGRF following multiple sessions of real-time biofeedback. We included a small sample of individuals on average 48 months following ACLR. It remains unknown if our results translate to the larger population of individuals with ACLR. Additionally individuals still undergoing structured rehabilitation following ACLR (i.e. <6 months following ACLR) may acquire and recall various loading conditions differently when provided real-time biofeedback during walking gait as compared to individuals in our study who underwent ACLR multiple year's prior.

In conclusion, individuals with ACLR demonstrate the least amount of error immediately upon receiving real-time biofeedback during symmetrical loading as compared to overloading and under-loading during walking gait. While individuals with ACLR demonstrated the greatest RMSE during the auqisition period of the under-loading condition as compared to the overloading and symmetrical loading conditions, there was no difference in RMSE when participants recalled each loading condition forty-five minutes following removal of the real-time biofeedback. Although difficulty was the greatest during the under-loading condition, perceived difficulty decreased over time across all loading conditions. Real-time biofeedback may be a beneficial therapeutic intervention to promote symmetrical peak vGRF between limbs in individuals with ACLR.

CHAPTER 6: MANUSCRIPT 2

Acute Alterations in Peak Vertical Ground Reaction Force During Walking Gait Result in Changes in Lower Extremity Kinetics and Kinematics in Individuals with Anterior Cruciate Ligament Reconstruction

OVERVIEW

Context: Altering mechanical loading during walking gait may improve self-reported function and influence tissue metabolism in individuals with anterior cruciate ligament reconstruction (ACLR). However, acutely altering mechanical loading during walking gait in individuals with ACLR may result in changes in additional lower extremity kinetics and kinematics that may increase loading of the articular cartilage. **Objective:** To determine differences in lower extremity kinetics and kinematics during walking gait individuals with ACLR when provided real-time biofeedback during walking gait that cued a 5% increase in peak vGRF (i.e. overloading), a 5% decrease in peak vGRF (i.e under-loading), and symmetrical peak vGRF between limbs (i.e. symmetrical loading). **Design:** Single-blind, crossover study. **Setting:** Research laboratory. **Patients or Other Populations:** 30 individuals with ACLR. **Interventions:** Participants completed three testing sessions separated by at least 7 days. During each session, one of three loading conditions was completed during 20 minutes of treadmill walking. Loading conditions included 1) a 5% increase (i.e. overloading) in peak vGRF, 3) a 5% decrease (i.e. under-loading) in peak vGRF, and 4) symmetrical peak vGRF between limbs. Lower extremity biomechanical outcomes were assessed during the first ($Acquisition_1$) and final

minutes ($Acquisition_{19}$) of the administration of real-time biofeedback. Lower extremity biomechanical outcomes were also assessed during the first minute ($Recall_1$) following removal of the real-time biofeedback and following 45 minutes of rest ($Recall_{45}$). **Main Outcome Measures:** Peak vGRF, vGRF instantaneous loading rate, peak internal knee extension moment (KEM) during the first half of the stance phase of walking gait. **Results:** Data were analyzed using separate 4 x 4 (condition x time) repeated measures ANOVAs. Individuals with ACLR demonstrated significantly greater peak vGRF during the overloading condition and significantly lesser peak vGRF during the under-loading condition as compared to the control. During the overloading condition participants also demonstrated significantly greater peak KEM and knee flexion excursion as compared to the under-loading condition. **Conclusions:** Real-time biofeedback is effective at eliciting acute changes in lower extremity kinetics and kinematics in individuals with ACLR.

INTRODUCTION

Approximately one-third of individuals undergoing ACLR develop PTOA within the first decade following reconstruction.[13,15,231,232] Aberrant walking gait biomechanics that develop following ACLR are hypothesized to contribute to the onset of PTOA in these individuals.[17,23,25,34,157,233] Despite therapeutic rehabilitation, altered lower extremity kinetics and kinematics have been demonstrated to persist at the time participants return to unrestricted physical activity,[29,30] as well as for years following ACLR.[31,32] Alterations in lower extremity kinetics and kinematics have been hypothesized to lead to PTOA onset.[17,23,34] Mechanical loading specifically has been associated with cartilage metabolism[26,27] and PTOA development in individuals with ACLR.[44] Targeting mechanical loading through therapeutic interventions

may improve long-term outcomes following ACLR. However, eliciting changes in mechanical loading may subsequently alter sagittal plane kinematic patterns at the knee,[226] further increasing the mechanical load applied to the articular cartilage.[157]

Individuals with ACLR commonly demonstrate inter-limb asymmetries during walking gait. Conflicting evidence suggests both increased[169,234] and decreased[44,147] mechanical loading occurs in the ACLR limb compared to the contralateral limb during walking gait. Both excessive[135,136] and insufficient mechanical[26,27] loading may lead to PTOA development.[17,25,34] In animal models, greater magnitude of loading and rate of loading result in depleted proteoglycan content[135] and greater fissuring of the articular surface,[136] respectively. In individuals with ACLR specifically, lesser mechanical loading in the ACLR limb is associated with greater concentrations of plasma markers of inflammation (i.e. interleukin-6) and enzymes linked to cartilage degradation (i.e. matrix metalloproteinase-3) within the first 6 months following ACLR,[27] and cartilage turnover ratios chronically following ACLR.[26] Additionally, individuals developing PTOA within 5 years following ACLR demonstrate lesser tibiofemoral contact force in the ACLR limb at the time of return to physical activity compared to those who do not develop PTOA.[44]

Individuals with ACLR also demonstrate persistent alterations in sagittal plane kinematics during walking gait.[235] Greater knee flexion at initial contact[60,157] and lesser peak knee flexion[29,32,157] during walking gait is commonly reported in the ACLR limb compared to contralateral and healthy control limbs. Greater knee flexion at initial contact shifts tibiofemoral cartilage contact points to areas of cartilage that may not be conditioned to tolerate the large magnitudes that occur during repetitive loading.[157] Lesser knee motion during the stance phase of gait may decrease the ability for the quadriceps to attenuate energy in the tissues of the knee

joint.[130] Alterations in sagittal plane kinematics may result from deficits in passive range of motion,[155] or subconscious efforts to alter mechanical loading due to metabolic changes within the articular cartilage[23] or fear of movement and re-injury.[236] In order to develop effective clinical strategies to reduce the risk of PTOA following ACLR, it is imperative to understand how eliciting changes in mechanical loading may influence acute changes in kinetics and kinematics that may impact articular cartilage metabolism.

The peak vertical ground reaction force (vGRF) is an indication of the vertical force applied to the entire lower extremity during stance,[55,57] and may influence knee-specific compressive force.[237] Real-time biofeedback has previously been successful at altering peak vGRF during walking gait in individuals with total hip arthroplasty,[55] yet it remains unknown if real-time biofeedback can acutely manipulate peak vGRF during walking gait in individuals with ACLR. Additionally, it remains unknown if acutely manipulating peak vGRF results in changes in additional kinetic and kinematic outcomes. Therefore, the purpose of this study was to utilize real-time biofeedback to acutely elicit overloading (i.e. increased vGRF), under-loading (i.e. decreased vGRF) and symmetrical loading between limbs during walking gait in individuals with ACLR, and determine subsequent changes in lower extremity kinetics and kinematics known to influence PTOA development. Secondarily, as effective interventions are capable of eliciting long-term changes in movement patterns, we sought to determine if individuals with ACLR recall symmetrical loading, overloading, and under-loading following removal of the real-time biofeedback.

METHODS

Design

For this crossover study, each participant completed four testing sessions (control, symmetrical, overloading, under-loading) that were conducted at the same time of day (mean time difference between sessions = 0.29±0.48 hours) with at least a 7-day interval between each session (mean = 8.83±2.29 days). We blinded the participants to the symmetrical, overloading, under-loading loading condition implemented via real-time biofeedback. The biofeedback targets for the three loading conditions were determined during the control session, which was always the first session conducted for each participant. No biofeedback was provided during the control session.

The order of loading conditions elicited via real-time biofeedback during the remaining three testing sessions was block randomized prior to participant enrollment. All testing procedures followed the same order for each session, which consisted of five 60-second walking trials (Figure 9A). First, the baseline trial was collected, during which participants were instructed to walk in a normal gait pattern. Immediately following the baseline trial real-time biofeedback was displayed to the participant during each 20-minute loading condition. Acquisition of each loading condition (i.e. symmetrical loading, overloading, under-loading) was assessed during the first minute of the intervention ($Acquisition_1$) and during the final minute (i.e. minute 19) of the intervention ($Acquisition_{19}$). Recall was assessed during the first minute following removal of the real-time biofeedback ($Recall_1$) and following 45 minutes of rest ($Recall_{45}$). Lower extremity kinetic (peak vGRF, instantaneous vGRF loading rate, peak internal knee extension moment [KEM]) and kinematic outcomes (knee flexion excursion) were collected during each 60-second walking trial. The university's Institutional Review Board

approved all methods and all participants provided written informed consent prior to participation.

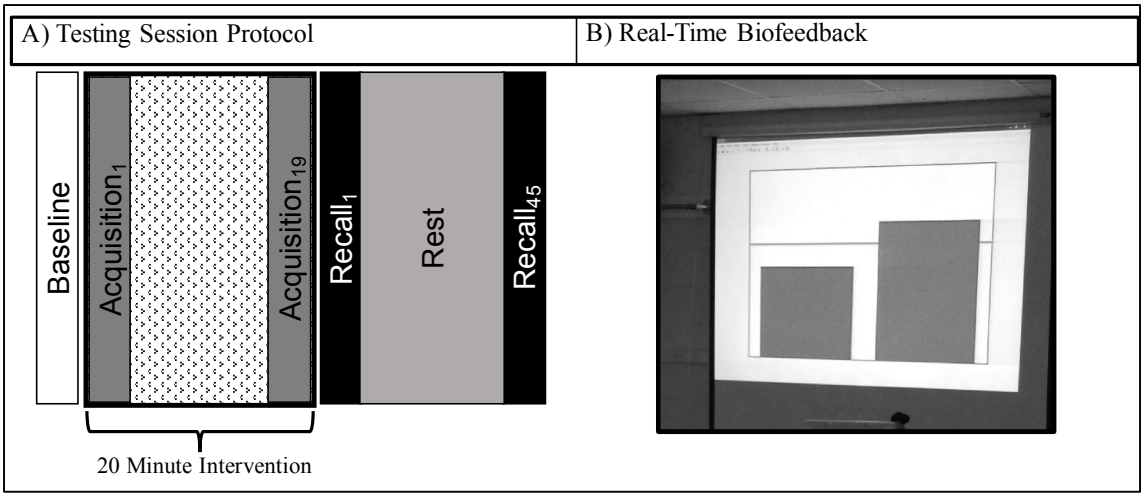


Figure 9A. Aim 2 Testing Protocol. All four testing sessions were conducted in the same order, which consisted of five 60-seconds trials. Participants were instructed to walk in a normal gait pattern during the baseline trial. Immediately following the baseline trial real-time biofeedback was displayed to the participant during the 20-minute intervention. No biofeedback was provided during the control session. Acquisition of each loading condition (i.e. symmetrical loading, overloading, under loading) was assessed during the first Acquisition was assessed during the first minute of the intervention ($Acquisition_1$) and during the final minute (i.e. minute 19) of the intervention ($Acquisition_{19}$). Immediate recall was assessed during the first minute following removal of the real-time biofeedback ($Recall_1$) and following 45 minutes of rest ($Recall_{45}$). **Figure 9B.** The real-time biofeedback displayed a blue bar graph for each limb, which represented the magnitude of the first peak of the vGRF. A red target line was placed in the center of the screen, and participants were instructed to alter their movement in order to match each blue bar (i.e. peak vGRF) to the red line during each step.

Participants

We recruited a convenient sample of individuals between 18-35 years of age who underwent a primary, unilateral ACLR using either a patellar tendon or hamstring autograft from the university community. All participants were participating in unrestricted physical activity as allowed by their orthopaedic physician, which included at least 30 minutes of physical activity three times per week. We excluded individuals: 1) with a history of musculoskeletal injury to either leg (e.g. ankle sprain, muscle strain) within 6 months prior to participation in the study, 2) a history of lower extremity surgery other than ACLR, 3) with a history of knee osteoarthritis or

current symptoms related to knee osteoarthritis (e.g. pain, swelling, stiffness), 4) who were currently pregnant or planning to become pregnant while enrolled in the study, 5) with a history of cardiovascular restrictions that limited the participant's ability to participate in physical activity. Participants were asked to self-report age, sex, ACL graft type, and the date of ACL injury and ACLR. Height and weight were measured in the laboratory prior to testing. All participants completed the subjective portion of the International Knee Documentation Committee index to evaluate self-reported disability and the Tegner Activity Scale to measure level of physical activity. We estimated we would detect a moderate effect (Cohen's d = 0.50) for each kinetic and kinematic outcome between loading conditions, which we determined from our pilot data collected for this study in three healthy individuals. Therefore, in order to detect a moderate effect size of 0.50 with 80% power and an alpha level of 0.05 we would need to enroll 29 participants (G*Power Statistical Power Analysis Software v3.1[223]).

Collection of Kinematics and Kinetic Data

All kinematics and kinetics were collected with a 14-camera motion capture system (Motion Analysis Corporation, Santa Rose, CA) and a dual-belt, force-sensing treadmill (Bertec, Columbus, OH) with two 70 x 40 inch force plates (Model S020008). Kinematic data were sampled at 100Hz and filtered using a 4th order low-pass Butterworth filter with a cut-off frequency of 6Hz. Kinetic data were sampled at 1000Hz and filtered using a 4th order low-pass Butterworth filter with a cut-off frequency of 100Hz. Participants were outfitted with 15 anatomical retro-reflective markers on the lower extremities (1st metatarsal, 5th metatarsal, calcaneus, lateral malleolus, lateral epicondyle, anterior superior iliac spine, posterior superior iliac spine, and 2nd sacral vertebrae) with 14 additional tracking markers affixed using rigid

clusters to the thigh and shank bilaterally. A static trial was collected with an additional 4 markers (bilateral medial malleolus, medial epicondyle), and used to determine knee and ankle joint centers. Hip joint centers were calculated from a leg circumduction task.[238] During the first control session, prior to treadmill walking, self-selected walking speed was determined using timing gates during 5 over-ground walking trials (Brower TC-Gate; Brower Timing Systems, Draper, Utah) and used to set the speed of the treadmill for the subsequent 4 testing sessions. Before beginning the first testing session (control session) participants walked on the treadmill for 5 minutes to allow for acclimation to treadmill walking.[50]

The static calibration trial and functional hip joint centers were used to scale a seven segment, 18 degree-of-freedom model of the pelvis and left and right lower extremities.[211] The filtered marker and force data were used to estimate sagittal plane knee angle (flexion [+]) and internal sagittal plane knee moment (extension [-]) using previously described inverse dynamics calculations.[212] All outcomes were identified during the first 50% of the stance phase of gait, which we determined as the interval from initial contact (vGRF $\geq$ 20N) to toe-off (vGRF $\leq$ 20N) and stride-averaged across the 60-second trial using a custom-built MATLAB program. The body mass of each participant was converted to Newtons (N) and used to normalize peak vGRF (xBW) and instantaneous vGRF loading rate (xBW/seconds). Instantaneous vGRF loading rate was determined by calculating the first derivative of the slope from the force-time curve. Peak internal KEM was normalized to the product of bodyweight and height (xBW*meters). Knee flexion excursion was calculated from sagittal plane knee angle at initial contact to peak knee flexion angle.

Real-Time Biofeedback Conditions

During the baseline trial of the control session a custom MATLAB (Mathworks, Inc, Natick, MA) program processed and extracted left and right limb peak vGRF from the first 50% of the stance phase, which was used to determine the biofeedback targets for the three loading conditions (symmetrical loading, overloading, and under-loading) conducted in the subsequent sessions.

For the symmetrical loading, overloading, and under-loading sessions a 72 inch projection screen directly in front of the treadmill displayed the real-time biofeedback (Figure 9B). A second custom MATLAB script continuously computed the average of the previous four peak vGRF during the first 50% of stance phase, which was visually displayed as right and left blue bar graphs on the projection screen, with a red target line across the center. The target line for the symmetrical loading condition corresponded to the mean peak vGRF between the ACLR and contralateral limb collected during the baseline trial of the control session. The target line for the overloading condition corresponded to a 5% increase in the baseline peak vGRF for the ACLR limb and the contralateral limb. The target line for the under-loading condition corresponded to a 5% decrease in baseline peak vGRF. The target line was always displayed in the center of the screen for each loading condition to maintain participant blinding to condition. Target values for the overloading (5% above baseline peak vGRF) and under-loading (5% below baseline peak vGRF) conditions were determined individually for left and right leg based on the baseline value of each limb. Therefore, the overloading and under-loading conditions did not specifically cue inter-limb symmetry, rather a relative change in magnitude in each limb.

Before completing the real-time biofeedback intervention a study investigator (BALH) conducted a brief presentation with each participant explaining the peak vGRF and how the

biofeedback continuously displayed peak vGRF. Participants were instructed to match the height of each blue bar (i.e. peak vGRF) to the target line as close as possible during each loading condition and utilize any movement strategy possible to manipulate peak vGRF. During the initial presentation all participants were provided one strategy that focused on manipulating the vertical displacement of their center of mass (COM). Specifically, that increasing or decreasing the vertical displacement of their COM may result in a subsequent increase or decrease in peak vGRF, respectively. We provided one strategy to maximize the success of participants consistently reaching the target. Real-time biofeedback was not provided during the assessment of recall ($Recall_1$, $Recall_{45}$), and participants were instructed to "Walk in the same manner as when attempting to match each blue bar to the target line."

Statistical Analysis

We calculated percent change scores from baseline at each time point ($Acquisition_1$, $Acquisition_{19}$, $Recall_1$, $Recall_{45}$) within each testing session using Equation 1 for each kinetic and kinematic outcome.

Equation 1:

$$Percent\ Change = \left[\frac{(Post - Pre)}{Pre}\right] * 100$$

First, with all participants included, we conducted separate 4x4 (condition x time) repeated measures ANOVAs to determine differences in percent change from baseline between loading conditions (symmetrical, overloading, under-loading, control) at each time point ($Acquisition_1$, $Acquisition_{19}$, $Recall_1$, $Recall_{45}$). As main effects for condition were compared across four time points (0.05/4), and main effects for time were compared across four conditions (0.05/4) we adjusted our P-value for comparing main effects was $P = 0.0125$.[213] Dependent samples t-tests were used for *post hoc* analyses if a significant condition x time interaction was

determined. We adjusted the P-value for multiple comparisons as our total number of family-wise comparisons was 16 (four conditions X four time points) therefore our adjusted P-value for our dependent samples t-tests was set at P = 0.003. As a *post hoc* analysis we determined the influence of outliers on our results. Outliers for each outcome measure were identified via box plots, and were defined as any data point >3 standard deviations away from the mean during two or more time points within each loading condition. Outliers were first removed from each outcome, and then we conducted separate 4 x 4 (condition x time) repeated measures ANOVAs and Bonferroni post hoc analyses as previously described above.

We calculated six Cohen's *d* effect sizes with corresponding 95% confidence intervals (95% CI) at each time point to determine the magnitude of differences in percent change scores between each loading condition.[214] Cohen's *d* effect sizes were classified as strong ≤ 0.80, moderate $0.79 - 0.50$, and small ≥ 0.49.[214] All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Version 21, IBM Corp., Somers, NY).

RESULTS

We enrolled 30 individuals with unilateral ACLR (Table 6).

Kinetic Outcomes

Peak Vertical Ground Reaction Force

We found a significant condition x time interaction for the percent change in peak vGRF ($F_{9,253}$=3.282, P=0.001; Figure 10). At $Acquisition_1$, participants demonstrated significantly greater peak vGRF during the overloading condition as compared to the symmetrical loading condition (t_{29} =-5.263, P<0.001; Table 7), under-loading condition (t_{29} =4.419, P<0.001) and the control (t_{29} =-9.487, P<0.001). Participants demonstrated significantly lesser peak vGRF during

the under-loading condition as compared to the symmetrical loading condition (t_{29}=-10.908, P<0.001) and the control (t_{29}=4.47, P<0.001).

At Acquisition$_{19}$, participants demonstrated greater peak vGRF during the overloading condition as compared to the symmetrical loading (t_{29}=-5.078, P<0.001; Table 7), under-loading (t_{29}=-11.65, P<0.001) and the control conditions(t_{29}=-5.584, P<0.001). Participants demonstrated a significant decrease in peak vGRF during the under-loading condition as compared to the symmetrical loading (t_{29}=5.246, P<0.001) and the control conditions(t_{29} =5.5.645, P<0.001).

At Recall$_{1,}$ participants demonstrated significantly greater peak vGRF during the overloading condition as compared to the symmetrical loading condition (t_{29}=-4.584, P<0.001; Table 7), under-loading condition (t_{29}=-5.119, P<0.001) and the control conditions (t_{29}=-4.965, P<0.001). Participants demonstrated significantly lesser peak vGRF during the under-loading condition as compared to the symmetrical loading condition (t_{29}=5.119, P<0.001) and the control condition (t_{29}=5.263, P<0.001).

At Recall$_{45}$, participants demonstrated significantly greater peak vGRF during the overloading condition as compared to the symmetrical loading condition (t_{29}=-3.488, P<0.001; Table 7), under-loading (t_{29}=-9.13, P<0.001) and the control condition (t_{29}=-4.17, P<0.001). Participants demonstrated significantly lesser peak vGRF during the under-loading condition as compared to the symmetrical loading condition (t_{29}=5.771, P<0.001) and the control (t_{29}=6.334, P<0.001). Peak vGRF was not significantly different between the symmetrical loading condition and the control condition at any time point (P>0.003).

One outlier was identified for peak vGRF. Removal of this participant did not change our results as previously described (Figure 10). All between condition effect sizes were strong and

demonstrated conclusive confidence intervals not crossing zero at each time point, except when the control condition was compared to the symmetrical condition (Table 8).

Instantaneous Vertical Ground Reaction Force Loading Rate

Instantaneous vGRF loading rate demonstrated a significant main effect for condition ($F_{3,87}$ =10.282, P<0.001; Figure 11). Across all time points, participants demonstrated significantly greater instantaneous vGRF during the overloading condition as compared to the symmetrical loading condition (P=0.007) and the control condition (P<0.001). The change in instantaneous vGRF loading rate was not significantly different between any other conditions (P>0.05).

No outliers were found for instantaneous vGRF. We found strong effect sizes with conclusive confidence intervals for differences between the overloading and control conditions across all time points (Table 8). No other between condition effect sizes were strong across all time points.

Peak Internal Knee Extension Moment

There were significant condition main effects for peak KEM ($F_{3,87}$=13.247, P<0.001) and time ($F_{3,87}$=7.409, P<0.001; Figure 12). Across all time points, peak KEM was significantly greater in the overloading condition as compared to the the under-loading condition (P< 0.001) and the control condition (P= 0.001). Peak KEM was not significantly different between any other conditions. Across all conditions, participants demonstrated lesser peak KEM at $Acquisition_1$ as compared to $Acquisition_1$ (P=0.01) and $Recall_1$ (P=0.006). Additionally, peak KEM was greater at $Recall_1$ as compared to $Recall_{45}$ (P=0.012). Peak KEM was not significantly different between any other time points (P>0.0125).

We determined there were two outliers for the peak KEM variable. Removal of these outliers did not change our results as compared to when all participants included in the analysis (Figure 12). Between condition effect sizes were strong and demonstrated conclusive confidence intervals at $Acquisition_1$, $Acquisition_{19}$ and $Recall_1$ indicating 1) greater peak KEM during overloading compared to control, 2) greater peak KEM during overloading compared to symmetrical loading, and 3) greater peak KEM during overloading compared to under-loading (Table 8). At $Recall_1$ between condition effect sizes were strong and demonstrated conclusive confidence intervals indicating 1) greater peak KEM during overloading compared to control, and 2) greater peak KEM during overloading compared to under-loading. No other between condition effect sizes were strong across all time points.

Knee Kinematics

Knee Flexion Excursion

With all participants included, knee flexion excursion demonstrated a significant main effect for time ($F_{3,87}$=13.157, P<0.001; Figure 13). Across conditions, knee flexion excursion was significantly greater at $Acquisition_{19}$ as compared $Acquisition_1$ (P=0.007) and $Recall_{45}$ (P=0.001). Participants demonstrated greater knee flexion excursion at $Retention_1$ as compared to $Retention_{45}$ (P<0.001). Additionally, flexion excursion was significantly greater at $Recall_1$ as compared to $Acquisition_1$ (P=0.003) and compared to $Recall_{45}$ (P<0.001). Knee flexion excursion was not significantly greater at $Acquisition_{19}$ as compared to $Retention_1$ (P>0.003).

Four outliers were determined for knee flexion excursion. Following removal of the outliers, we determined a significant time x condition interaction ($F_{9,250}$=2.228, P=0.021; Figure 13). At $Acquisition_1$ knee flexion excursion was not significantly different between any of the loading conditions (P>0.003).

At $Acquisition_{19}$, participants demonstrated significantly greater knee flexion excursion during the overloading condition as compared to the symmetrical loading condition ($t_{,25} = -7.332$, $P<0.001$). The change in knee flexion excursion was not significantly different between any other conditions at $Acquisition_{19}$ ($P>0.003$).

At $Recall_1$, participants demonstrated significantly greater knee flexion excursion during the overloading condition as compared to the symmetrical loading condition ($t_{25} = -5.361$, $P<0.001$) and the under-loading condition ($t_{25} = -3.577$, $P=0.001$). Knee flexion excursion was not significantly different between any other conditions at $Recall_1$ ($P>0.003$).

At $Recall_{45}$, participants demonstrated significantly greater knee flexion excursion during the overloading condition as compared to the symmetrical loading condition ($t_{25} = -3.803$, $P=0.001$) and the under-loading condition ($t_{25} = -3.580$, $P=0.001$). Knee flexion excursion was not significantly different between any other conditions at $Recall_{45}$ ($P>0.003$).

Table 6. Manuscript 2 Participant Demographics

Sex	21 Female; 9 Male
Age (years)	20.43±2.91
Height (cm)	172.70±10.81
Mass (kg)	73.16±16.10
BMI	24.42±4.25
Time since Surgery (months)	47.83±26.97
Graft Type	HS = 16; PT = 14
IKDC	86.49±9.51
Tegner	7.47±1.33
ACLR Limb Baseline Biomechanical Outcomes	
Peak vGRF (xBW)	1.11±0.06
Peak vGRF Limb Symmetry Index (%)	98.55±2.75
Instantaneous vGRF Loading Rate (xBW/sec)	132.92±30.74
Peak KEM (xBW * height)	-0.05±0.01
Knee Flexion Excursion (degrees)	13.49±2.75

Data presented as mean ± standard deviation. HS = hamstring tendon autograft; PT = patellar tendon autograft. Limb symmetry index calculated as ACLR limb relative to uninjured limb, with values <100 indicating lesser peak vGRF on ACLR limb compared to contralateral limb.

Table 7. Changes in Biomechanical Outcomes

	Peak vGRF									
	All Participants n=30	Outliers Removed n=29	All Participants n=30	Outliers Removed n=29	All Participants n=30	Outliers Removed n=29	All Participants n=30	Outliers Removed n=29	All Participants n=30	Outliers Removed n=29
	$Acquisition_1$		$Acquisition_{19}$		$Recall_1$		$Recall_{45}$		Condition Mean	
Symmetrical Loading	0.97±3.61	0.48±2.54	1.31±3.77	0.79±2.47	1.96±4.06	1.44±2.89	1.25±3.81	0.79±2.89	1.38±3.45	0.88±2.14
Overloading	4.34±2.33	4.23±2.29	5.04±2.33	4.99±2.49	6.16±3.99	6.02±3.99	5.65±5.49	5.81±15.51	5.30±2.94	5.26±2.98
Under-loading	-2.19±2.34	-2.15±2.37	-2.31±2.28	-2.33±2.32	-2.44±2.90	-2.39±2.94	-4.04±3.93	-3.88±3.39	-2.84±2.20	-2.77±2.19
Control	-0.09±1.08	-0.10±1.09	1.35±2.37	1.37±2.41	1.62±2.46	1.64±2.51	1.03±2.00	1.05±2.04	0.98±1.64	0.992±1.66
Time Point Mean	0.76±1.45	0.62±1.25	1.26±1.41	1.13±1.24	1.83±1.73	1.67±1.55	0.982.16	0.94±2.19		
	Instantaneous vGRF Loading Rate									
	All Participants n=30	Outliers Removed	All Participants n=30	Outliers Removed	All Participants n=30	Outliers Removed	All Participants n=30	Outliers Removed	All Participants n=30	Outliers Removed
	$Acquisition_1$		$Acquisition_{19}$		$Recall_1$		$Recall_{45}$		Condition Mean	
Symmetrical Loading	4.71±11.17		1.18±13.01		0.98±13.66		4.08±10.76		2.73±10.77	
Overloading	13.52±16.54		11.27±21.31		10.71±17.75		16.66±19.92		13.04±14.51	
Under-loading	1.36±10.41	N/A	-1.57±14.38	N/A	0.33±16.34	N/A	-0.63±18.37	N/A	-0.13±14.08	N/A
Control	-0.31±3.26		-3.11±6.12		-2.76±5.58		0.71±5.78		-1.37±3.53	
Time Point Mean	4.82±5.84		1.94±8.84		2.32±8.49		5.20±6.64			

Table 7. Changes in Biomechanical Outcomes Continued

Peak Internal KEM

	All Participants n=30	Outliers Removed n=28	All Participants n=30	Outliers Removed n=28	All Participants n=30	Outliers Removed n=28	All Participants n=30	Outliers Removed n=28	All Participants n=30	Outliers Removed n=28
	Acquisition$_1$		Acquisition$_{19}$		Recall$_1$		Recall$_{45}$		Condition Mean	
Symmetrical Loading	1.66±8.81	0.96±8.63	9.61±20.82	5.95±13.88	7.71±14.84	5.43±8.83	3.49±16.12	1.54±9.73	5.62±12.67	3.42±7.42
Overloading	12.13±11.10	11.25±9.74	14.34±13.91	12.34±8.74	19.13±16.81	17.09±14.24	11.72±15.65	12.14±15.96	14.33±11.32	13.21±9.99
Under-loading	-2.54±7.54	-2.49±7.69	-1.70±13.81	-1.36±14.24	-1.60±14.49	-0.95±14.80	-5.80±15.80	-4.21±14.70	-2.91±10.94	-2.26±11.00
Control	0.11±4.23	-0.14±4.16	7.72±12.61	5.81±8.72	8.54±12.98	6.36±8.45	1.14±8.90	0.43±8.78	4.39±8.28	3.11±6.19
Time Point Mean	2.84±5.76	2.40±5.30	7.49±8.47	5.69±4.91	8.45±8.87	6.93±6.94	2.64±8.16	2.47±7.23		

Knee Flexion Excursion

	All Participants n=30	Outliers Removed n=26	All Participants n=30	Outliers Removed n=26	All Participants n=30	Outliers Removed n=26	All Participants n=30	Outliers Removed n=26	All Participants n=30	Outliers Removed n=26
	Acquisition$_1$		Acquisition$_{19}$		Recall$_1$		Recall$_{45}$		Condition Mean	
Symmetrical Loading	4.49±12.88	2.11±9.67	10.88±17.88	6.34±9.88	13.03±21.99	7.04±11.76	6.45±25.26	0.70±10.63	8.71±17.52	4.05±8.61
Overloading	10.44±15.29	9.26±14.76	18.29±16.12	20.11±14.67	21.76±15.03	22.28±14.48	12.70±18.62	12.73±16.90	15.80±12.32	16.10±11.68
Under-loading	3.81±18.73	1.03±14.84	13.83±43.96	6.36±29.04	12.23±46.38	4.05±28.05	3.35±46.80	-6.62±25.08	8.31±36.17	1.20±21.76
Control	2.69±6.51	2.80±6.82	13.67±14.04	14.84±14.49	14.30±14.78	14.98±15.65	3.99±10.70	4.06±11.24	8.66±1.80	9.17±10.39
Time Point Mean	5.36±9.55	3.80±7.90	14.17±13.41	11.91±12.34	15.33±14.26	12.09±11.74	6.62±13.84	2.72±9.61		

Table 8. Between Condition Effect Sizes for Lower Extremity Kinetics and Kinematics

Peak Internal KEM

	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Acquisition 1						Acquisition 19					
Conditions Compared	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	-0.22	-0.73	0.29	-0.16	-0.68	0.36	-0.11	-0.61	0.40	-0.01	-0.54	0.51
Control vs Overloading	-1.41	-1.98	-0.85	-1.50	-2.09	-0.91	-0.49	-1.01	0.02	-0.74	-1.28	-0.20
Control vs Under loading	0.43	-0.08	0.94	0.37	-0.15	0.90	0.70	0.18	1.22	0.60	0.06	1.13
Symmetrical vs Overloading	-1.03	-1.57	-0.49	-1.10	-1.66	-0.54	-0.26	-0.77	0.24	-0.54	-1.08	-0.01
Symmetrical vs Under-loading	0.51	-0.01	1.02	0.42	-0.11	0.95	0.63	0.11	1.15	0.51	-0.02	1.04
Overloading vs Under-loading	1.53	0.95	2.10	1.54	0.95	2.14	1.14	0.60	1.69	1.14	0.58	1.71

Knee Flexion Excursion

	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Acquisition 1						Acquisition 19					
	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	-0.17	-0.68	0.33	0.08	-0.46	0.63	0.17	-0.34	0.68	0.68	0.12	1.23
Control vs Overloading	-0.65	-1.17	-0.13	-0.59	-1.15	-0.04	-0.30	-0.81	0.21	-0.36	-0.90	0.19
Control vs Under loading	-0.08	-0.58	0.43	0.15	-0.39	0.70	0.00	-0.51	0.50	0.35	-0.20	0.90
Symmetrical vs Overloading	-0.42	-0.93	0.10	-0.59	-1.15	-0.04	-0.43	-0.94	0.08	-1.08	-1.67	-0.50
Symmetrical vs Under-loading	0.04	-0.47	0.55	0.08	-0.46	0.63	-0.09	-0.59	0.42	-0.01	-0.56	0.53
Overloading vs Under-loading	0.37	-0.14	0.88	0.57	0.02	1.13	0.13	-0.37	0.64	0.58	0.02	1.13

Table 8. Between Condition Effect Sizes for Lower Extremity Kinetics and Kinematics Continued

Acquisition											
Peak vGRF											
	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Acquisition 1						Acquisition 19					
Conditions Compared	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	-0.08	-0.58	0.43	-0.30	-0.82	0.22	0.01	-0.49	0.52	0.35	-0.17	0.87
Control vs Overloading	-1.87	-2.47	-1.26	-2.38	-3.05	-1.71	-1.70	-2.29	-1.11	-1.45	-2.03	-0.87
Control vs Under loading	1.50	0.92	2.07	1.09	0.54	1.65	1.55	0.98	2.13	1.54	0.96	2.13
Symmetrical vs Overloading	-1.12	-1.67	-0.58	-1.52	-2.11	-0.94	-1.29	-1.84	-0.73	-1.76	-2.37	-1.15
Symmetrical vs Under loading	1.03	0.49	1.56	1.06	0.51	1.61	1.15	0.60	1.69	1.14	0.59	1.70
Overloading vs Under loading	2.80	2.09	3.51	2.70	1.99	3.41	3.30	2.52	4.08	3.00	2.25	3.75
Instantaneous vGRF Loading Rate											
	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Acquisition 1						Acquisition 19					
Conditions Compared	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	-0.60	-1.12	-0.08				-0.42	-0.93	0.10			
Control vs Overloading	-1.15	-1.69	-0.60				-0.91	-1.44	-0.37			
Control vs Under loading	-0.21	-0.72	0.29	N/A			-0.14	-0.64	0.37	N/A		
Symmetrical vs Overloading	-0.62	-1.13	-0.10				-0.56	-1.08	-0.05			
Symmetrical vs Under loading	0.31	-0.20	0.82				0.20	-0.31	0.71			
Overloading vs Under loading	0.87	0.34	1.40				0.70	0.18	1.22			
Peak Internal KEM											
	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Acquisition 1						Acquisition 19					
Conditions Compared	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	-0.22	-0.73	0.29	-0.16	-0.68	0.36	-0.11	-0.61	0.40	-0.01	-0.54	0.51
Control vs Overloading	-1.41	-1.98	-0.85	-1.50	-2.09	-0.91	-0.49	-1.01	0.02	-0.74	-1.28	-0.20
Control vs Under loading	0.43	-0.08	0.94	0.37	-0.15	0.90	0.70	0.18	1.22	0.60	0.06	1.13

Table 8. Between Condition Effect Sizes for Lower Extremity Kinetics and Kinematics Continued

Recall

Peak vGRF

	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Recall 1						Recall 45					
	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	-0.06	-0.56	0.45	0.07	-0.44	0.59	-0.07	-0.58	0.43	0.10	-0.41	0.62
Control vs Overloading	-0.89	-1.42	-0.36	-1.30	-1.86	-0.73	-1.10	-1.65	-0.56	-1.55	-2.13	-0.96
Control vs Under-loading	1.28	0.73	1.84	1.46	0.88	2.03	1.60	1.02	2.19	1.57	0.98	2.15
Symmetrical vs Overloading	-1.46	-2.03	-0.89	-1.30	-1.86	-0.73	-0.92	-1.45	-0.39	-1.48	-2.06	-0.90
Symmetrical vs Under-loading	1.54	0.97	2.12	1.30	0.73	1.86	1.35	0.79	1.91	1.34	0.77	1.91
Overloading vs Under-loading	3.00	2.26	3.74	2.37	1.70	3.04	2.00	1.38	2.62	2.48	1.79	3.16

Instantaneous vGRF Loading Rate

	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Recall 1						Recall 45					
	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	-0.35	-0.86	0.16				-0.39	-0.90	0.13			
Control vs Overloading	-1.01	-1.55	-0.47				-1.07	-1.61	-0.53			
Control vs Under-loading	-0.25	-0.76	0.26				0.10	-0.41	0.60			
Symmetrical vs Overloading	-0.61	-1.12	-0.09	N/A			-0.78	-1.30	-0.25	N/A		
Symmetrical vs Under-loading	0.04	-0.46	0.55				0.31	-0.20	0.82			
Overloading vs Under-loading	0.60	0.08	1.12				0.89	0.36	1.42			

Table 8. Between Condition Effect Sizes for Lower Extremity Kinetics and Kinematics Continued

Peak Internal KEM

	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Recall 1						Recall 45					
	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	0.07	-0.44	0.57	0.11	-0.42	0.63	-0.18	-0.69	0.33	-0.12	-0.64	0.41
Control vs Overloading	-0.69	-1.21	-0.17	-0.90	-1.45	-0.35	-0.82	-1.35	-0.29	-0.90	-1.45	-0.35
Control vs Under-loading	0.74	0.21	1.26	0.60	0.06	1.13	0.53	0.02	1.05	0.38	-0.15	0.91
Symmetrical vs Overloading	-0.71	-1.23	-0.19	-0.97	-1.52	-0.42	-0.51	-1.03	0.00	-0.79	-1.33	-0.25
Symmetrical vs Under-loading	0.63	0.11	1.14	0.52	-0.02	1.05	0.57	0.06	1.09	0.45	-0.08	0.99
Overloading vs Under-loading	1.30	0.75	1.86	1.22	0.65	1.80	1.10	0.56	1.64	1.05	0.49	1.61

Knee Flexion Excursion

	All Participants Included n=30			Outliers Removed n=29			All Participants Included n=30			Outliers Removed n=29		
	Recall 1						Recall 45					
	Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI		Effect Size	95% CI	
Control vs Symmetrical	0.07	-0.44	0.57	0.56	0.01	1.12	-0.13	-0.63	0.38	0.30	-0.24	0.85
Control vs Overloading	-0.49	-1.01	0.02	-0.48	-1.03	0.07	-0.57	-1.08	-0.05	-0.59	-1.15	-0.04
Control vs Under-loading	0.06	-0.45	0.57	0.47	-0.08	1.03	0.02	-0.49	0.52	0.54	-0.01	1.09
Symmetrical vs Overloading	-0.46	-0.97	0.06	-1.14	-1.72	-0.55	-0.28	-0.79	0.23	-0.84	-1.41	-0.27
Symmetrical vs Under-loading	0.02	-0.48	0.53	0.14	-0.41	0.68	0.08	-0.42	0.59	0.37	-0.17	0.92
Overloading vs Under-loading	0.27	-0.24	0.78	0.80	0.24	1.37	0.26	-0.25	0.77	0.89	0.32	1.46

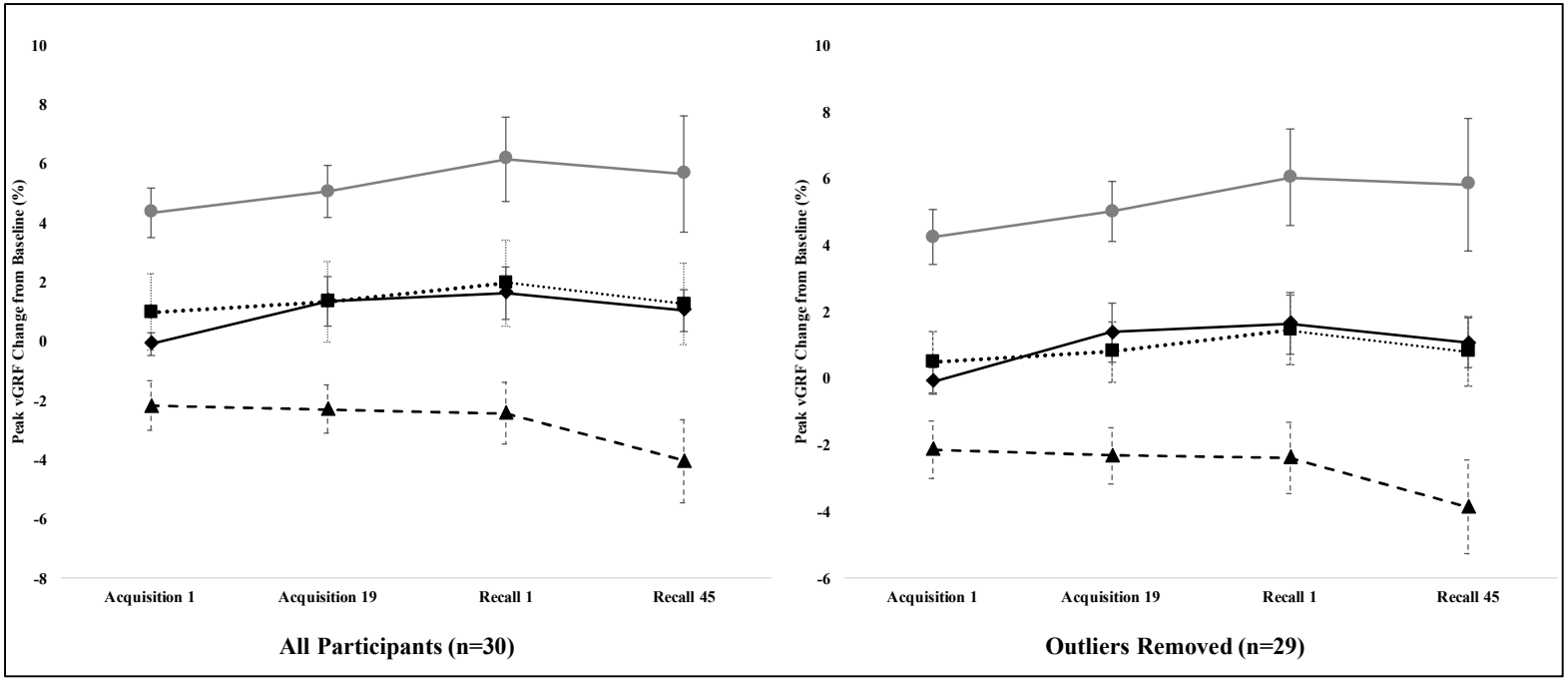


Figure 10. Change in Peak Vertical Ground Reaction Force Between Loading Conditions. Data presented as mean change from baseline peak vGRF at each time point, with corresponding 95% confidence intervals.

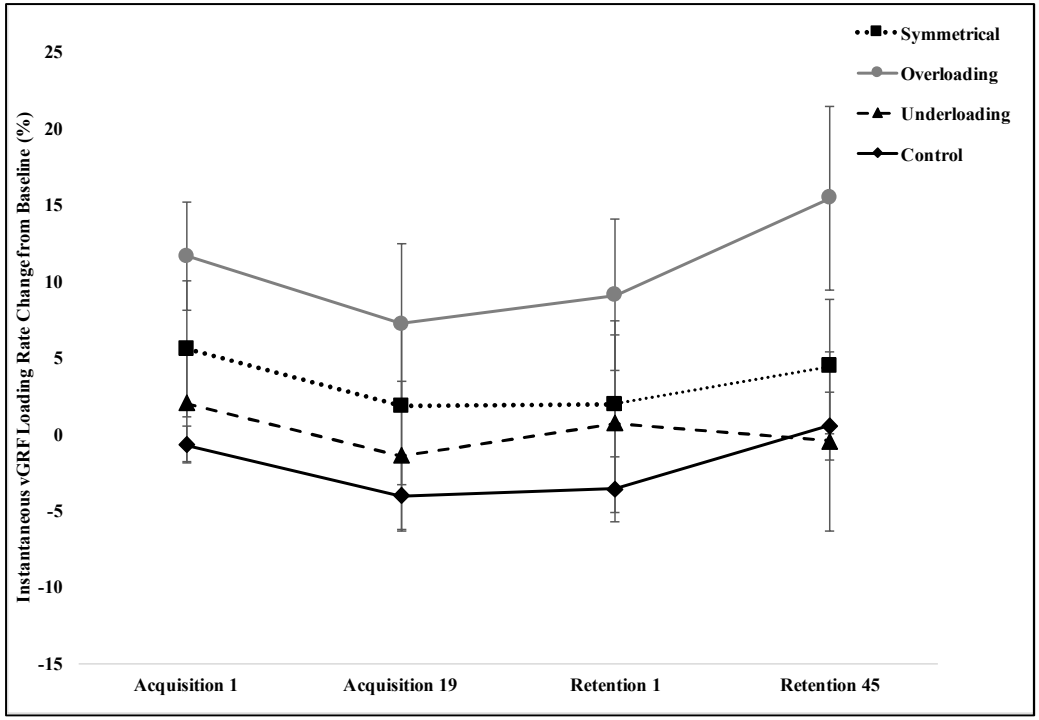


Figure 11. Change in Instantaneous Peak Vertical Ground Reaction Force Loading Rate Between Loading Conditions. Data presented as mean change from baseline peak vGRF at each time point, with corresponding 95% confidence intervals.

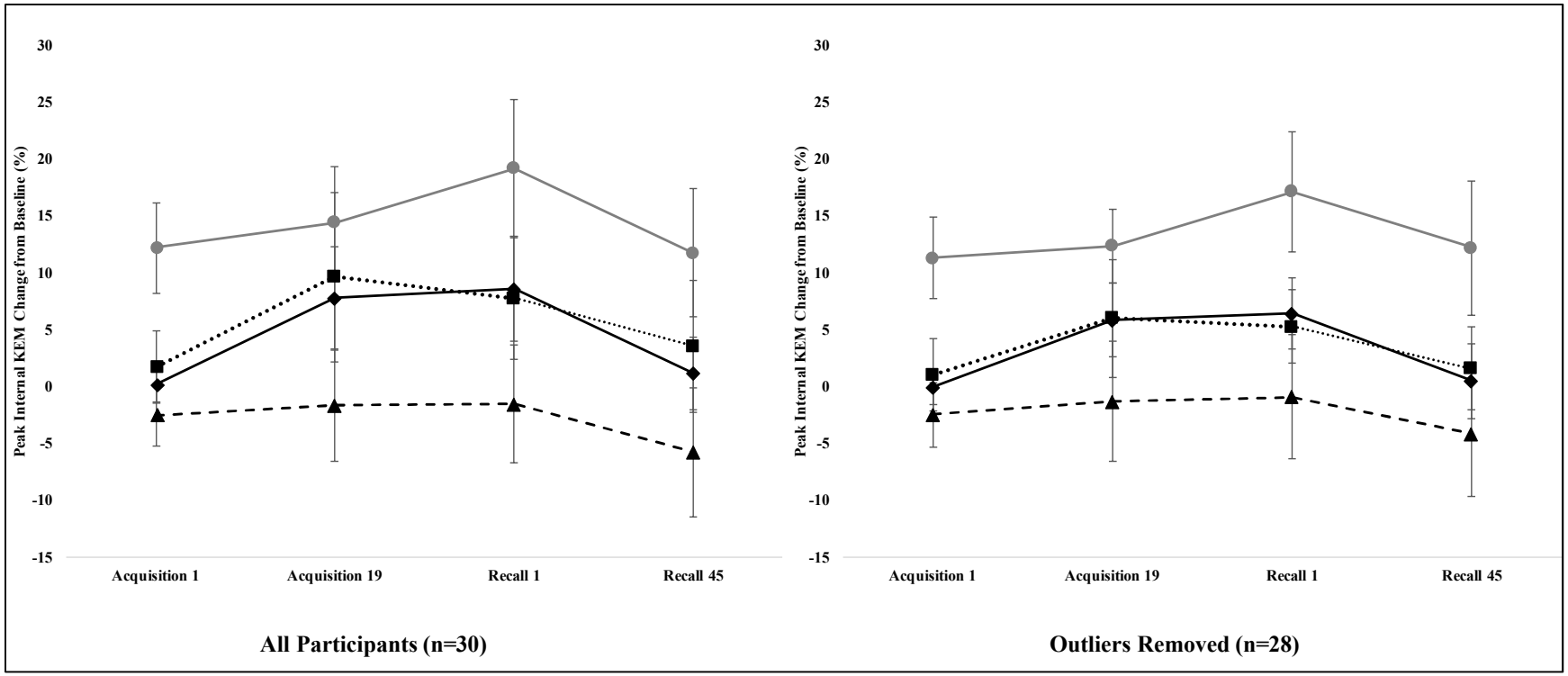


Figure 12. Change in Peak Internal Knee Extension Moment Between Loading Conditions. Data presented as mean change from baseline peak vGRF at each time point, with corresponding 95% confidence intervals.

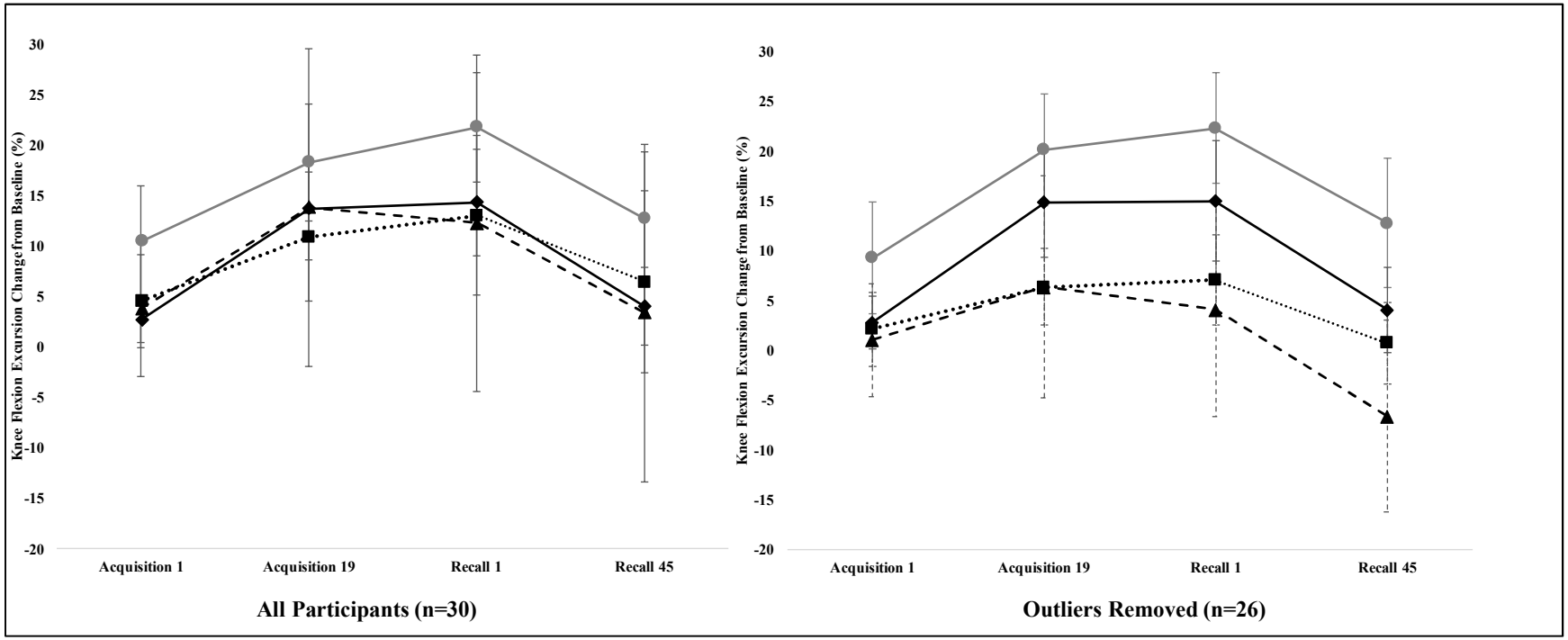


Figure 13. Change in Knee Flexion Excursion Between Loading Conditions. Data presented as mean change from baseline peak vGRF at each time point, with corresponding 95% confidence intervals.

DISCUSSION

Overall, our study provides evidence that individuals with ACLR can significantly increase and decrease peak vGRF when provided real-time biofeedback during walking gait. Moreover, individuals with ACLR can recall significant increases and decreases in peak vGRF forty-five minutes following removal of real-time biofeedback. During the overloading condition (i.e. 5% increase in peak vGRF) participants demonstrated greater peak KEM and knee flexion excursion as compared to the under-loading condition that persisted following removal of real-time biofeedback. Participants also demonstrated greater instantaneous vGRF during the overloading condition as compared to the control and symmetrical loading conditions. Our results indicate real-time biofeedback may be a beneficial intervention for altering peak vGRF during walking gait in individuals with ACLR.

In our study, individuals with ACLR were able to acquire and recall an average 5% increase in peak vGRF during walking gait during the overloading condition (Table 2). This is not surprising as our real-time biofeedback intervention was intended to elicit a 5% change in peak vGRF from baseline. However, during the under-loading condition our participants were able to acquire and recall an average 2.24% and 3.14% decrease in peak vGRF, respectively (Table 2). Individuals with ACLR have been reported to demonstrate lesser peak vGRF[26] and peak KEM[61,235] in the reconstructed limb compared to the contralateral limb and the limbs of healthy controls. Twenty (66%) of our participants demonstrated lesser peak vGRF on the injured limb compared to the contralateral limb at baseline (mean baseline peak vGRF limb symmetry index = 98.55±2.72; range = 93.51 to 104.01). Therefore, a further decrease in vGRF in the ACLR limb may have been difficult to achieve in our cohort. We chose not to provide participants with knee-specific specific strategies to increase or decrease peak vGRF to capture

the naturally emerging changes in kinetics and kinematics. Providing a specific strategy, or providing the biofeedback for longer than 20 minutes in our study may have influenced participant's ability to elicit larger decreases in peak vGRF.

We also demonstrated individuals with ACLR also recalled an average 5.81% increase in peak vGRF and a 3.88% decrease in peak vGRF forty-five minutes following the real-time biofeedback conditions eliciting overloading and under-loading, respectively. The ability to recall changes peak vGRF during walking gait in individuals with ACLR is promising, as greater peak vGRF is associated with lesser serum collagen turnover three years following ACLR.[26] However, it remains unknown if changes in peak vGRF can be recalled in longer long-term follow-ups. It is unlikely a single bout of real-time biofeedback would elicit long-term changes in peak vGRF. Long-term changes in mechanical loading in individuals with ACLR may be feasible with further development of real-time biofeedback interventions.

Individuals with ACLR demonstrated an average 13.22% increase in peak KEM and an average 13.73% increase in knee flexion excursion across all time points during the overloading condition (Table 2). Conversely, peak KEM was reduced by 2.64% and knee flexion excursion was reduced 1.16% across all time points during the under-loading condition (Table 2). During stance, eccentric action of the quadriceps may act to attenuate energy through controlled flexion of the knee.[64,104] A stiffer knee motion, or reduced knee flexion excursion, during the stance phase of gait may decrease the ability for the quadriceps to attenuate energy through the tissues of the knee joint.[130] While the overall force (i.e. greater peak vGRF) applied to the entire limb increased during the overloading condition, the subsequent increases in peak KEM and knee flexion excursion may have enhanced the ability of the knee to attenuate load during the overloading condition. It could be hypothesized that individuals with ACLR who demonstrate

greater peak vGRF during walking gait adopt a protective strategy, such as increasing peak KEM or knee flexion excursion, to better attenuate greater loading during walking gait. These adaptations may allow individuals with ACLR to accept greater overall mechanical loading during walking gait, and may partly explain the associations between greater mechanical loading and lesser cartilage turnover that has been demonstrated previously in individuals with an ACLR.[26] Conversely, knee joint torque increases as the net quadriceps demand increases during walking gait through either increased peak vGRF or greater knee flexion excursion.[239] Individuals with an ACLR commonly demonstrate persistent reductions in quadriceps strength[128,240] and neuromuscular control in the ACLR limb.[127 241] Individuals presenting with deficits in neuromuscular control of the quadriceps may not have the capacity to increase KEM to match the demands of increasing peak vGRF as in the overloading condition in our study. The inability to appropriately increase KEM in response to an increase in peak vGRF may increase stress on additional passive structures of the knee such as the articular cartilage.[104,242,243] Future research is necessary in order to determine the influence of quadriceps neuromuscular control on the ability to increase KEM in response to greater peak vGRF during walking gait.

While the overloading condition resulted in significant increases in peak KEM and knee flexion excursion, individuals with ACLR also demonstrated an average 10.81% increase in instantaneous vGRF loading rate all time points during the overloading condition. Greater instantaneous vGRF loading rate in individuals with ACLR has been reported in the reconstructed limb as compared to the contralateral limb[234] and a non-injured control group.[107] However, greater loading rate may provide a protective benefit to articular cartilage in individuals with ACLR, as recent evidence[27] suggests greater instantaneous vGRF loading rate associates with lesser plasma concentrations of interleukin-6 enzymes linked to cartilage

degradation. Determining the optimal range in which loading rate is beneficial for joint health is important for determining what is protective vs detrimental to overall health.

It is important to note that during the overloading condition the changes in our kinematic outcomes were greater (mean range = -6.62% - 22.28%, Table 2) than the 5% change in peak vGRF our real-time biofeedback intervention cued. Therefore, subtle changes in the overall load applied to the lower extremity may result in substantial knee-specific biomechanical alterations during walking gait. We determined outliers influenced our results for knee flexion excursion, but did not influence the results for any kinetic outcome (peak vGRF, instantaneous vGRF loading rate, peak KEM). While individuals with ACLR demonstrate consistent changes in kinetics when provided real-time biofeedback they likely adopt individualized kinematic patterns to alter loading during walking gait.

Individuals with ACLR demonstrating greater asymmetries in kinetic outcomes between limbs are less likely to meet return to physical activity criteria[29,33] and demonstrate poorer self-reported[29] outcomes than individuals with ACLR who do not demonstrate kinetic asymmetries. Our third loading condition in this study aimed to elicit peak vGRF symmetry between the ACLR and contralateral limb. We did not determine significant differences between the symmetrical loading condition and the control condition for any kinetic or kinematic outcome. While the overloading and under-loading conditions elicited a 5% change from baseline peak vGRF in each participant, the change in peak vGRF from baseline elicited during the symmetrical loading condition was dependent upon the magnitude of inter-limb asymmetry during baseline time point of the control condition. The mean percent change from baseline peak vGRF during the symmetrical loading condition was 0.84±1.53%, with a maximum change from baseline of 3.56%. The small amount of change elicited during the symmetrical loading

condition likely yielded changes in our additional outcomes that were statistically not significant from the control condition. Although not statistically different from the control, slight changes in kinetics and kinematics may influence PTOA development following ACLR.[157] Utilizing real-time biofeedback to promote inter-limb symmetry may be more beneficial for individuals with ACLR demonstrating greater asymmetry than our cohort, particularly acutely following ACLR when inter-limb asymmetries are greater.[32]

While this study improves our understanding of the changes in lower extremity kinetics and kinematics in response to alterations in peak vGRF driven by real-time biofeedback, there are limitations that can inform future research. Although we determined changes in our kinematic outcomes during the under-loading condition, these changes were not statistically significant as compared to the control condition. Large inter-subject variability in changes from baseline during the under-loading condition likely precluded detection of significant mean differences due to our relatively small sample of 30 individuals with ACLR. However, a wide range of changes in peak knee flexion (-47.11% to 25.12%) and knee flexion excursion (-54.06% to 93.28%) may suggest that individuals with ACLR adopt various kinematic strategies when attempting to decrease mechanical loading in the ACLR limb. Further investigation determining patient-specific adaptations to altered mechanical loading is warranted. A variety of factors may influence loading following ACLR, which we did not assess in this study, including quadriceps strength[104] and kinesiophobia.[236] The participants in this study demonstrated high levels of self-reported function (mean IKDC = 86.5); it remains unknown how individuals with lower self-reported function and quadriceps weakness may respond to real-time biofeedback eliciting a change in peak vGRF. Additionally we only assessed changes in knee specific kinetics and kinematics. Altering peak vGRF may also elicit kinematic changes at the hip and ankle, and

these changes should be investigated in the future. The development of PTOA following ACLR is multi-faceted, and does not solely involve alterations in loading.[25] Metabolic alterations, such as increased inflammation and altered cartilage metabolism may also influence PTOA development, and may further PTOA progression when combined with alterations in mechanical loading.[16] Future research should evaluate if real-time biofeedback cuing a change in peak vGRF also results in acute metabolic changes at the level of the articular cartilage.

In conclusion, this study demonstrates real-time biofeedback cuing a change in peak vGRF results in significant increases and significant decreases in peak vGRF during walking gait in individuals with ACLR. Additionally, significant increases and decreases in peak vGRF can be recall forty-five minutes following removal of the biofeedback. Cueing an increase in peak vGRF during the overloading condition resulted in significant increases in instantaneous vGRF loading rate and peak KEM compared to normal walking. Further research is needed to determine if real-time biofeedback can elicit long-term changes in lower extremity kinetics and kinematics and cartilage metabolism in individuals with ACLR.

CHAPTER 7: MANUSCRIPT 3

Acutely Altering Peak Vertical Ground Reaction Force During Walking Gait Influences Cartilage Metabolism in Individuals with Anterior Cruciate Ligament Reconstruction

OVERVIEW

Context: Mechanical loading during walking following anterior cruciate ligament reconstruction (ACLR) interacts with cartilage metabolism, and may lead to deleterious changes in cartilage composition and structure. Acutely altering mechanical loading may influence cartilage metabolism following ACLR, and provide a therapeutic target for limiting cartilage degradation following joint injury. **Objective:** To determine if acutely increasing, decreasing, and promoting symmetrical peak vertical ground reaction force (vGRF) during walking gait results in different metabolic responses quantified through serum concentrations of cartilage oligomeric matrix protein (COMP). **Design:** Single-blind, crossover study. **Setting:** Research laboratory. **Patients or Other Populations:** Thirty individuals with unilateral ACLR. **Interventions:** Participants completed four testing sessions separated by at least 7 days. During each session, one of four loading conditions was completed during the 20 minutes of walking. Loading conditions included 1) control consisting of normal walking, 2) a 5% increase (i.e. overloading) in peak vGRF, 3) a 5% decrease (i.e. under-loading) in peak vGRF, and 4) symmetrical peak vGRF between limbs. Serum samples were collected before and immediately following each loading condition. **Main Outcome Measures:** The change in serum COMP concentration ($COMP_{CHANGE}$) was determined between baseline and immediately following each loading

condition. **Results:** Data were analyzed using separate one-way repeated measures (condition x time) ANOVAs. Individuals with ACLR demonstrating an increase in $COMP_{CHANGE}$ during the control condition demonstrated a significant decrease in $COMP_{CHANGE}$ during the overloading condition as compared to the control. Additionally, individuals demonstrating a decrease in $COMP_{CHANGE}$ during the control condition demonstrated a significant increase in $COMP_{CHANGE}$ during the under-loading condition as compared to the control. **Conclusions:** Manipulation of kinetics during walking gait may acutely influence cartilage metabolism in individuals with ACLR.

INTRODUCTION

One in three individuals undergoing anterior cruciate ligament reconstruction (ACLR) develop radiographic posttraumatic knee osteoarthritis (PTOA) following reconstruction.[13] The complex interaction between aberrant mechanical loading and altered tissue metabolism that occurs following ACLR perpetuates a series of maladaptive processes that culminates in the development of PTOA.[23-25,35] Individuals with ACLR commonly demonstrate persistent inter-limb asymmetries in loading during walking gait, with lesser mechanical loading in the ACLR limb compared to the contralateral limb.[27,29,32,33,44,147] Maintaining appropriate mechanical loading of the articular cartilage is imperative for maintaining joint health,[34-36] therefore chronic reductions in mechanical loading following ACLR may result in changes in tissue metabolism that lead to cartilage degradation.[26,27] Determining the acute metabolic response to alterations in mechanical loading may identify effective therapeutic targets that maintain homeostatic tissue metabolism following ACLR.

Animal models demonstrate that alterations in mechanical loading, both excessive[37,38] and insufficient loading,[36,39] lead to articular cartilage degradation consistent with the onset of PTOA. Rabbit models suggest that energy exerted to the articular cartilage with greater loading rates, applied with a single strike to the articular cartilage, results in greater fissuring of the extraceuluar matrix and increased chondrocyte death as compared to a conditions that applied force to the cartilage with lesser loading rates.[37,38] However, due to the repetitive nature of walking gait, subtle changes in mechanical loading applied over a long duration may also lead to degradation of the articular cartilage.[17,35] Removal of sufficient mechanical loading results in a pro-inflammatory response that increases the expression of matrix metalloproteinase-1 (MMP).[41,42] Greater MMP concentration signals degradation of the articular cartilage.[43] Reduced mechanical loading that occurs within 6-months following ACLR may be involved in signaling alterations in the inflammatory response that ultimately results in cartilage turnover.[41,42]

At 6-months following ACLR individuals demonstrating a lesser peak external knee adduction moment during walking gait in the reconstructed limb also demonstrate greater concentrations of interleukin-6, a pro-inflammatory cytokine.[27] Additionally, lesser instantaneous loading rate of the peak vertical ground reaction force (vGRF) associateed with greater plasma MMP-3 concentrations in individuals with ACLR.[27] A separate study determined individuals developing radiographic PTOA within 5 years of ACLR demonstrated lesser tibiofemoral contact force in the ACLR limb during walking gait at the time of return to physical activity as compared to individuals who did not develop PTOA.[44] While there is growing evidence that long-term alterations in mechanical loading may influence tissue metabolism following ACLR,[26,27,44] it remains unknown if acute reductions in mechanical loading can acutely influence tissue metabolism in individuals with ACLR.

Assessing changes in serum concentrations of cartilage oligomeric matrix protein (COMP) following alterations in mechanical loading may provide insight into the interaction between mechanical loading and altered tissue metabolism. Serum COMP concentrations increase following acute bouts of loading,[45,46,48,244] and greater increases in COMP may indicate a greater metabolic response to alterations in mechanical loading.[48,49] Lesser peak vGRF ACLR limb during walking gait three years following injury associates with greater serum type-II collagen turnover.[26] However it remains unknown if eliciting acute changes in peak vGRF results in a metabolic response in individuals with ACLR. The purpose of this study was to determine if serum COMP responded differently following 20-minutes of walking in individuals with a unilateral ACLR during an overloading (i.e. increased vGRF), under-loading (i.e. decreased vGRF) and symmetrical loading condition compared to a control condition, in which participants walked normally. We hypothesized individuals with ACLR would demonstrate a greater serum COMP response (i.e. greater metabolic response) during the under-loading condition, and a lesser serum COMP response (i.e. lesser metabolic response) during the overloading condition, as compared to the serum COMP response during normal walking.

METHODS

Design

For this crossover study, each participant completed four testing sessions (control, symmetrical, overloading, under-loading) that were conducted at the same time of day (mean time difference between sessions = 0.29±0.48 hours) with at least a 7-day interval between each session (mean = 8.83±2.29 days). We blinded the participants to the symmetrical, overloading, under-loading loading condition implemented via real-time biofeedback. The biofeedback targets for the three loading conditions were determined during the control session, which was always

the first session conducted for each participant. No biofeedback was provided during the control session. The order of loading conditions elicited via real-time biofeedback during the remaining three testing sessions was block randomized prior to participant enrollment. All testing procedures followed the same order for each session (Figure 14A). Upon arrival to the laboratory participants rested quietly for 30 minutes prior to collection of the first blood sample ($COMP_{PRE}$). Next, participants walked on the treadmill for one minute to allow for collection of a baseline walking gait trial and then began the 20-minute loading condition (control, symmetrical, overloading, under-loading). A second blood sample was collected immediately following completion of the loading condition ($COMP_{POST}$). During the first control session, prior to treadmill walking, self-selected walking speed was determined using timing gates during 5 over-ground walking trials (Brower TC-Gate; Brower Timing Systems, Draper, Utah) and used to set the speed of the treadmill for the subsequent 4 testing sessions. Before the

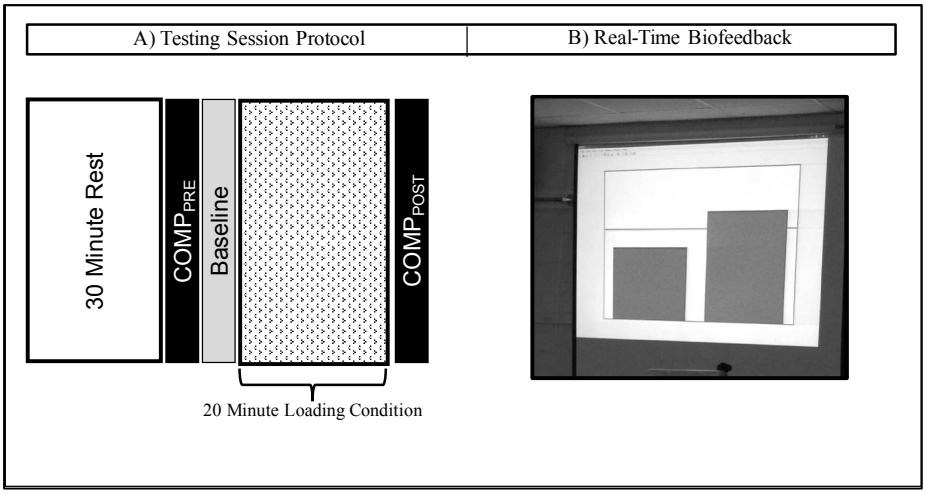


Figure 14A. All four testing sessions were conducted in the same order. Upon arrival to the laboratory participants rested for 30 minutes. A baseline blood sample was collected following the 30-minute rest to assess baseline concentration of cartilage oligomeric matrix protein ($COMP_{PRE}$). A baseline walking trial was then collected and participants were instructed to walk in a normal gait pattern. After the baseline trial was collected real-time biofeedback was displayed to the participant during the 20-minute loading condition. No biofeedback was provided during the control session. Immediately upon completion of the loading condition a second blood sample was collected ($COMP_{POST}$). **Figure 1B.** The real-time biofeedback displayed a blue bar graph for each limb, which represented the magnitude of the first peak of the vGRF. A red target line was placed in the center of the screen, and participants were instructed to alter their movement in order to match each blue bar (i.e. peak vGRF) to the red line during each step.

resting period at beginning the first testing session (control session) participants walked on the treadmill for 5 minutes to allow for acclimation to treadmill walking.[50] The Institutional Review Board at the University of North Carolina at Chapel Hill approved all methods and all participants provided written informed consent prior to participation.

Participants

We recruited a convenient sample of individuals between 18-35 years of age who underwent a primary, unilateral ACLR using either a patellar tendon or hamstring autograft from the university community. All participants were participating in unrestricted physical activity as allowed by their orthopaedic physician, which included at least 30 minutes of physical activity three times per week. We excluded individuals: 1) with a history of musculoskeletal injury to either leg (e.g. ankle sprain, muscle strain) within 6 months prior to participation in the study, 2) a history of lower extremity surgery other than ACLR, 3) with a history of knee osteoarthritis or current symptoms related to knee osteoarthritis (e.g. pain, swelling, stiffness), 4) who were currently pregnant or planning to become pregnant while enrolled in the study, 5) with a history of cardiovascular restrictions that limited the participant's ability to participate in physical activity. Participants were asked to self-report age, sex, ACL graft type, and the date of ACL injury and ACLR. Height and weight were measured in the laboratory prior to testing. All participants completed the subjective portion of the International Knee Documentation Committee index to evaluate self-reported disability and the Tegner Activity Scale to measure level of physical activity. A previous study[46] assessing changes in serum COMP concentration determined moderate effects (Cohen's $d = 0.5$) in serum COMP concentration following an acute bout of running and a loading protocol consisting of deep knee bends in healthy individuals. Therefore, we estimated that we would need 29 participants to detect a moderate effect between

conditions (Cohen's $d = 0.5$) with 80% power with an alpha level of 0.05. (G*Power Statistical Power Analysis Software v3.1[223])

Collection and Analysis of Cartilage Oligomeric Matrix Protein

Five milliliters of antecubital venous blood were collected via a standard vacutainer serum collection tube with a 21-gauge needle. Serum tubes were placed on ice until centrifuged at 4°C for 10 minutes at 3000*g*.[26] Serum was pipetted equally into two 1.5mL cryovials and stored in a -80°C freezer for batch analysis following completion of the study. Serum COMP concentration was assessed via a commercially available specific enzyme-linked immunosorbant assay (ELISA) (BosterBio, Pleasanton, CA) with an assay detection sensitivity of <10pg/ml. Unknown samples were diluted 33x and all assays were performed in triplicate determinations for standards and unknowns and demonstrated inter- and intra-assay variability <10%.

Real-Time Biofeedback Conditions

During the baseline trial of the control session a custom MATLAB (Mathworks, Inc, Natick, MA) program processed and extracted left and right limb peak vGRF from the first 50% of the stance phase, which was used to determine the biofeedback targets for the three loading conditions conducted in the subsequent sessions.

For the symmetrical loading, overloading, and under-loading sessions a 72 inch projection screen directly in front of the treadmill displayed the real-time biofeedback (Figure 14B). A second custom MATLAB script continuously computed the average of the previous four peak vGRF during the first 50% of stance phase, which was visually displayed as right and left blue bar graphs on the projection screen, with a red target line across the center. The target line for the symmetrical loading condition corresponded to the mean peak vGRF between the ACLR and contralateral limb collected during the baseline trial of the control session. The target line for

the overloading condition corresponded to a 5% increase in the baseline peak vGRF for the ACLR limb and the contralateral limb. The target line for the under-loading condition corresponded to a 5% decrease in baseline peak vGRF. The target line was always displayed in the center of the screen for each loading condition to maintain participant blinding to condition. Target values for the overloading (5% above baseline peak vGRF) and under-loading (5% below baseline peak vGRF) were determined individually for left and right leg based on the baseline value of each limb. Therefore, the overloading and under-loading conditions did not specifically cue inter-limb symmetry, rather a relative change in magnitude in each limb.

Before completing the real-time biofeedback intervention a study investigator (BALH) conducted a brief presentation for each participant explaining the peak vGRF and how the biofeedback continuously displayed peak vGRF. Participants were instructed to match the height of each blue bar (i.e. peak vGRF) to the target line as close as possible during each loading condition and utilize any movement strategy possible to manipulate peak vGRF. During the initial presentation all participants were provided one strategy that focused on manipulating the vertical displacement of their center of mass (COM). Specifically, that increasing or decreasing the vertical displacement of their COM may result in a subsequent increase or decrease in peak vGRF, respectively. We provided one strategy to maximize the success of participants consistently reaching the target.

Statistical Analysis

We obtained blood samples from both of the baseline and posttest time points in 26 of the 30 participants. We imputed missing serum COMP concentrations using univariate linear regression equations from serum COMP concentrations analyzed from the same individuals at

other time points to predict missing values. Missing data points and corresponding regression equations are presented in Table 1.

We calculated percent change scores from $COMP_{PRE}$ to $COMP_{POST}$ to determine the change in COMP concentration ($COMP_{CHANGE}$) during each testing session using Equation 1.

Equation 1:

$$Percent\ Change = \left[\frac{(Post-Pre)}{Pre}\right] * 100$$

First, normality was assessed using the Shapiro-Wilk test and skewness and kurtosis values, and outliers were identified via box plots as any data point greater than three standard deviations from the mean. We conducted a one-way repeated measures ANOVA to determine differences in $COMP_{CHANGE}$ between loading conditions (symmetrical, overloading, under-loading, control). Bonferroni adjusted pairwise comparisons were used if significant a main effect was determined ($P \leq 0.05$).[213]

We calculated six Cohen's *d* effect sizes[214] with corresponding 95% confidence intervals (95% CI) to determine the magnitude of difference in $COMP_{CHANGE}$ between each loading condition. Cohen's *d* effect sizes were classified as strong ≤ 0.80, moderate $0.79 - 0.50$, and small ≥ 0.49.[214] All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Version 21, IBM Corp., Somers, NY).

Post-hoc Analysis

Individuals with previous knee injury demonstrate a heterogeneous COMP response to acute bout of running.[245] To better understand if our participant's COMP response during the control condition influenced $COMP_{CHANGE}$ during each loading condition (i.e. symmetrical loading, overloading, and under-loading) we conducted a *post-hoc* analysis in which we

separated our cohort into two sub-groups based on $COMP_{CHANGE}$ during the control condition. One sub-group consisted of individuals demonstrating an increase in $COMP_{CHANGE}$ (percent change > 0%) following the control session, and the second sub-group consisted of individuals demonstrating a decrease in $COMP_{CHANGE}$ (percent change ≤ 0%) during the control session. We then completed the same 4 x 1 repeated measures ANOVA with Bonferroni corrected pairwise comparisons within each group as previous described.

RESULTS

Thirty individuals with ACLR were enrolled in this study (Table 9.) No outliers were determined for $COMP_{CHANGE}$ following any loading condition. Within our entire cohort, $COMP_{CHANGE}$ was not significantly different between loading conditions ($F_{3,118} = 1.506$, P=0.219, Figure 15). Effect sizes for $COMP_{CHANGE}$ between conditions were small and demonstrated inconclusive confidence intervals crossing zero when comparing all loading conditions (Table 10).

Post-hoc Analysis

Twenty-four participants demonstrated an increase in $COMP_{CHANGE}$ during the control condition. In the sub-group of participants demonstrating an increase in $COMP_{CHANGE}$ during the control session we determined $COMP_{CHANGE}$ was significantly different between conditions ($F_{3,94} = 3.388$, P = 0.023; Figure 16). Participants demonstrated a significantly lesser $COMP_{CHANGE}$ during the overloading condition as compared to the control (P=0.020). The $COMP_{CHANGE}$ was not significantly different between any other loading conditions (P>0.05). There was a strong effect for $COMP_{CHANGE}$ with conclusive confidence intervals not crossing zero indicating less $COMP_{CHANGE}$ during the overloading condition compared to the control (Table 11). There was a moderate effect for $COMP_{CHANGE}$ with conclusive confidence intervals

not crossing zero indicating less $COMP_{CHANGE}$ during the symmetrical loading condition as compared to the control.

We determined six participants demonstrated a decrease in $COMP_{CHANGE}$ during the control condition. In the cohort of participants demonstrating a decrease in $COMP_{CHANGE}$ during the control condition we determined $COMP_{CHANGE}$ significantly differed across loading conditions ($F_{3,24} = 8.853$, $P = 0.031$; Figure 17). Participants demonstrated a significantly greater $COMP_{CHANGE}$ during the under-loading condition as compared to the control condition ($P=0.034$). The $COMP_{CHANGE}$ was not significantly different between any other loading conditions ($P>0.05$). Between condition effect sizes for $COMP_{CHANGE}$ were strong and with conclusive confidence intervals not crossing zero that demonstrated the: 1) $COMP_{CHANGE}$ was increased during the symmetrical loading condition as compared to the control, 2) $COMP_{CHANGE}$ was increased during the overloading loading condition as compared to the control, 3) $COMP_{CHANGE}$ was increased during the under-loading condition as compared to the control, 4) symmetrical loading and overloading, and 5) symmetrical loading and under-loading.

Table 9. Regression Equations used to Predict Missing COMP Concentration Data

Independent Variable	Predictor Variable	Regression Equation	R^2 Value	P-Value
Control $COMP_{POST}$	Control Pre COMP	$COMP_{POST} = [0.891*COMP_{PRE}]+ 33.42$	0.781	P<0.001
Symmetrical loading $COMP_{PRE}$	Under-loading $COMP_{PRE}$	Symmetrical Loading $COMP_{PRE} = [0.948*$Under-loading $COMP_{PRE}]+ 12.71$	0.804	P<0.001
Symmetrical loading $COMP_{POST}$	Symmetrical loading $COMP_{PRE}$	Symmetrical Loading $COMP_{POST} = [0.994*$Symmetrical Loading $COMP_{PRE}]+ 11.54$	0.957	P<0.001
Overloading $COMP_{PRE}$	Symmetrical loading $COMP_{PRE}$	Overloading $COMP_{PRE} = [0.796*$Symmetrical loading $COMP_{PRE}]+ 36.34$	0.606	P<0.001
Overloading $COMP_{POST}$	Overloading $COMP_{PRE}$	Overloading $COMP_{POST} = [0.912*$Overloading $COMP_{PRE}]+ 20.782$	0.872	P<0.001
Under-loading $COMP_{PRE}$	Symmetrical loading $COMP_{PRE}$	Under-loading $COMP_{PRE} = [0.848*$Symmetrical loading $COMP_{PRE}]+ 20.411$	0.844	P<0.001
Under-loading $COMP_{POST}$	Under-loading $COMP_{PRE}$	Under-loading $COMP_{POST} = [0.913*$Under-loading $COMP_{PRE}]+ 28.627$	0.863	P<0.001

Table 10. Manuscript 3 Participant Demographics

Sex (% female)	21 Female; 9 Male
Age (years)	20.43±2.91
Height (cm)	172.70 (10.81)
Mass (kg)	73.16 (16.10)
BMI	24.42 (4.25)
Time since Surgery (months)	47.83 (26.97)
Graft Type (%PT)	HS = 16 (53%) ; PT = 14 (47%)
IKDC	86.4887 (9.51)
Tegner	7.47 (1.33)
Change in peak vGRF (%)	
Control	1.35±2.37
Symmetrical Loading	1.31±3.77
Overloading	5.04±2.33
Under-loading	-2.31±2.28

Data Presented as mean ± standard deviation.

Table 11. Cartilage Oligomeric Matrix Protein Concentrations (ng/mL)

	All Participants Included (n=30)		
	Pre COMP	**Post COMP**	**Percent Change**
Symmetrical Loading	158.52±63.98	169.13±66.37	7.79±14.50
Overloading	162.72±64.58	168.71±62.89	6.20±16.06
Under-loading	154.84±60.08	170.04±58.87	14.34±21.76
Control	149.50±53.17	166.63±53.49	13.84±19.45
	Participants with Decreased Control Response (n=6)		
	Pre COMP	**Post COMP**	**Percent Change**
Symmetrical Loading	176.38±94.92	182.51±101.26	2.30±5.18
Overloading	166.79±89.85	187.65±87.60	15.79±14.17
Under-loading	153.01±80.04	182.85±81.35	26.95±23.65
Control	169.14±48.69	154.57±75.71	-8.74±9.77
	Participants with Increased Control Response (n=24)		
	Pre COMP	**Post COMP**	**Percent Change**
Symmetrical Loading	154.06±55.54	164.79±57.16	9.16±15.80
Overloading	161.14±59.13	163.97±56.59	3.80±15.86
Under-loading	155.31±56.18	166.83±54.65	11.19±20.58
Control	144.59±45.74	169.65±48.10	19.48±17.02

Data presented as mean ± standard deviation

Table 12. Between Condition Effect Sizes for Cartilage Oligomeric Matrix Protein

All Participants Included (n=30)

	Effect Size	**95% Confidence Interval**	
Control vs Symmetrical Loading	0.35	-0.16	0.86
Control vs Overloading	0.42	-0.09	0.93
Control vs Under-loading	-0.02	-0.53	0.48
Symmetrical vs Overloading	0.10	-0.40	0.61
Symmetrical vs Under-loading	-0.35	-0.86	0.16
Overloading vs Under-loading	-0.42	-0.93	0.09

Participants with Decreased Control Response (n=6)

	Effect Size	**95% Confidence Interval**	
Control vs Symmetrical Loading	-1.39	-1.96	-0.83
Control vs Overloading	-1.99	-2.61	-1.37
Control vs Under-loading	-1.95	-2.56	-1.33
Symmetrical vs Overloading	-1.25	-1.80	-0.69
Symmetrical vs Under-loading	-1.42	-1.99	-0.85
Overloading vs Under-loading	-0.57	-1.08	-0.05

Participants with Increased Control Response (n=24)

	Effect Size	**95% Confidence Interval**	
Control vs Symmetrical Loading	0.60	0.08	1.12
Control vs Overloading	0.94	0.41	1.47
Control vs Under-loading	0.43	-0.08	0.95
Symmetrical vs Overloading	0.32	-0.19	0.83
Symmetrical vs Under-loading	-0.11	-0.61	0.40
Overloading vs Under-loading	-0.40	-0.91	0.11

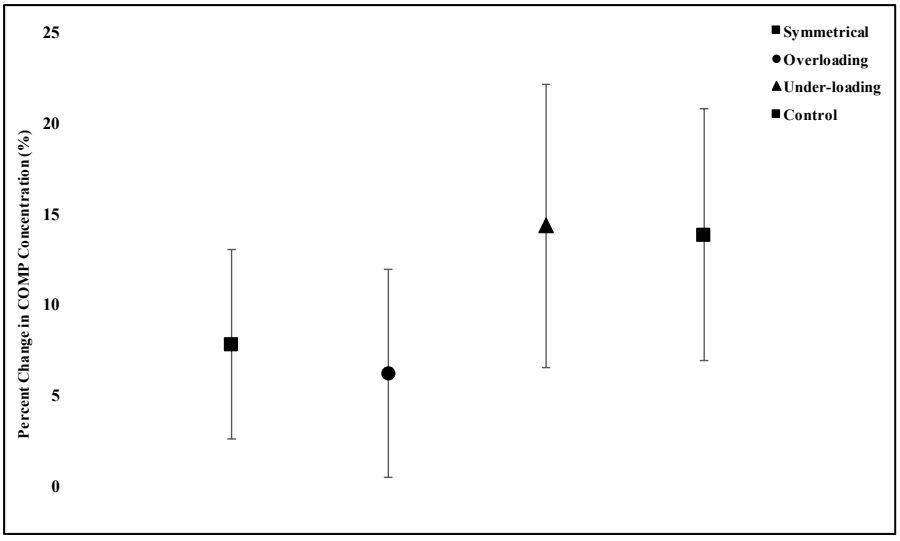


Figure 15. Changes in COMP Concentration in Entire Cohort. Data presented as mean percent change with corresponding 95% confidence intervals.

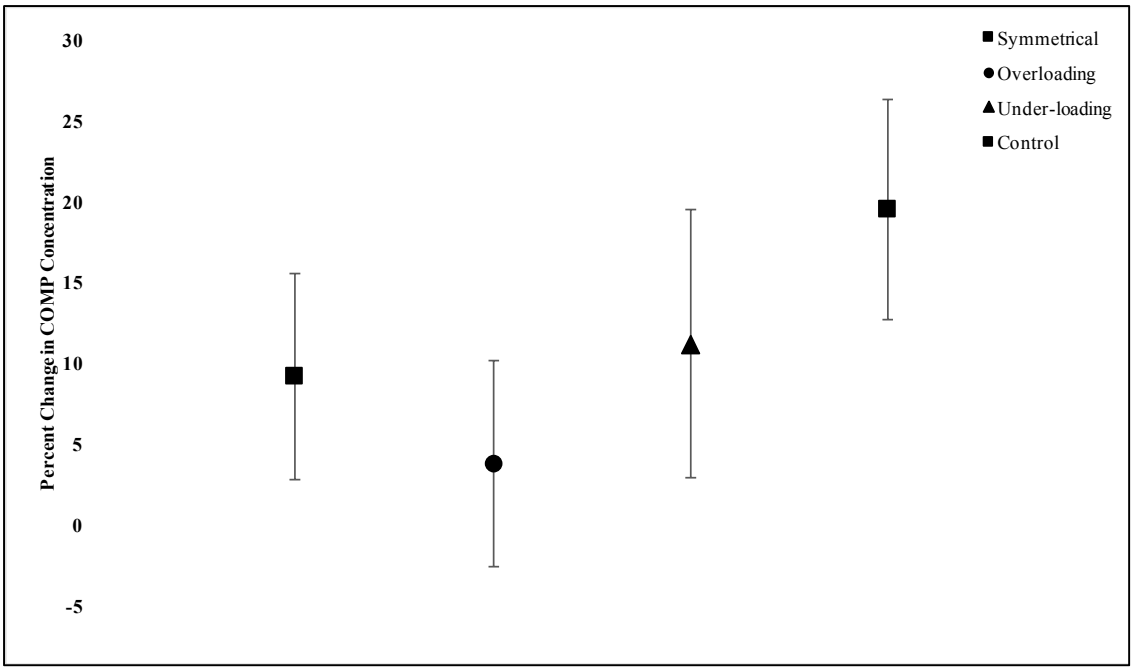


Figure 16. Changes in COMP Concentration in Individuals Demonstrating an Increase in COMP During the Control Condition. Data presented as mean percent change with corresponding 95% confidence intervals.

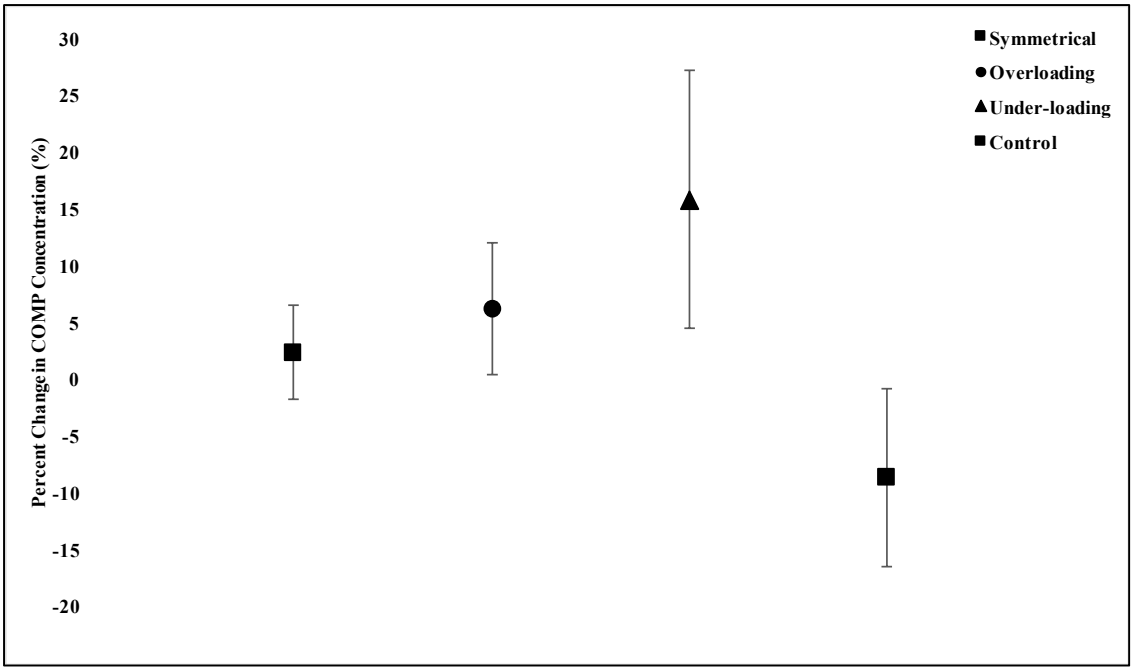


Figure 17. Changes in COMP Concentration in Individuals Demonstrating a Decrease in COMP During the Control Condition. Data presented as mean percent change with corresponding 95% confidence intervals.

DISCUSSION

$COMP_{CHANGE}$ was not statistically different between the loading conditions (i.e. control, symmetrical loading, overloading, and under-loading) when evaluating the entire cohort, yet we identified two cohorts of individuals who either increased or decreased in $COMP_{CHANGE}$ during the control session. The results of our post hoc analyses, which separately evaluated individuals who increased in $COMP_{CHANGE}$ and those who decreased in $COMP_{CHANGE}$ during the control condition, demonstrate that cartilage metabolism can be acutely influenced by manipulating the magnitude of peak vGRF during 20 minutes of walking at a self-selected speed. Consistent with our hypothesis, 20 minutes of overloading peak vGRF during walking decreased $COMP_{CHANGE}$ in individuals who typically demonstrated an increase in $COMP_{CHANGE}$ during 20 minutes of normal walking. Furthermore, 20 minutes of under-loading increased $COMP_{CHANGE}$ in individuals who do not typically demonstrate increased $COMP_{CHANGE}$ during 20 minutes of normal walking. These results are significant as they suggest that manipulation of kinetics during walking gait may be able to influence cartilage metabolism in individuals who are at increased risk of developing PTOA.

Our cohort demonstrated a heterogeneous COMP response following 20 minutes of normal walking (i.e. control condition) at a self-selected speed, with 24 participants demonstrating an increase in $COMP_{CHANGE}$ (percent change > 0%) and 6 participants demonstrating a decrease in $COMP_{CHANGE}$ (percent change $\leq$0%). Cohorts of indiviudals with knee osteoarthritis[244] and a previous history of knee injury[245] demonstrate a similar heterogeneous COMP response across all patients following walking and running, respectively. Greater concentrations in serum COMP following an acute bout of walking may indicate a greater metabolic response to a standardized mechanical load.[48,188,246] Our results suggest the

metabolic response to mechanical loading may not be consistent across all individuals with ACLR. The metabolic response to loading is influenced, in part, by the health of the articular cartilage.[35,112] Previous evidence[129] demonstrats that indiviudals with an ACLR have greater type-II collagen turnover as compared to healthy controls, and greater type-II collagen turnover may indicate underlying structural changes within the articular cartilage.[247] Alterations in tissue metabolism, such as greater type-II collagen turnover, may reduce the ability of the articular cartilage to appropriately attenuate loading resulting in a greater increase in $COMP_{CHANGE}$ following walking.

While more than half of individuals with ACLR demonstrate radiographic PTOA within two decades following reconstruction,[13,14,217] the adaptive mechanisms that allowing some indiviudals to avoid the development of early OA remains unknown.[24] We determined only six of our thirty participants demonstrated a $COMP_{CHANGE}$ decrease following the control condition. A decrease in $COMP_{CHANGE}$ following loading may indicate increased ability of the articular cartilage to withstand loading,[48] or that 20 minutes of walking did not place an abnormal amount of stress on the articular cartilage. While we are unable to determine the specific mechanism by which these six individuals demonstrated a decrease in $COMP_{CHANGE}$ following the control condition, it could be hypothesized these individuals adopted a lower extremity loading pattern that was advantageous for maintaining cartilage health, such as an increased vGRF.[26] Healthy articular cartilage is conditioned to the loading it normally withstands, with thicker articular corresponding to the specific region of cartilage where the greatest amount of loading is applied.[34,139,157] Individuals with ACLR who accept greater mechanical loading during walking gait, or an increased vGRF, may demonstrate a decrease in $COMP_{CHANGE}$ during the control condition.

Animal models of acute injury support the hypothesis that OA may be influenced by excessive mechanical loading to joint tissues. The application of a single blunt impact to the patellofemoral joint of rabbits results in greater number and depth of surface fissures in the articular cartilage when compared to patellofemoral joints not subjected to a blunt impact.[38] Additionally, greater loading rate of a single blunt impact results in greater fissuring of the retropatellar cartilage in rabbits as compared to a lower loading rate.[37] In our cohort of individuals who increased COMP following the control condition (n=24), we determined a significantly lesser $COMP_{CHANGE}$ during the overloading condition (mean $COMP_{CHANGE}$ = 3.80±15.86%) as compared to the control condition (mean $COMP_{CHANGE}$ = 19.48±17.02%). Our real-time biofeedback cued a small (i.e. 5%) change in peak vGRF over 20 minutes of walking, rather than a substantial change in magnitude or rate of loading. Our results suggest small increases in mechanical loading applied over 20 minutes of walking may promote a lesser metabolic response as compared to 20 minutes of normal walking in individuals with ACLR.

We also determined a significantly greater $COMP_{CHANGE}$ during the under-loading condition (mean $COMP_{CHANGE}$ = 26.95±23.65%) as compared to the control condition (mean $COMP_{CHANGE}$ = -8.74±9.77%) in the cohort of individuals demonstrating a decrease in $COMP_{CHANGE}$ following the control condition (n=6). Our results are similar to previous cross-sectional studies[26,27] in individuals with ACLR that demonstrate lesser peak vGRF instantaneous loading rate and lesser peak vGRF associate with greater MMP-3 concentration and and type-II collagen turnover, respectively. Insufficient mechanical loading results in cartilage atrophy[40] and degradation of articular cartilage through an inflammatory response resulting in increased expression of MMPs.[41,42] Lesser mechanical loading during walking gait following ACLR may result in a greater inflammatory response that promotes increased cartilage turnover. The

application of cyclical mechanical loading occurring within a physiological boundary demonstrates anti-inflammatory benefits,[248] and application of cyclical loading following periods of unloading reverses the increase in MMP-1 that occurs following unloading.[41] The acute increase in peak vGRF during the overloading condition may have induced an acute anti-inflammatory response at the level of the articular cartilage,[248] resulting in a lesser $COMP_{CHANGE}$ during the overloading condition compared to the control condition.

While our study provides evidence acutely altering peak vGRF during walking gait results in changes in serum COMP concentrations in individuals with ACLR, there are limitations that can inform the development of future research. We chose to assess $COMP_{CHANGE}$ in our study as it has been reported previously to increase following acute loading.[48,244,249] While greater $COMP_{CHANGE}$ following loading may indicate greater collagen turnover,[48] we are unable to specifically determine if acutely altering peak vGRF would result in an acute change in concentrations of biomarkers of cartilage breakdown relative to synthesis, pro- or anti-inflammatory cytokines, or MMPs; all of which may adversely affect cartilage health. While our loading conditions (symmetrical loading, overloading and under-loading) were randomized, the control session was always completed first. The initial adjustment to treadmill walking during the first testing session may have influenced the $COMP_{CHANGE}$ during the control condition.

While we were able to determine acute $COMP_{CHANGE}$ immediately following altering peak vGRF during walking gait, it remains unknown how alterations in peak vGRF may influence cartilage health over a greater follow-up duration. Assessing the delayed response of COMP following alterations in joint loading may provide more insight into long-term consequences of aberrant mechanical loading.[47] Age[244] and level of physical activity[250] associate with serum COMP concentrations. Our relatively small cohort of individuals with ACLR was

young (mean age = 20.43±2.91years) and physically active at least three times per week. It remains unknown if our results are generalizable to the larger population of individuals with ACLR. Alterations in kinetics and kinematics also occur following ACLR,[61,235] and may lead to PTOA development. Further research is needed to determine if biomechanical outcomes other than peak vGRF influence $COMP_{CHANGE}$ during walking gait in individuals with ACLR.

Overall, there were no differences in $COMP_{CHANGE}$ when real-time biofeedback was provided to cue acute changes in peak vGRF during walking gait in our entire cohort of individuals with an ACLR. After separately evaluating individuals who increased in $COMP_{CHANGE}$ and those who decreased in $COMP_{CHANGE}$ during the control condition, we determined that cartilage metabolism can be acutely influenced by manipulating the magnitude of peak vGRF during 20 minutes of walking at a self-selected speed. Individuals who increased in $COMP_{CHANGE}$ demonstrated a significant decrease in $COMP_{CHANGE}$ during the overloading condition that cued a 5% increase in peak vGRF. Individuals who decreased in $COMP_{CHANGE}$ demonstrated a significant increase in $COMP_{CHANGE}$ during the under-loading condition that cued a 5% decrease in peak vGRF. Our results provide evidence greater mechanical loading, rather than lesser mechanical loading, may be beneficial for maintaining joint health following ACLR.

CHAPTER 8: MANUSCRIPT 4

Lesser Peak Vertical Ground Reaction Force is Associated with a Greater Increase in Serum Cartilage Oligomeric Matrix Protein following Twenty Minutes of Walking in Individuals with Anterior Cruciate Ligament Reconstruction

OVERVIEW

Context: Mechanical loading during walking gait associates with cartilage metabolism in individuals with anterior cruciate ligament reconstruction (ACLR). Therapeutic interventions that target mechanical loading may be beneficial for cartilage metabolism, however it remains unknown if peak vertical ground reaction force (vGRF) associates with a change in cartilage metabolism during walking gait and different loading conditions during walking gait. **Objective:** To determine if baseline peak vGRF associates with a change in serum cartilage oligomeric matrix protein (COMP) following acute bouts of altered loading. **Design:** Single-blind, crossover study. **Setting:** Research laboratory. **Patients or Other Populations:** 30 individuals with ACLR. **Interventions:** Participants completed four testing sessions separated by at least 7 days. During each session, one of four loading conditions was completed during 20 minutes of treadmill walking. Loading conditions included 1) control consisting of normal walking, 2) a 5% increase (i.e. overloading) in peak vGRF, 3) a 5% decrease (i.e. under-loading) in peak vGRF, and 4) symmetrical peak vGRF between limbs. A 60-second walking trial was collected before each loading condition, and blood samples were collected before and immediately following each loading condition. **Main Outcome Measures:** The change in serum

COMP concentration ($COMP_{CHANGE}$) was determined from baseline to immediately following each loading condition. **Results:** Individuals with ACLR demonstrating a lesser peak vGRF also demonstrated a greater increase in $COMP_{CHANGE}$ following 20 minutes of normal walking during the control condition. Baseline peak vGRF did not associate with $COMP_{CHANGE}$ following the symmetrical loading, overloading, or under-loading conditions. **Conclusions:** Lesser mechanical loading associates with a greater metabolic response following 20 minutes of walking, and baseline peak vGRF does not determine the serum COMP response that occurs during acute bouts of altered mechanical loading in individuals with ACLR.

INTRODUCTION

Greater than 50% of individuals with an anterior cruciate ligament reconstruction (ACLR) develop radiographic post-traumatic knee osteoarthritis (PTOA) within the first two decades following ACLR.[13,14,217] Individuals with PTOA are on average a decade younger at the time of PTOA diagnosis, and endure more years lived with disability compared with individuals diagnosed with idiopathic osteoarthritis.[12,16] Identifying therapeutic targets capable of influencing tissue metabolism is imperative for developing effective rehabilitation strategies that may slow the progression to PTOA following ACLR. Current rehabilitation paradigms are effective at allowing a majority of individuals to return to physical activity following ACLR,[28] yet they do not alleviate aberrant mechanical loading[61,235,251] and altered tissue metabolism[176,252,253] that chronically persist following ACLR. Therapeutic strategies that target aberrant mechanical loading may be a clinically feasible approach to restoring homeostatic tissue metabolism following ACLR.

Aberrant mechanical loading and altered tissue metabolism interact following ACLR;[23-25] there is recent evidence that lesser mechanical loading associates with tissue metabolism[26,27] and PTOA onset.[44] Specifically, individuals who have ungone ACLR in the previous 6 months, demonstrating lesser peak vertical ground reaction force (vGRF) instantaneous loading rate and external knee adduction moment (KAM) dunring walking gait also demonstrated greater plasma concentrations of matrix metalloproteinase-3 and interleukin-6, respectively.[27] Moreover, individuals three years following ACLR who demonstrate lesser peak vertical ground reaction force (vGRF) during walking gait also demonstrate greater serum type-II collagen turnover.[26] Previous work in other orthopedic populations (i.e. total knee and hip arthoplasty) has demonstrated peak vGRF can be manipulated during walking gait and other function tasks in clinical settings.[55,57] The peak vGRF indicates the magnitude of loading applied to entire lower extremity during stance, [55,57] and also contributes to other kinetic outcomes (i.e. peak KAM, internal knee extension moment) hypothesized to influence the development of PTOA.[60,61] Specifically targeting peak vGRF may result in simultaneous changes in additional kinetic outcomes across multiple planes of motion that may also influence tissue metabolism. Therefore, therapeutic interventions that target peak vGRF may be beneficial for promoting homeostatic tissue metabolism following ACLR, and may be applicable in a clinical setting. While peak vGRF during walking gait associates with type-II collagen turnover in individuals with ACLR (37.95±29.27 months following ACLR),[26] it remains unknown if peak vGRF associates with acute changes in tissue metabolism following a single bout of cyclical loading.

Assessing changes in cartilage oligomeric matrix protein (COMP) may provide insight into the acute metabolic response to cyclical loading in individuals with ACLR. Serum COMP concentrations increase following a single bout of cyclical loading as compared to resting

levels.[45,46,48,244,249] COMP fragments released into the synovial fluid are indicative of the metabolic processes that occur within the extracelluar cartlage matrix,[246] therefore a greater increase in serum COMP likely reflects a greater metabolic response to a standardized mechanical load.[48,188,246] Determining changes in serum COMP while acutely altering peak vGRF during walking gait in individuals with ACLR may provide insight into the magnitude of loading that elicits a favorable metabolic response. However, healthy articular cartilage becomes conditioned to the mechanical loading experienced while walking over time.[34,35,157] Thicker areas of articular cartilage correspond to the location at which large magnitude loads are typically applied during walking gait.[139,157] An individual's natural peak vGRF during walking gait may influence the metabolic response to acute alterations in mechanical loading. If peak vGRF does associate with an acute metabolic response following altered loading it may identify the individuals who will most likely to benefit from therapeutic interventions that target mechanical loading. Therefore, the primary purpose of this study was to determine if peak vGRF during walking gait associates with acute changes in serum COMP concentration following 20-minutes of walking in individuals with ACLR. Secondarily, we sought to determine if baseline vGRF associates with changes in COMP concentration when provided real-time biofeedback cuing an increase in bilateral peak vGRF (i.e. overloading), a decrease in bilateral peak vGRF (i.e. under-loading) and peak vGRF symmetry between limbs during walking gait in individuals with unilateral ACLR. We hypothesized individuals with ACLR demonstrating a lesser baseline peak vGRF would demonstrate greater increases in serum COMP following 20 minutes of walking. Additionally, we hypothesized individuals demonstrating a lesser baseline peak vGRF would demonstrate a smaller change in serum COMP (i.e. more favorable tissue metabolism) following the overloading condition.

METHODS

Design

For this crossover study, each participant completed four testing sessions (control, symmetrical, overloading, under-loading) that were conducted at the same time of day (mean time difference between sessions = 0.29±0.48 hours) with at least a 7-day interval between each session (mean = 8.83±2.29 days). We blinded the participants to the symmetrical, overloading, under-loading loading condition implemented via real-time biofeedback. The biofeedback targets for the three loading conditions were determined during the control session, which was always

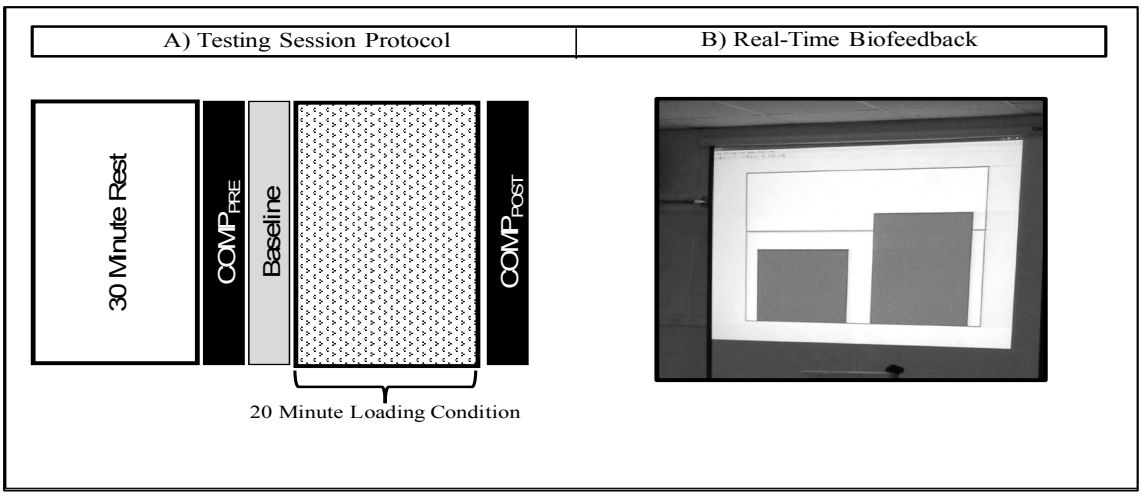


Figure 18A. Aim 4 Testing Protocol. All four testing sessions were conducted in the same order. Upon arrival to the laboratory participants rested for 30 minutes. A baseline blood sample was collected following the 30-minute rest to assess baseline concentration of cartilage oligomeric matrix protein ($COMP_{PRE}$). A baseline walking trial was then collected and participants were instructed to walk in a normal gait pattern. After the baseline trial was collected real-time biofeedback was displayed to the participant during the 20-minute loading condition. No biofeedback was provided during the control session. Immediately upon completion of the loading condition a second blood sample was collected ($COMP_{POST}$). **Figure 1B.** The real-time biofeedback displayed a blue bar graph for each limb, which represented the magnitude of the first peak of the vGRF. A red target line was placed in the center of the screen, and participants were instructed to alter their movement in order to match each blue bar (i.e. peak vGRF) to the red line during each step.

the first session conducted for each participant. No biofeedback was provided during the control session. The order of loading conditions elicited via real-time biofeedback during the remaining three testing sessions was block randomized prior to participant enrollment. All testing procedures followed the same order for each session (Figure 18A). Upon arrival to the laboratory

participants rested quietly for 30 minutes prior to collection of the first blood sample ($COMP_{PRE}$). Next, participants walked on the treadmill for one minute to allow for collection of a baseline walking gait trial and then began the 20-minute loading condition (control, symmetrical, overloading, under-loading). A second blood sample was collected immediately following completion of the loading condition ($COMP_{POST}$). The Institutional Review Board at the University of North Carolina at Chapel Hill approved all methods and all participants provided written informed consent prior to participation.

Participants

We recruited a convenient sample of individuals between 18-35 years of age who underwent a primary, unilateral ACLR using either a patellar tendon or hamstring autograft from the university community. All participants were participating in unrestricted physical activity as allowed by their orthopaedic physician, which included at least 30 minutes of physical activity three times per week. We excluded individuals: 1) with a history of musculoskeletal injury to either leg (e.g. ankle sprain, muscle strain) within 6 months prior to participation in the study, 2) a history of lower extremity surgery other than ACLR, 3) with a history of knee osteoarthritis or current symptoms related to knee osteoarthritis (e.g. pain, swelling, stiffness), 4) who were currently pregnant or planning to become pregnant while enrolled in the study, 5) with a history of cardiovascular restrictions that limited the participant's ability to participate in physical activity. Participants were asked to self-report age, sex, ACL graft type, and the date of ACL injury and ACLR. Height and weight were measured in the laboratory prior to testing. All participants completed the subjective portion of the International Knee Documentation Committee index to evaluate self-reported disability and the Tegner Activity Scale to measure level of physical activity. We estimated we would detect a moderate association ($r = 0.59$)

between peak vGRF and changes in serum COMP concentration, which we determined from our previous study that determined a significant association between peak vGRF and serum type-II collagent turnover in individuals with ACLR.[26] Therefore, we would need to include 20 participants in order to detect statistical significance for a two-tailed bivariate association of this magnitude (-0.59 or 0.59) with 80% power and an alpha level of 0.05. (G*Power Statistical Power Analysis Software v3.1[223])

Collection and Analysis of Baseline Peak Vertical Ground Reaction Force

Kinetic data was collected with a dual-belt, force-sensing treadmill (Bertec, Columbus, OH). Kinetic data were sampled at 1000Hz and filtered using a 4th order low-pass Butterworth filter with a cut-off frequency of 100Hz. During the first control session, prior to treadmill walking, self-selected gait speed was determined using timing gates during 5 over-ground walking trials (Brower TC-Gate; Brower Timing Systems, Draper, Utah). This over-ground walking speed was used to set the speed of the treadmill, which remained consistent during all 4 testing sessions. Before beginning the first testing session (control session) participants walked on the treadmill for 5 minutes to allow for acclimation to treadmill walking.[50]

Peak vGRF was identified during the first 50% of the stance phase of gait, which we determined as the interval from initial contact (vGRF $\geq$ 20N) to toe-off (vGRF $\leq$ 20N) and stride-averaged across the 60-second trial using a custom-built MATLAB program. The body mass of each participant was converted to Newtons (N) and used to normalize peak vGRF (xBW).

Collection and Analysis of Cartilage Oligomeric Matrix Protein

Five milliliters of antecubital venous blood were collected via a standard vacutainer serum collection tube with a 21-gauge needle. Serum tubes were placed on ice until centrifuged

at 4°C for 10 minutes at 3000*g*.[26] Serum was pipetted equally into two 1.5mL cryovials and stored in a -80°C freezer for batch analysis following completion of the study. Serum COMP concentration was assessed via a commercially available specific enzyme-linked immunosorbant assay (ELISA) (BosterBio, Pleasanton, CA) with an assay detection sensitivity of <10pg/ml. Unknown samples were diluted 33x. All assays were performed in triplicate determinations for standards and unknowns and demonstrated inter- and intra-assay variability <10%.

Real-Time Biofeedback Conditions

During the baseline trial of the control session a custom MATLAB (Mathworks, Inc, Natick, MA) program processed and extracted left and right limb peak vGRF from the first 50% of the stance phase, which was used to determine the biofeedback targets for the three loading conditions (symmetrical loading, overloading, and under-loading) conducted in the subsequent sessions.

For the symmetrical loading, overloading, and under-loading sessions a projection screen directly in front of the treadmill displayed the real-time biofeedback (Figure 18B). A second custom MATLAB script continuously computed the average of the previous four peak vGRF during the first 50% of stance phase, which was visually displayed as right and left blue bar graphs on the projection screen, with a red target line across the center. The target line for the symmetrical loading condition corresponded to the mean peak vGRF between the ACLR and contralateral limb collected during the baseline trial of the control session. The target line for the overloading condition corresponded to a 5% increase in the baseline peak vGRF for the ACLR limb and the contralateral limb. The target line for the under-loading condition corresponded to a 5% decrease in baseline peak vGRF. The target line was always displayed in the center of the screen for each loading condition to maintain participant blinding to condition. Target values for

the overloading (5% above baseline peak vGRF) and under-loading (5% below baseline peak vGRF) were determined individually for left and right leg based on the baseline value of each limb. Therefore, the overloading and under-loading conditions did not specifically cue inter-limb symmetry, rather a relative change in magnitude in each limb.

Before completing the real-time biofeedback intervention a study investigator (BALH) conducted a brief presentation with each participant explaining the peak vGRF and how the biofeedback continuously displayed peak vGRF. Participants were instructed to match the height of each blue bar (i.e. peak vGRF) to the target line as close as possible during each loading condition and utilize any movement strategy possible to manipulate peak vGRF. During the initial presentation all participants were provided one strategy that focused on manipulating the vertical displacement of their center of mass (COM). Specifically, that increasing or decreasing the vertical displacement of their COM may result in a subsequent increase or decrease in peak vGRF, respectively. We provided one strategy to maximize the success of participants consistently reaching the target.

Statistical Analysis

We calculated percent change scores from $COMP_{PRE}$ to $COMP_{POST}$ to determine the change in COMP concentration ($COMP_{CHANGE}$) during each testing session using Equation 1.

Equation 1:

$$Percent\ Change = \left[\frac{(Post-Pre)}{Pre}\right] * 100$$

First, normality was assessed using the Shapiro-Wilk test and skewness and kurtosis values, and outliers were identified via box plots as any data point greater than three standard deviations from the mean. For our primary analysis we conducted bivariate two-tailed Pearson Product-Moment correlations between baseline peak vGRF and $COMP_{CHANGE}$ within each

loading condition. It is known that gait speed associates with peak vGRF,[215] and time since ACLR associates with serum biomarkers of cartilage degradation;[176] therefore, we secondarily conducted partial correlations between peak vGRF and $COMP_{CHANGE}$ while independently controlling for gait speed and time since ACLR. Correlation coefficients were interpreted as negligible (0.0 – 0.3), low (0.31 – 0.5), moderate (0.51 – 0.7), high (0.71 – 0.9) and very high (0.9-1.0).[216] Statistical significance was determined *a priori* as $P \leq 0.05$, and all statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Version 21, IBM Corp., Somers, NY).

Post-hoc Analysis

Individuals with previous knee injury demonstrate a heterogeneous COMP response to an acute bout of running.[245] Following our a priori analysis we determined 6 participants demonstrated a decreased $COMP_{CHANGE}$ during the control condition and 22 participants demonstrated an increased $COMP_{CHANGE}$ following the control condition. To determine if $COMP_{CHANGE}$ during the control condition (i.e. increased $COMP_{CHANGE}$ or decreased $COMP_{CHANGE}$) influences the association between peak vGRF and $COMP_{CHANGE}$ across all loading conditions, we chose to remove the 6 participants demonstrating a decrease in COMP concentration following the control condition and then conducted Pearson product moment correlations in the 22 participants demonstrating an inceased $COMP_{CHANGE}$ following the control condition.

RESULTS

We enrolled 30 individuals with unilateral ACLR (Table 12). Complete $COMP_{PRE}$ and $COMP_{POST}$ data were available for 26 participants during each session, and complete vGRF data

was obtained from all 30 participants. The number of participants included in each analysis is reported in Table 13.

During the control condition, participants demonstrating a greater baseline peak vGRF also demonstrated a lesser $COMP_{CHANGE}$ ($r = -0.437$; $P = 0.020$; Figure 19). The association between baseline peak vGRF and $COMP_{CHANGE}$ during the control condition remained significant when controlling for gait speed (partial $r_{25} = -0.467$; $P = 0.014$) and time since reconstruction (partial $r_{25} = -0.428$; $P = 0.026$). Baseline peak vGRF was not significantly associated with $COMP_{CHANGE}$ during the symmetrical loading condition ($r = 0.002$; $P = 0.993$; Figure 20), during the overloading condition ($r = 0.129$; $P = 0.504$; Figure 21), or during the under-loading condition ($r = 0.215$; $P = 0.272$; Figure 22).

Post-hoc Analysis

Following removal of the 6 participants demonstrating a decrease in COMP concentration following the control condition, the association between baseline peak vGRF and $COMP_{CHANGE}$ during the control condition was no longer statistically significant ($r = -0.073$; $P = 0.784$). The association between baseline peak vGRF and $COMP_{CHANGE}$ remained statistically non-significant during the symmetrical loading condition ($r = 0.009$; $P = 0.968$), during the overloading condition ($r = 0.002$; $P = 0.992$), or during the under-loading condition ($r = 0.139$; $P = 0.559$).

Table 13. Manuscript 4 Participant Demographics

Sex	21 Female; 9 Male
Age (years)	20.43±2.91
Height (cm)	172.70±10.81
Mass (kg)	73.16±16.10
BMI	24.42±4.25
Time since Surgery (months)	47.83±26.97
Graft Type	HS = 16; PT = 14
IKDC	86.49±9.51
Tegner	7.47±1.33
ACLR Limb Baseline peak vGRF (xBW)	
Control Condition	1.11±0.06
Symmetrical Loading	1.12±0.06
Overloading	1.11±0.04
Under-loading	1.12±0.06
$COMP_{CHANGE}$ (%)	
Control Condition	13.96±20.11
Symmetrical Loading	7.82±14.76
Overloading	5.98±16.98
Under-loading	14.09±22.45

Data presented as mean ± standard deviation. Peak vertical ground reaction force (vGRF) was normalized to body weight (xBW). The percent change in cartilage oligomeric matrix protein ($COMP_{CHANGE}$) was calculated from baseline to immediately post-loading condition.

Table 14. Correlations between Baseline peak vGRF and $COMP_{CHANGE}$ during each Loading Condition

	n	Association between baseline peak vGRF and $COMP_{CHANGE}$	Partial correlation between baseline peak vGRF and $COMP_{CHANGE}$ accounting for gait speed	Partial correlation between baseline peak vGRF and $COMP_{CHANGE}$ accounting for time since ACLR
Control	28	r = -0.437; P = 0.020	r = -0.467; P = 0.014	r = -0.428; P = 0.026
Symmetrical Loading	29	r = 0.002; P = 0.993	r = 0.143; P = 0.468	r = 0.016; P = 0.936
Overloading	29	r = 0.129; P = 0.504	r = 0.134; P = 0.498	r = 0.133; P = 0.501
Under-loading	28	r = 0.215; P = 0.272	r = 0.276; P = 0.163	r = 0.204; P = 0.307

vGRF = vertical ground reaction force; $COMP_{CHANGE}$ = percent change in cartilage oligomeric matrix protein from baseline.

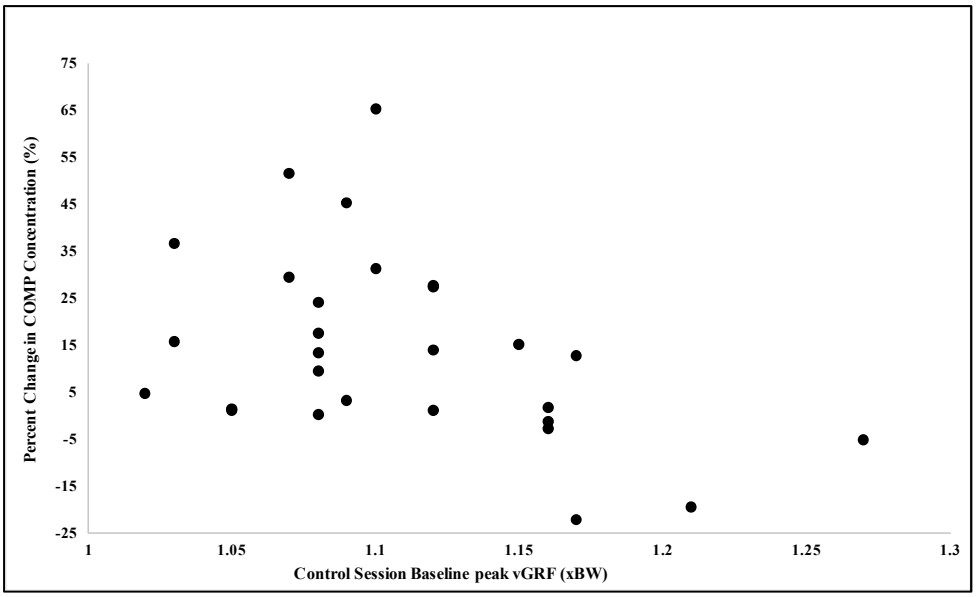


Figure 19. Association between Baseline peak vGRF and $COMP_{CHANGE}$ during the Control Condition. participants demonstrating a greater baseline peak vGRF also demonstrated a lesser $COMP_{CHANGE}$ (r = -0.437; P = 0.020).

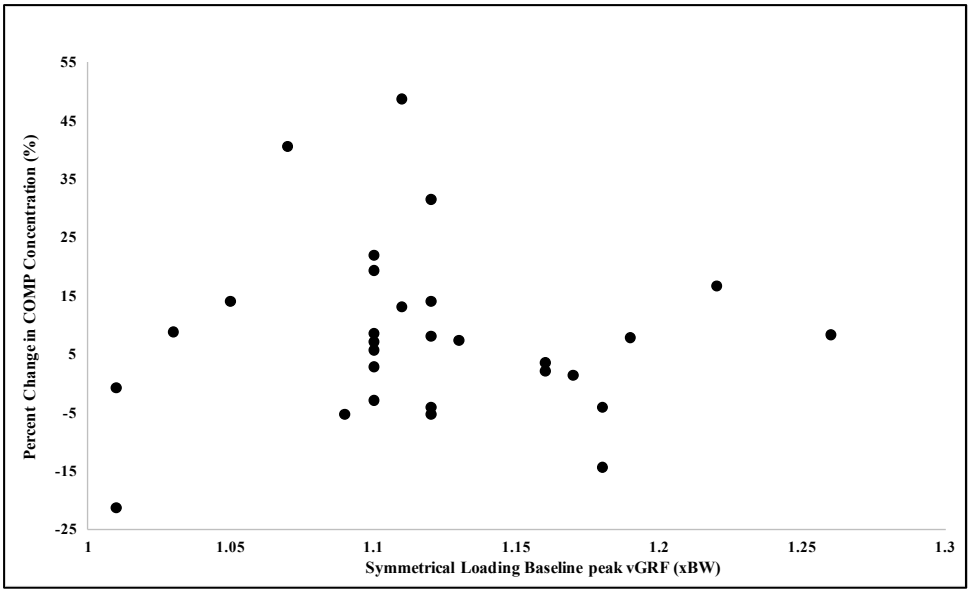


Figure 20. Association between Baseline peak vGRF and $COMP_{CHANGE}$ during the Control Condition. The association between baseline peak vertical ground reaction force (vGRF) and cartilage oligomeric matrix protein (COMP) following the overloading condition was not significant (r = 0.129; P = 0.504).

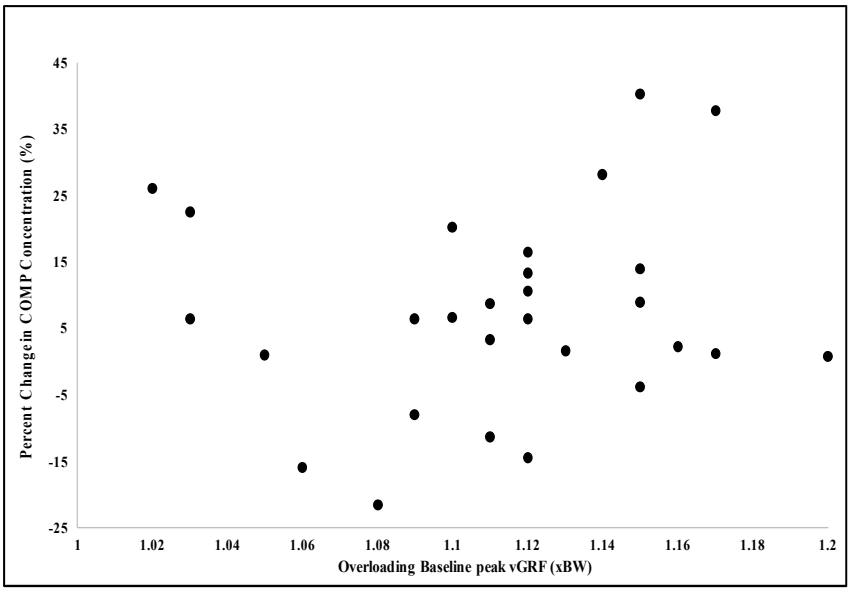


Figure 21. Association between Baseline peak vGRF and $COMP_{CHANGE}$ during the Overloading Condition. The association between baseline peak vertical ground reaction force (vGRF) and cartilage oligomeric matrix protein (COMP) following the overloading condition was not significant (r = 0.002; P = 0.993).

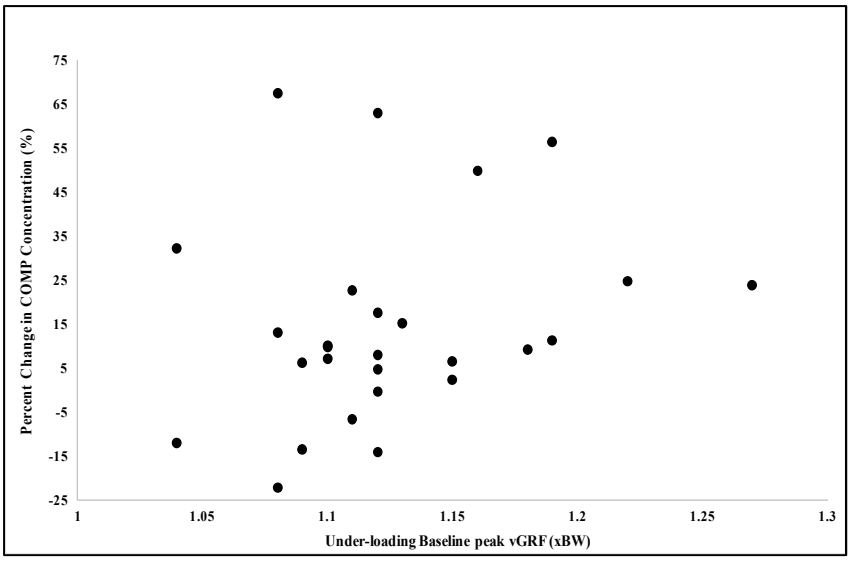


Figure 22. Association between Baseline peak vGRF and $COMP_{CHANGE}$ during the Under-loading Condition. The association between baseline peak vertical ground reaction force (vGRF) and cartilage oligometic matrix protein (COMP) following the under-loading condition was not significant (r = 0.215; P = 0.272).

DISCUSSION

The main finding of the current study is that individuals with ACLR who demonstrate a lesser peak vGRF during walking gait also demonstrate a greater increase in $COMP_{CHANGE}$ during 20 minutes of walking at a self-selected speed. The association between peak vGRF and $COMP_{CHANGE}$ during the control condition remained significant even after controlling for gait speed and time since ACLR. Additionally, baseline peak vGRF does not significantly associate with the response in $COMP_{CHANGE}$ during separate conditions that incoporated real-time biofeedback that cued an increase in peak vGRF (i.e. overloading), a decrease in peak vGRF (i.e. under-loading) and symmetrical peak vGRF between limbs. Overall, the association between lesser peak vGRF and greater change in serum COMP following an acute bout of walking provides evidence lesser mechanical loading over a standard period of time associates with acute changes in tissue metabolism that may lead to breakdown of articular cartilage following ACLR. The lack of association between baseline peak vGRF and $COMP_{CHANGE}$ during any altered loading condition (i.e. symmetrical, overloading, under-loading) may indicate that the COMP response to acutely altering peak vGRF may not be influenced by the baseline magnitude of peak vGRF that individuals with an ACLR demonstrate during normal walking.

Our study is the first to evaluate the association between baseline peak vGRF and $COMP_{CHANGE}$ following an acute bout of normal walking (i.e. control condition) in individuals with ACLR. Our results are similar to previous cross-sectional studies,[26,27] which demonstrated that lesser mechanical loading in the ACLR limb interacts with tissue metabolism following ACLR. At 6-months following ACLR, lesser peak vGRF instantaneous loading rate during walking gait associates with greater plasma concentration of matrix metalloproteinase 3 (MMP-3),[27] an enzyme involved in the signaling of cartilage degradation. Additionally, lesser internal

knee abduction moment in individuals with ACLR associated with greater concentrations of interleukin-6,[27] a pro-inflammatory cytokine. At an average of approximately 3 years following ACLR, lesser peak vGRF in the ACLRr limb associated with greater serum type-II collagen turnover ratio.[26] Taken together, the results of this study as well as others[26,27] indicate lesser mechanical loading associates with unfavorable tissue metabolism in individuals with ACLR.

Healthy articular cartilage becomes conditioned to mechanical loading applied over time, and maintenance of mechanical loading is necessary for maintaining tissue homeostasis.[34-36,157,248] Insufficient mechanical loading may influence articular cartilage degradation through increased expression of MMPs.[36,41,42] Chronic reductions in peak vGRF of the ACLR limb during walking following reconstruction may result in up-regulation of MMPs, resulting in an imbalance of cartilage degradation relative to synthesis over time. Cartilage response to loading is also dependent on the health of the articular cartilage.[35,112] Individuals with ACLR demonstrate greater type-II collagen turnover as compared to healthy controls.[177] Greater collagen turnover following ACLR may reduce the ability of the articular cartilage to withstand mechanical loading, resulting in a greater $COMP_{CHANGE}$ following 20 minutes of walking. Additionally, following removal of the 6 participants demonstrating a decreased $COMP_{CHANGE}$ during the control condition, the association between peak vGRF and $COMP_{CHANGE}$ during the control condition was no longer statistically significant. Therefore, the association between baseline peak vGRF and $COMP_{CHANGE}$ during the control condition may be driven by an individual's metabolic response to normal walking. It currently remains unknown why a small cohort of individuals in our study demonstrated a decreased $COMP_{CHANGE}$ following 20 minutes of normal walking at a self-selected speed. Future research should investigate the mechanisms

that may result in a decreased $COMP_{CHANGE}$ following walking, as well as how the metabolic response to an acute bout of loading may influence long-term cartilage health following ACLR.

Contrary to our hypothesis, baseline peak vGRF did not significantly associate with $COMP_{CHANGE}$ during the symmetrical loading condition ($r = 0.002$; $P = 0.993$), the overloading condition ($r = 0.002$; $P = 0.993$) or the under-loading condition ($r = 0.215$; $P = 0.272$). It is possible that peak vGRF does not determine how serum COMP responds following acute bouts of altered loading. Small alterations in peak vGRF (i.e. 5% increase, 5% decrease, symmetrical vGRF) may result in greater changes in additional kinetic (i.e. peak KAM, peak internal knee extension moment) or kinematic (i.e. knee flexion excursion) outcomes that are more strongly associated with serum COMP response. Wellsandt et al[44] determined individuals who develop radiographic PTOA within 5 years following ACLR demonstrated lesser tibiofemoral contact force and lesser external knee adduction moments in the injured limb at 6 months following ACLR compared to those that did not develop PTOA. The peak vGRF is an indication of the magnitude of force applied to the entire lower extremity.[55,57] Acute alterations in knee-specific outcomes of mechanical loading, such as tibiofemoral contract force, may demonstrate a stronger association with changes in serum COMP than peak vGRF. Further research is needed to determine biomechanical outcomes that are most predictive of acute changes in tissue metabolism following bouts of loading in individuals with ACLR.

While this study provides further evidence that lesser mechanical loading during walking gait is linked to potential deleterious responses in tissue metabolism following ACLR, there are limitations that should be addressed in order to better inform future research. We chose to assess serum COMP concentrations, as COMP is a biochemical marker of cartilage metabolism that has been previously demonstrated to increase following acute bouts of mechanical

loading.[45,46,48,244,249] A greater COMP response may indicate greater cartilage turnover, however an up-regulation of cartilage synthesis may also occur following our acute bout of loading, suggesting that increased COMP may indicate an increase in overall cartilage metabolism rather than increased cartilage breakdown, specifically. We did not determine if additional metabolic processes (i.e. inflammation, cartilage synthesis) may have also occurred simultaneously in response to acute loading. Additionally, we assessed COMP concentration in serum, which can be influenced by systemic alterations in tissue metabolism rather than knee specific alterations in tissue metabolism. The cross-sectional design of our study prohibits us from determining the causal nature of the association between peak vGRF and acute changes in tissue metabolism to a standard 20 minutes of walking. Further research is needed to determine the long-term consequences of alterations in tissue metabolism that occur following acute bouts of loading.

In conclusion, we determined individuals with lesser baseline peak vGRF during walking gait demonstrated a greater increase in serum $COMP_{CHANGE}$ following an acute bout of their normal walking. These results provide evidence that lesser mechanical loading interacts with tissue metabolism following an acute bout of loading. Additionally, baseline peak vGRF does not associate with the response in $COMP_{CHANGE}$ following real-time biofeedback that cued a 5% increase in peak vGRF, a 5% decrease in peak vGRF and symmetrical peak vGRF. Therefore baseline peak vGRF does not seem to influence the serum COMP response that occurs during acute bouts of altered mechanical loading in individuals with ACLR.

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