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Joint Loading Factors of Articular Cartilage Structure in Healthy and ACL-Injured Knees

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JOINT LOADING FACTORS OF ARTICULAR CARTILAGE STRUCTURE IN HEALTHY AND ACL-INJURED KNEES

by

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University of Nebraska, 2020

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Articular cartilage structure and chondrocyte health are sensitive and reliant on dynamic joint loading during activities. The risk of osteoarthritis (OA) is high after anterior cruciate ligament (ACL) injury, but mechanisms underlying its development are poorly understood. The overall goals of this work were 1) to determine the association between measures of individual and cumulative knee joint loading with T2 relaxation times in the knee cartilage of young individuals without injury and 2) to determine if these same knee joint loading factors are associated with cartilage T2 relaxation time one month after ACL injury. The central hypotheses was that lower measures of knee joint loading would be associated with higher (worse) T2 relaxation time throughout the articular cartilage of knees with and without ACL injury. Individuals without a history of knee injury and with an acute ACL injury in the past month served as participants for this study. Participants completed magnetic resonance imaging with T2 mapping, biomechanical gait analysis, and one week of accelerometry during daily living to measure T2 relaxation time, knee joint angles and moments, and daily physical activity levels, respectively. Individual loading factors and cumulative knee joint loading were correlated with higher T2 relaxation times in the articular cartilage of uninjured knees. Altered knee joint adduction moment impulse, less knee flexion excursion, and higher daily physical activity were associated with prolonged T2 relaxation time one month after ACL injury. Gait biomechanics and daily PA may be modifiable targets to alter OA development acutely after ACL injury.

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%	percentage
°	degree
ACL	anterior cruciate ligament
ASIS	anterior superior iliac spine
BMI	body mass index
BW	bodyweight
CB3	Center for Brain, Biology and Behavior
CI	confidence interval
IC	initial contact
KAM	knee adduction moment
KFA	knee flexion angle
KFM	knee flexion moment
kg	kilogram
LFC	lateral femoral condyle
LTC	lateral tibial condyle
m	meter
MET	metabolic equivalent
MFC	medial femoral condyle
mm	millimeter
MRI	magnetic resonance imaging
MTC	medial tibial condyle
N	newton
NAPL	Nebraska Athletic Performance Laboratory

NCI	National Cancer Institute
NHANES	National Health and Nutrition Examination Survey
OA	osteoarthritis
OAI	Osteoarthritis Initiative
p	p-value
PA	physical activity
PASE	Physical Activity Scale for the Elderly
PSIS	posterior superior iliac spine
PTOA	post-traumatic osteoarthritis
R	Pearson correlation coefficient
s	second
SD	standard deviation
TKA	total knee arthroplasty
UTE	ultrashort echo time
vGRF	vertical ground reaction force
yrs	years

Chapter 1

INDIVIDUAL AND CUMULATIVE MEASURES OF KNEE JOINT LOAD ASSOCIATED WITH T2 RELAXATION OF KNEE CARTILAGE IN YOUNG, UNINJURED INDIVIDUALS: A PILOT STUDY

1.1 Abstract

Background: Articular cartilage structure and chondrocyte health are sensitive and reliant on dynamic joint loading during activities. The purpose of this study was to determine the association between measures of individual and cumulative knee joint loading with T2 relaxation times in the knee cartilage of young individuals without knee injury.

Methods: Twelve participants (17-30 years old) with no history of knee injury or surgery completed MRI, physical activity (PA), and biomechanical gait testing. T2 relaxation times were calculated in the cartilage within the patella and lateral and medial compartments. Accelerometry was used to measure mean daily step counts, minutes of PA, and % sedentary time over 7 days. Vertical ground reaction force, external knee joint moments and peak knee flexion angle were measured during stance phase of gait using three-dimensional motion capture. Cumulative knee joint loading was calculated as daily step count by external knee joint moment impulse. The relationship between measures of knee joint loading and T2 relaxation time was assessed using Pearson correlations.

Results: Higher T2 relaxation times in the femoral and tibial cartilage were consistently correlated to greater body mass, daily step counts, moderate and vigorous PA, and peak knee joint moments (r= 0.10 to 0.84). Greater cumulative knee flexion and adduction loading was associated with higher T2 relaxation time in the femoral and tibial cartilage (r= 0.16 to 0.65).

Conclusion: Preliminary findings suggest that individual loading factors and cumulative knee joint loading are associated with higher T2 relaxation times in the articular cartilage of young, healthy knees.

1.2 Introduction

Osteoarthritis (OA) is a degenerative joint disease that is the leading sources of chronic pain and disability in the United States and many other countries.[1,2] Knee OA accounts for over 80% of the total burden due to OA.[2] Further, post-traumatic knee OA that results from joint injury or trauma accounts for 13% of the OA prevalence in the United States.[3] OA is a disease that is characterized by deterioration of articular cartilage. Articular cartilage is composed mostly of water, collagen, proteoglycans and chondrocytes, and provides a smooth surface between joints to allow for transmission of loads to underlying subchondral bone with low friction.[4] Due to the limited healing capacity of articular cartilage,[4] early detection of damage is necessary for implementing strategies to prevent its breakdown.

Radiography and magnetic resonance imaging (MRI) are commonly used to detect features of OA. Radiographic changes in osteophyte and cyst formation, subchondral sclerosis, and joint space narrowing are most commonly used to diagnose structural OA, but these are late stage manifestations of the disease.[5] Thus, changes detected on radiographs are likely an indication of irreversible damage and loss of greater than 10% of total cartilage volume.[6] T2 relaxation time is a quantitative MRI method that can assess articular cartilage structure and matrix organization.[5,7,8] It is dependent on water and collagen architecture as well as collagen organization.[9] Increases in T2 relaxation time have been used to measure cartilage breakdown after knee injuries including anterior cruciate ligament (ACL) injury, meniscus repair, meniscectomy, and cartilage repair.[10-13] Understanding factors that influence quantitative MRI markers of cartilage health such as T2 relaxation time will inform interventions to prevent OA both within and outside of the context of joint injury.

In a healthy knee, dynamic mechanical loading during activities such as walking is protective to articular cartilage by facilitating joint metabolism and synthesis of proteoglycans and collagen within its extracellular matrix.[14] In uninjured individuals, greater medial knee joint loading, as estimated by a higher external knee adduction moment during walking, is associated with thicker articular cartilage in the medial tibiofemoral compartment.[15-17] Knee flexion angle at initial contact during the stance phase of gait is correlated to the locations of thickest cartilage in the medial tibiofemoral compartment.[18] Levels of physical activity (PA) have also been shown to affect cartilage structure. Middle-aged adults without knee OA who report moderate levels PA demonstrate smaller increases in T2 relaxation times than those participating in the highest and lowest levels of PA.[19] A lack of mechanical stimulation results in articular cartilage that is thinner and softer and may be more susceptible to damage.[20,21] This combined evidence suggests articular cartilage structure is influenced by cumulative joint loading, which integrates both magnitude and frequency of knee joint loading. Cumulative joint loading has been used by Maly and colleagues using the product of knee adduction moment impulse and steps per day to differentiate individuals with and without knee OA.[22] Voinier et al. used combinations of daily step counts with body mass index (BMI) to examine correlation of cumulative load and cartilage damage on MRI in middle-aged and older adults.[23] However, the relationship between measures of cumulative knee joint loading with articular cartilage structure in young, uninjured individuals is unknown. An understanding of the relationship between joint loading and uninjured articular cartilage health will provide insight into factors potentially responsible for cartilage breakdown and PTOA development after knee joint injury. Because knee joint loading can be modified, these factors may serve as interventional targets to delay or prevent PTOA in young individuals after knee injury.

The purpose of this study was to determine the association between individual (i.e., knee joint moments, PA levels, body mass) and cumulative measures of knee joint loading with T2 relaxation times in the articular cartilage of the knee in young, uninjured individuals. We hypothesized that knee joint moments during walking, number of steps per day, total minutes of moderate and vigorous intensity PA, and body mass would be associated with T2 relaxation times throughout the knee's articular cartilage.

1.3 Methods

1.3.1 Participants

Young, healthy individuals with no previous history of lower extremity injury or surgery between the ages of 17-30 were eligible for this study. Older individuals were excluded due to higher baseline risk of early knee OA. Exclusion criteria included recent history of knee pain (<3 months), history of inflammatory disease, immune compromise, body mass index over 30 kg/m^2, chronic use of nonsteroidal anti-inflammatory drugs, history of cortisone injection during prior 3 months, current pregnancy, or having contraindications to MRI. The University of Nebraska Medical Center Institutional Review Board approved this study and all subjects provided written informed consent.

1.3.2 Magnetic Resonance Imaging (MRI) Acquisition and T2 Relaxation Time

MRI acquisition was performed at the Center for Brain, Biology, and Behavior (CB3) at the University of Nebraska – Lincoln by a single technician. Participants were in a non-weightbearing position (supine) for 30 minutes prior to the knee scan to unload the articular cartilage due to acute effects of loading on T2 relaxation time.[24] MRIdata were acquired on a 3-Tesla Siemens Skyra MRI scanner using a 15 channel transmit /receive knee coil (Siemens

Medical Solutions USA, Inc., Malvern, PA, USA). A randomization table used to determine if the left or right knee was scanned for this study. Participants were positioned in a supine position with the knee in 0° of knee flexion and neutral rotation. For T2 mapping, a multi-echo, multi-slice sequence was acquired with the parameters: TR= 2700 ms; TE= 11.1, 22.2, 33.3, 44.4, 55.5, 66.6, 77.7, 88.8, 99.9, 111.0 ms; FOV: 120 mm x 120 mm; acquisition matrix=269x384; number of slices= 22; slice thickness= 3.0 mm; slice gap= 0.48 mm; pixel size= 0.3 mm x 0.3 mm;; number of excitations [NEX]= 1; scan duration: 12:11 min.

T2 maps were generated by fitting the multi-echo MRI data at each pixel to an exponential signal equation $S_i = S_0 \exp(-TE_i/T_2)$, using Levenberg-Marquardt nonlinear least squares algorithm. Here S_i is the signal at echo time TE_i, and S_0 is the signal at TE=0. Data corresponding to the first echo (TE=11.1 ms) were not used in the fitting procedure to minimize the errors due to stimulated echoes. The computer programs were written in Interactive Data Language (IDL; Harris Geospatial Solutions Inc, Broomfield, CO, USA).

Cartilage masks of the femoral, tibial and patellar articular cartilage were manually segmented using open source ITK-SNAP software[25] on images corresponding to TE=44.4 ms from multi-echo data used for generating T2 maps. Thus, the segmented masks exactly match the T2 maps for T2 relaxation time extraction from each region of interest. The 22 sagittal images were subdivided into medial and lateral compartments for both the femur (MFC and LFC, respectively) and the tibia (MTC and LTC, respectively) according to the center of the intercondylar notch. The femoral and tibial cartilage masks in each tibiofemoral compartment (LFC, MFC, LTC, MTC) were divided into anterior, weightbearing, and posterior regions as defined by the location of the meniscus horns in the sagittal plane (Figure 1.1). Axial MRIs were used to assist in identification of anterior and posterior meniscus horns. Anterior and posterior regions of the tibial cartilage contained very few pixels and thus were not included in

this analysis. The patellar cartilage was not subdivided into smaller regions of interest. A board-certified, fellowship-trained musculoskeletal radiologist who was blinded to all other participant data confirmed accurate location of segmentation masks on each slice as well as region of interest boundaries. Our lab has demonstrated reliable segmentation of both the femoral and tibial articular cartilage (intrarater intraclass correlation coefficients [ICC] [n=12]: femoral: 0.759; tibial: 0.775; interrater ICC [n=12]: femoral: 0.949; tibial: 0.930). The labeled cartilage masks were then applied to the corresponding T2 maps to extract mean T2 relaxation time within all 9 regions of interest using in-house computer software. Only pixels with T2 relaxation times between 10 ms and 90 ms were included in this analysis to remove any outliers due to fitting errors.[26]

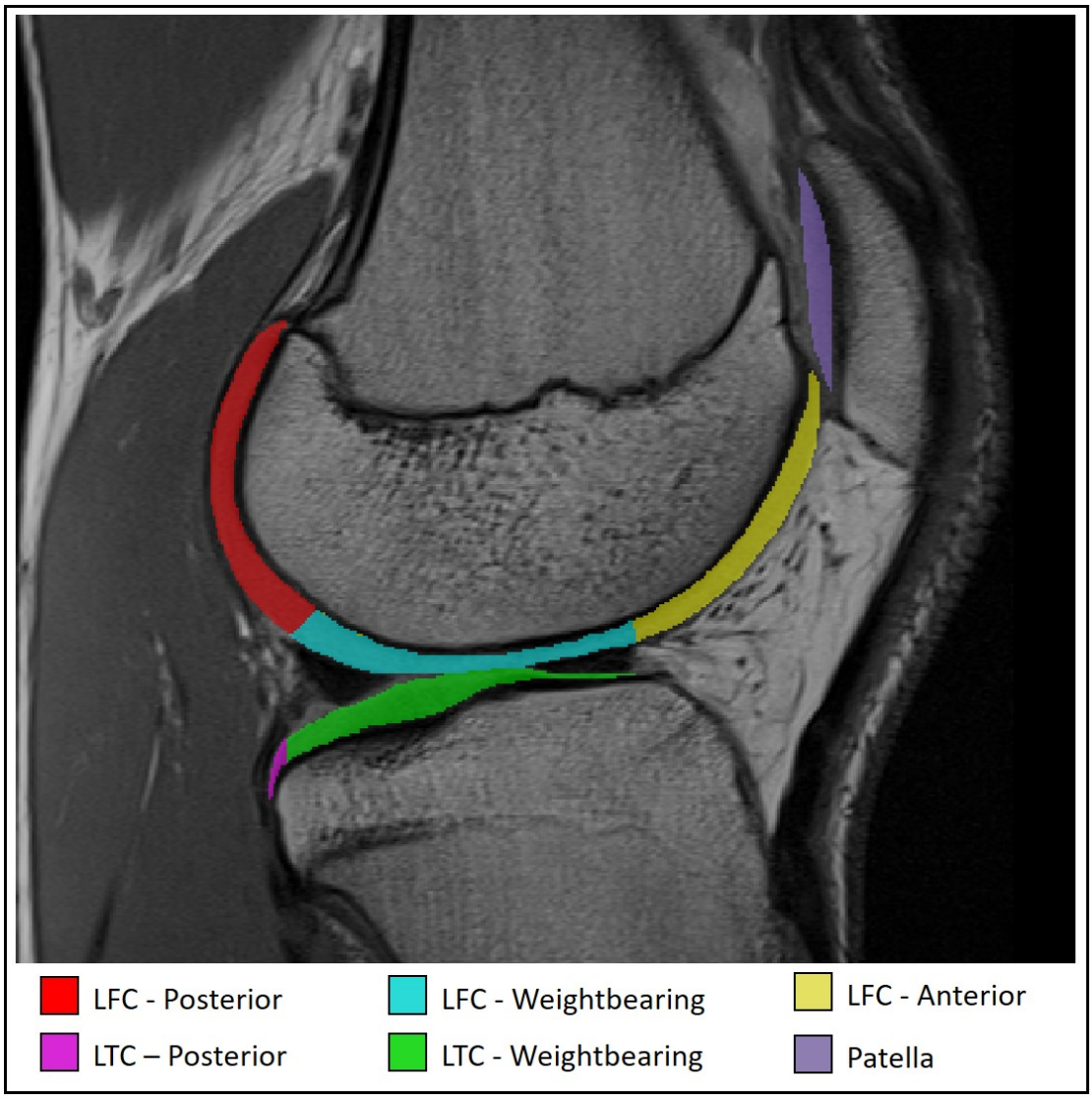

Figure 1.1: T2 Relaxation Time Regions of Interest. The femoral and tibial cartilage segmentation masks in each compartment (lateral compartment pictured above) were divided into anterior, weightbearing, and posterior regions as defined by the location of the meniscus horns in the sagittal plane. The anterior and posterior (not pictured) tibial cartilage was not used in analysis. The patellar cartilage comprised a single region.

1.3.3 Gait Biomechanics

Biomechanical gait analysis was performed at the Nebraska Athletic Performance Laboratory (NAPL) at the University of Nebraska – Lincoln. Passive, 14-millimeter retro-reflective markers were attached to bony landmarks of the bilateral lower extremities and trunk, including the first and fifth metatarsal heads (over their own shoes), superior and inferior posterior heel (over shoes), medial and lateral malleoli, medial and lateral femoral epicondyles, greater trochanters, anterior superior iliac spines (ASIS), iliac crests and acromion. Rigid shells each containing four markers were secured using elastic wraps and athletic tape to the lateral shanks, lateral thighs, and lower thoracic spine. A rigid shell containing three markers was secured to the posterior pelvis with the superior edge placed at the height of the posterior superior iliac spines (PSIS). This lower extremity marker set has previously been shown to be reliable in measuring knee flexion angles during standing activities.[27]

Three-dimensional kinematic data were collected using a 12-camera motion capture system (Qualysis AB, Sweden) sampled at 120 Hz. Kinetic data were collected using 2 embedded force plates (Bertec Corporation, Columbus, OH) sampled at 1080 Hz. A static trial was collected for one second with the participant standing in anatomical position. For gait trials, participants were asked to walk at a comfortable, self-selected walking speed across the embedded force plates. Gait speed was established during the first three trials and was maintained within 5% for all remaining walk trials using timing gates across a 5.4-meter walkway (Brower TCi System, Draper, UT). Participants completed eight trials for each limb with valid kinematic and kinetic data.

After completing marker labelling in Qualisys Track Manager, kinematic and kinetic data were imported into Visual3D software (C-Motion, Inc., Bethesda, MD). Custom, post-

processing scripts were used to construct subject-specific models and calculate joint angles and external joint moments. 3D marker trajectory and ground reaction force data were processed using a low-pass, fourth-order, bidirectional Butterworth filter with a cut-off frequency of 6 Hz. A subject-specific model was created from the static trial to determine segment lengths and joint centers. A threshold of 10 N was used to define the first and last frames of stance phase in each limb during walking trials. An inverse dynamics approach was used to calculate external knee joint moments in the sagittal and frontal planes during stance phase.[28] Vertical ground reaction force (vGRF) was normalized to bodyweight (BW). External knee moments were normalized to mass (kilograms) and height (meters). Bodyweight and mass were measured using the vGRF component during the 1-second static trial. Height was measured using a stadiometer. The vGRF and external knee flexion and adduction moments were integrated over stance phase using the trapezoidal rule to calculate joint moment impulses. The external knee joint moment impulse is directly proportional to the average knee joint moment and duration of stance phase. Biomechanical gait variables of interest were averaged over five gait trials and included mass as measured during the one-second static trial, peak vGRF during the first 50% of stance phase, impulse of vGRF during all of stance phase, peak knee flexion angle during weight acceptance of stance phase, peak external knee flexion moment, external knee flexion moment impulse during all of stance phase, peak external knee adduction moment during the first 50% of stance phase and external knee adduction moment during all of stance phase.

1.3.4 Physical Activity (PA)

PA data were collected using a 3-axis Actigraph accelerometer (wGT3X-BT; Actigraph Corporation, Pensacola, FL) that has previously been shown to reliably measure

step counts across gait speeds.[29] Participants were instructed to wear the accelerometer on a provided elastic belt at the top of the right iliac crest in line with the axilla consecutively during all waking hours (except when in water) for 1 week following MRI and biomechanical gait analysis. Uniform instructions were provided in a handout detailing appropriate wear and positioning of the accelerometer. We used previously reported criteria established by Troiano and colleagues and the National Cancer Institute (NCI) to define valid accelerometer wear time.[30] Wear criteria consisted of at least 10 hours per day on at least 4 of the 7 days, as this is the minimum time required to reliably estimate PA behaviors.[30-32] Non-wear days were excluded from analysis. Activity counts, which represent the weighted sum of the number of accelerations, were used as outputs over 1-minute intervals to identify accelerometer wear and PA intensity. Daily non-wear periods were defined as intervals of at least 90 minutes of zero activity counts that contained no more than two minutes of activity counts less than 100.[32] Activity count outputs were used to define intensity levels of PA based on metabolic equivalents (METs) for each 1-minute of wear. Activity count cut points for each minute were 0-99 for sedentary activity, 100-2019 for light PA, 2020-5998 for moderate PA, and greater than or equal to 5999 for vigorous PA.[30] Variables of interest for this study were average steps per day, percentage of day during accelerometer wear in sedentary activity and average daily minutes of light, moderate, and vigorous PA. All PA activity was analyzed within Actilife 6 software (Actigraph Corporation, Pensacola, FL).

1.3.5 Cumulative Knee Joint Loading

Daily cumulative knee joint loading was defined by the average daily step count measured by accelerometry multiplied by the average normalized (mass x height) external knee joint moment impulse measured during one stance phase of gait. Cumulative knee flexion load was calculated as the product of mean daily step count and mean external knee

flexion moment impulse. Cumulative knee adduction load was calculated as the product of mean daily step count and mean external knee adduction moment impulse. Cumulative knee flexion and adduction loads were examined because both knee flexion and adduction moments have been found to predict joint contact forces in the knee.[33]

1.3.6 Statistical Analysis

Continuous data were described using means, standard deviations and 95% confidence intervals. Nominal data were described using counts and proportions. Pearson correlation tests were used to identify associations between mass, height, PA levels, gait biomechanics, and cumulative knee joint loading with T2 relaxation times in each articular cartilage region of interest. Pearson correlation coefficient (r) of 0.1 to less than 0.3 and -0.1 to greater than -0.3 was categorized as weak association, 0.3 to less than 0.5 and -0.3 to greater than -0.5 as moderate association, and 0.5 to 1.0 and -0.5 to -1.0 as strong association. A p-value less than 0.05 was considered statistically significant.

1.4 Results

Of the 12 participants included in this study, 5 (41.7%) were female and 7 (58.3%) were male. Nine participants (75%) were white, one (8.3%) was Asian, one (8.3%) was Hispanic, Latino or Spanish, and one (8.3%) did not report race or ethnicity. Mean demographics, body size, PA levels, gait biomechanics, and measures of cumulative knee joint loading are presented in Table 1.1. Mean T2 relaxation times in each articular cartilage region of interest are presented in Table 1.2.

Table 1.1: Healthy Cohort Participant Characteristics. Mean age, mass, height, physical activity levels, gait biomechanics, and measures of cumulative knee joint loading for all 12 participants. Abbreviations: SD, standard deviation; CI, confidence interval; yrs, years; kg, kilogram; m, meter; %, percentage; PA, physical activity; °, degree; BW, bodyweight; s, second; N, newton.

	Mean (SD)	95% CI
Age (yrs)	22.9 (3.3)	20.8 - 24.9
Mass (kg)	69.8 (14.0)	60.9 - 78.7
Height (m)	1.76 (0.07)	1.72 - 1.80
Steps per day	8187.7 (3595.5)	5903.2 - 10472.2
Sedentary time (daily %)	63.8 (11.4)	56.5 - 71.0
Light PA (daily minutes)	1512.3 (537.9)	1170.5 - 1854.1
Moderate PA (daily minutes)	178.4 (136.8)	91.5 - 265.4
Vigorous PA (daily minutes)	29.7 (39.1)	4.9 - 54.5
Peak KFA (°)	20.6 (6.0)	16.7 - 24.4
Peak vGRF (BW)	1.15 (0.10)	1.09 - 1.22
vGRF Impulse (BW·s)	0.53 (0.03)	0.52 - 0.55
Peak KFM (N·m/kg·m)	0.34 (0.16)	0.24 - 0.45
KFM Impulse (N·m·s/kg·m)	0.05 (0.03)	0.03 - 0.06
Cumulative KFM Load (daily N·m·s/kg·m)	393.7 (294.0)	206.9 - 580.5
Peak KAM (N·m/kg·m)	0.24 (0.09)	0.18 - 0.30
KAM Impulse (N·m·s/kg·m)	0.08 (0.02)	0.07 - 0.10
Cumulative KAM Load (daily N·m·s/kg·m)	650.2 (317.9)	448.2 - 858.1

Table 1.2: T2 Relaxation Time in Healthy Cohort. Mean T2 relaxation times (milliseconds) in each articular cartilage region of interest for all 12 participants. Abbreviations: SD, standard deviation; CI, confidence interval; LFC, lateral femoral condyle; MFC, medial femoral condyle; LTC, lateral tibial condyle; MTC, medial tibial condyle.

	Mean (SD)	95% CI
LFC – Anterior	54.3 (2.6)	52.6 - 56.0
LFC – Weightbearing	50.2 (2.7)	48.5 - 51.9
LFC – Posterior	49.8 (3.1)	47.9 - 51.8
LTC – Weightbearing	45.4 (4.0)	42.9 - 48.0
MFC – Anterior	55.0 (5.1)	51.8 - 58.3
MFC – Weightbearing	50.1 (3.8)	47.6 - 52.5
MFC – Posterior	46.8 (5.3)	43.4 - 50.1
MTC – Weightbearing	46.0 (3.8)	43.5 - 48.4
Patella	44.4 (2.2)	43.0 - 45.8

1.4.1 Gait Biomechanics

Greater knee joint moments and angles were consistently associated with greater articular cartilage T2 relaxation times. Associations with gait biomechanics were stronger in the weightbearing and posterior regions of femoral and tibial cartilage compared to the anterior femoral and patellar cartilage (Figure 1.2). Peak knee flexion angle demonstrated the strongest positive associations with T2 relaxation times in the medial femoral and tibial cartilage. Greater peak knee adduction moment and impulse, which are surrogate measures for higher medial compartment and lower lateral compartment loading, were moderately to strongly associated with higher T2 relaxation times in the medial tibiofemoral compartment and lower T2 relaxation times in the lateral tibiofemoral compartment.

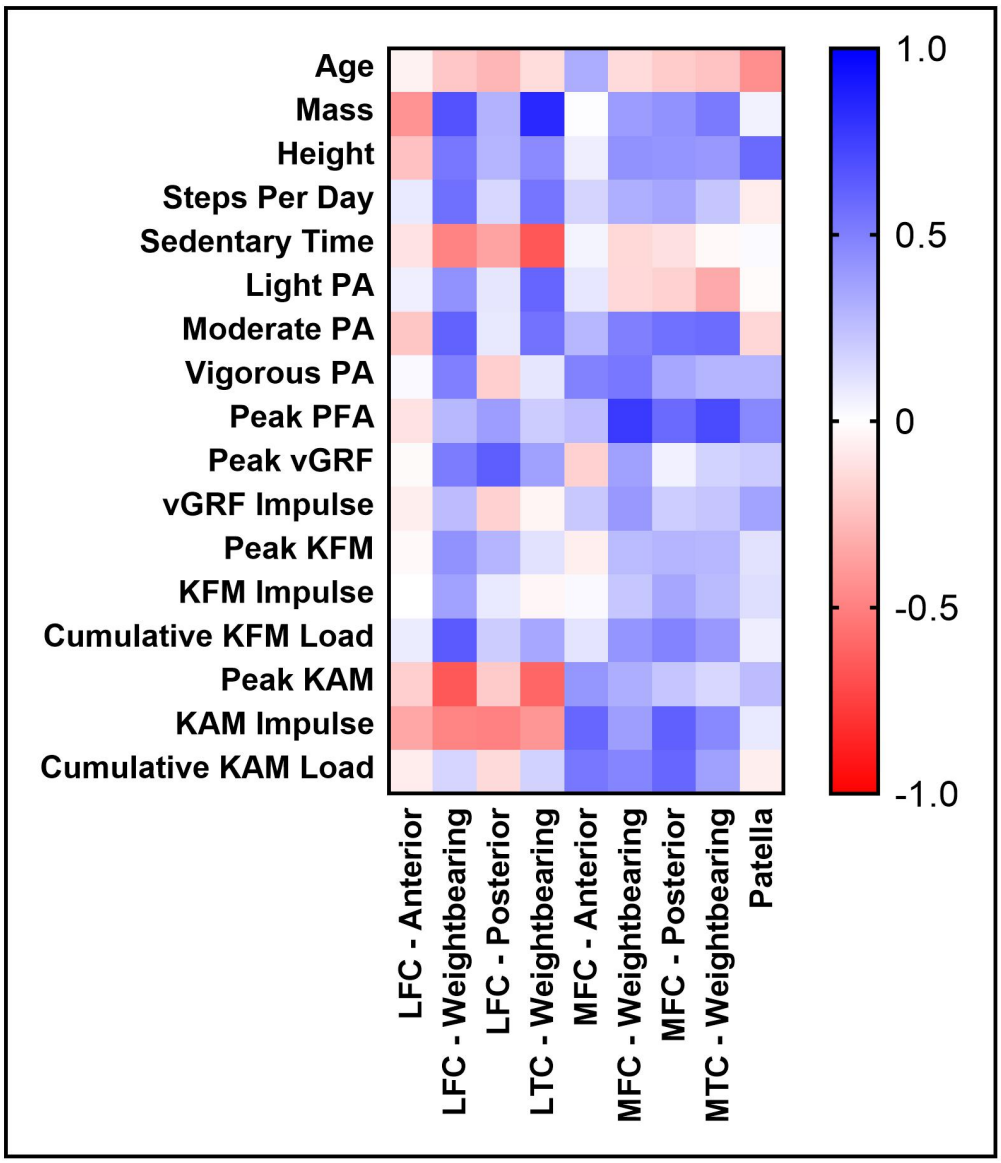

Figure 1.2: Correlation Matrix of Joint Loading and T2 Relaxation Time in Healthy Cohort. Generally, greater body size, higher physical activity levels, greater knee joint moments and angles, and greater cumulative knee joint loading (rows) were associated with higher T2 relaxation times throughout the femoral, tibial and patellar cartilage (columns). Pearson's correlation coefficients (r) are represented by blue and red shades, ranging from +1.0 to -1.0. Blue represents positive correlations. Red represents negative correlation. Darker shades of red and blue signify stronger correlations. Abbreviations: PA, physical activity; PFA, knee flexion angle; vGRF, vertical ground reaction force; KFM, knee flexion moment; KAM, knee adduction moment; LFC, lateral femoral condyle; LTC, lateral tibial condyle; MFC, medial femoral condyle; MTC, medial tibial condyle.

1.4.2 Physical Activity

All participants had a valid number of accelerometer days and wear time to be included in the analysis. Moderate and strong positive associations were consistently present between PA levels and T2 relaxation times in the femoral and tibial cartilage but not in the patellar cartilage (Figure 1.2). A higher daily step count and minutes of both moderate and vigorous PA was associated with higher T2 relaxation times in the weightbearing and posterior regions of bilateral femoral and tibial compartments. These relationships were not consistently present in the anterior femoral cartilage. A greater percentage of the day spent in sedentary activity was associated with lower T2 relaxation times in the medial and lateral tibiofemoral compartments.

1.4.3 Cumulative Knee Joint Loading

Peak knee flexion moment and impulse did not demonstrate strong positive correlation with T2 relaxation times in any articular cartilage regions of interest (Figure 1.2). However, greater cumulative knee flexion load (steps per day x knee flexion moment impulse) was moderately to strongly associated with higher T2 relaxation times across weightbearing and posterior regions in the medial and lateral tibiofemoral compartment. Similarly, greater cumulative knee adduction load (steps per day x knee adduction moment impulse) was moderately to strongly associated with higher T2 relaxation times in all regions within the medial tibiofemoral compartment.

1.4.4 Body Size and Age

Individual characteristics also influenced T2 relaxation times in the articular cartilage of the knee (Figure 1.2). Greater body mass and height was associated with lower T2

relaxation times only in the anterior lateral femoral cartilage and with higher T2 relaxation times in most other regions of the tibiofemoral and patellar compartments. Greater age was weakly associated with lower T2 relaxation times in all weightbearing regions and posterior femoral cartilage while a moderate negative correlation was present with patellar cartilage.

1.5 Discussion

This pilot study aimed to determine associations between individual joint loading variables (knee joint moments, PA levels, body mass) and cumulative knee joint loading variables with T2 relaxation time in the articular cartilage of the knee in young, uninjured individuals. Our results support our hypotheses; greater T2 relaxation time was consistently correlated to measures that influence overall knee joint loads, including greater knee joint moments during gait, daily step counts, moderate and vigorous PA, and body mass. Cumulative knee flexion and adduction loading, measures that combine average steps per day with the magnitude of knee joint moments during each step of gait, was associated with greater T2 relaxation time in the femoral and tibial cartilage. The correlations between these variables were consistently positive relationships, indicating greater magnitudes and frequencies of knee joint loading were associated with higher T2 relaxation times. In addition to our hypotheses, greater height was associated with higher T2 relaxation time while a higher percentage of daily sedentary time correlated with lower T2 relaxation time.

Articular cartilage structure and chondrocyte health are sensitive and reliant on dynamic joint loading.[34] Articular cartilage adapts to the chronic loading patterns placed upon it such as during walking.[35] Frequency of walking, intensity of walking, and biomechanical movement patterns during walking all contribute to the cumulative, chronic load experienced by the knee. It is well established that periods of acute compressive loading in tibiofemoral

joints without OA results in decreased T2 relaxation times that are most notable in the weightbearing regions.[24,36,37] The reduction in T2 relaxation times is likely the result of fluid moving out of the cartilage during periods of increased loading. However, less is known about the influence of chronic knee joint loading patterns, particularly in young individuals. The results of this study suggest that higher sources of knee joint load, including greater body mass, PA, and knee joint moments, are correlates of higher T2 relaxation times. Higher T2 relaxation times are thought to signal higher water content and poorer collagen matrix organization.[38] Thus, higher T2 relaxation times are frequently linked to signs of cartilage destruction and unhealthy tissue. However, the participants in this study had no risk factors for knee OA. Thus, higher T2 relaxation times in our study are likely not pathologically high. Further, systemic factors such as age, sex, race and genetics may establish an individual's baseline T2 relaxation profile.[20] It is possible that the higher T2 relaxation times in individuals with higher measures of knee joint loading are a normal, healthy response to the cumulative, daily loading withstood. Our findings that individual-specific profiles of body size, PA level, and knee joint loading patterns influence cartilage structure support the need for future studies to compare T2 relaxation time in a target knee (e.g. after joint injury) to the contralateral knee or as a percent change relative to baseline values to account for individual T2 differences.

1.5.1 Gait Biomechanics

Knee joint angles and moments during gait were correlates of T2 relaxation in the weightbearing regions of the femoral and tibial cartilage. Peak knee flexion angle during stance phase strongly correlated with T2 relaxation in the weightbearing regions of the medial tibiofemoral compartment. Meanwhile, vGRF strongly correlated with T2 relaxation in the weightbearing area of the lateral femur. The external knee adduction moment was more

strongly associated with T2 relaxation times than the external knee flexion moment. A higher peak knee adduction moment and impulse were correlated with higher T2 relaxation in the medial tibiofemoral compartment but lower T2 relaxation in the lateral tibiofemoral compartment. A higher external knee adduction moment is a surrogate measure for greater medial compartment joint loading relative to the lateral compartment. Therefore, these findings are consistent with our PA findings where higher loading (greater step counts, minutes of moderate and vigorous PA) was related to higher T2 relaxation times and lower loading (greater daily percentage of sedentary time) was related to lower T2 relaxation times.

The knee adduction moment has previously been shown to have a significant relationship with articular cartilage thickness in the medial compared to lateral tibiofemoral compartment.[16,39] Previous work detailing biomechanical movement patterns and T2 relaxation in the cartilage of the knee is limited. A small study by Souza et al. of 14 healthy participants with a similar age (22.7±3.3 years) to the current study found that the peak knee adduction moment during a drop jump predicted higher overall T2 relaxation in the femoral, tibial and patellar cartilage.[40] However, this study also reported that a higher knee flexion moment during a single hop correlated to lower T2 relaxation times. Further work is needed to determine if joint loading patterns in young, uninjured individuals during less frequent movement patterns such as jumping are consistent with loading patterns exhibited during higher frequency daily activities such as walking. In contrast to our findings and those of Souza et al.,[40] Van Rossom and colleagues reported that knee joint contact forces estimated through musculoskeletal modeling was negative correlated to whole joint T2 relaxation time in 15 healthy individuals (age = 30.7±5.8 years) also without knee injury.[41] Further work is warranted to determine the role of movement patterns and knee joint loading in maintaining healthy articular cartilage in children and young adults.

Knee joint loading did not demonstrate significant relationships with T2 relaxation times in the anterior femoral and patellar cartilage. The trochlea and patellar surfaces within the patellofemoral joint are subjected to higher levels of shear stress in comparison to higher compressive stress experienced in the weightbearing regions of the femoral and tibial cartilage.[20] As a result, proteoglycan concentration and collagen orientation differs across articular cartilage within the knee. The current study focused on walking which subjects the patellofemoral joint to relatively low levels of joint load and stress. Our findings may have differed if different movement types were studied. For example, higher self-reported levels of knee-bending activity has been linked to higher T2 relaxation in the patella.[42] Teng and colleagues have reported associations between higher peak knee flexion moment, knee flexion moment impulse, and peak patellofemoral joint stress and greater T2 relaxation in the trochlear and patellar cartilage in individuals over 35 years old (mean = 52.5 years) both with and without patellofemoral joint OA.[43,44] The increased age of this population is a risk factor for cartilage breakdown and may explain the conflicting findings compared to the current study.

1.5.2 Physical Activity

Daily step counts and minutes of moderate and vigorous PA were moderately to strongly correlated with T2 relaxation times in the tibiofemoral cartilage. This is the first study to investigate accelerometer-based measures of PA with T2 relaxation time in the articular cartilage of the knee in a young population (mean age = 23±3.3 years). Regular PA positively relates to the maintenance of healthy cartilage. Weightbearing PA promotes a healthy body weight and lower extremity muscle strength, both which decrease the risk of symptomatic knee OA.[45,46] Participation in vigorous sports activities results in thicker articular cartilage in

children.[47] Even activities such as long distance recreational running that places repetitive cyclic loads through the knee does not result in a higher risk for OA development.[48,49] Two previous studies did not find that PA associated with T2 relaxation times in the cartilage of the knee. Stahl and colleagues reported that T2 relaxation was not different in the tibiofemoral or patellofemoral cartilage between healthy individuals who reported a Tegner score of 1-5 compared to those with a Tegner score of 6-10.[50] Similarly, Hovis and colleagues reported that T2 times did not differ in the tibiofemoral or patellofemoral cartilage between healthy individuals who self-reported light or moderate-strenuous PA on the Physical Activity Scale for the Elderly (PASE).[42] The differences in age across these studies (current = 23±3.3 years; Stahl et al. = 33.7±9.4 years; Hovis et al. 50.7±2.7 years) as well as PA measurement methodology (current = accelerometry; Stahl et al. = Tegner score; Hovis et al. = PASE) may account for conflicting conclusions regarding the influence of PA on T2 relaxation time in the articular cartilage of the knee.

A greater daily percentage of sedentary activity was associated with lower T2 relaxation, most notably in the lateral tibiofemoral compartment. Proteoglycan synthesis and chondrocyte function is enhanced by regular dynamic loading of the joint, whereas joint unloading leads to articular cartilage breakdown.[20] Previous studies have investigated periods of non-weightbearing activity on measures of cartilage structure in the knee. Souza et al. reported that T2 relaxation increases of up to 12% in the weightbearing regions of the articular cartilage after 8 weeks of non-weightbearing resulting from distal lower extremity injury.[51] Vanwanseele et al. reported up to 13% thinning of the cartilage in the tibiofemoral and patellofemoral cartilage at 1 year following complete spinal cord injury.[21] Knee joint unloading was not as extreme in the current study. The average daily percentage of time spent

in sedentary activity was 63.8±11.4% (range = 41.2-81.3%). Further, the negative correlation between sedentary activity and T2 relaxation time was observed in young healthy knees with no evidence of joint disease. Further study is warranted to determine if lower habitual PA in young, uninjured individuals negatively influences articular cartilage and increases risk for later OA development.

1.5.3 Cumulative Knee Joint Loading

Cumulative knee joint loading has been shown to differentiate individuals with and without knee OA using step counts and external knee adduction moment impulse and also identify at-risk individuals for greater progression of knee cartilage damage using steps counts and BMI.[22,23] These previous works highlight the absence of a standard definition for cumulative knee joint loading. We used a combination of daily step counts and average knee moment impulse during each step of gait to reflect dynamic knee loading patterns needed to maintain articular cartilage structure. Our findings demonstrated weak relationships between the external knee flexion moment during gait and T2 relaxation time throughout the knee cartilage. However, the relationship was strengthened when knee flexion moment was combined with daily step counts within the measure of cumulative knee flexion moment load. This early evidence supports further exploration into the role of cumulative knee joint loading on cartilage integrity and early OA development in young, active individuals.

1.5.4 Body Size and Age

Body mass and height were positively correlated to higher T2 relaxation times in the cartilage of the knee, but age was not. Not all participants in this study may have been fully physically mature. One individual in this study was less than 20 years old, while the remaining were 20-30 years old. Height typically plateaus by 20 years of age, but body mass typically

increases throughout the second decade of life, indicating that participants in this study may not have reached musculoskeletal maturity.[52,53] In children, increases in height is associated with accrual of articular cartilage volume in the tibiofemoral joint.[47] T2 relaxation in the cartilage of the knee decreases with progressive maturation in children under 19 years of age.[54] Further, Wang and colleagues have demonstrated that increases in T2 relaxation times after a marathon are greater in runners with higher body weight and BMI but not correlated with age, height, or sex.[55] Taken together, these findings underscore the need to carefully consider the role of body size and maturation within compositional MRI analyses of cartilage in young, active populations.

There are several limitations to this study. Previous work has shown changes in quantitative MRI markers within the cartilage of the knee before and after a day of normal daily activities.[56] This study did not control for time of day of MRI testing which could have influenced T2 relaxation time. Further, PA was measured after completion of MRI assessment. PA behavior of participants before completion of the MRI could differ than the reported PA measurements in this study. Second, static knee joint alignment was not measured in this study but may associate with T2 relaxation in knee cartilage.[57] Third, the sample size of this analysis was small which limited multivariate regression modelling to identify the strongest loading predictors of T2 relaxation. It also limited our ability to compare T2 relaxation time by sex. Finally, we only accounted for current PA levels and not lifelong PA levels that have chronically loaded the knee joint.

1.6 Conclusion

In this pilot study, individual loading factors of greater body size, PA and knee joint moments during gait as well as cumulative knee joint loading integrating daily step counts and knee joint moments are associated with higher T2 relaxation times in the femoral and tibial articular cartilage of young, healthy knees. Our findings provide insight into the potential role of modifiable joint loading factors that may influence early cartilage breakdown after knee injury. Future studies measuring T2 relaxation changes in knee cartilage of adolescents and young adults should use the contralateral limb or percent change relative to baseline values to account for individual T2 differences related to factors such as body size and PA patterns.

1.7 Acknowledgements

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Chapter 2

KNEE JOINT UNLOADING AND DAILY PHYSICAL ACTIVITY ASSOCIATE WITH CARTILAGE T2 RELAXATION TIME ONE MONTH AFTER ACL INJURY

2.1 Abstract

Background: The risk of osteoarthritis (OA) is high after anterior cruciate ligament (ACL) injury, but mechanisms underlying its development are poorly understood. The purpose of this study was to determine if gait biomechanics and daily physical activity (PA) are associated with cartilage T2 relaxation time, a marker of cartilage breakdown, one month after ACL injury.

Methods: Twenty-three ACL-injured participants (15-35 years old) without acute or degenerative chondral lesions were included. Participants completed quantitative magnetic resonance imaging, three-dimensional gait analysis, and one week of PA accelerometry. Interlimb differences were calculated for gait biomechanics and T2 relaxation time. Multiple linear regression models adjusted for age, sex, and concomitant meniscus injury were used to determine the association between gait biomechanics and PA with T2 relaxation time.

Results: Altered knee joint adduction moment impulse, less knee flexion excursion and higher daily step counts accounted for an additional 45.9-71.5% of the variation in prolonged T2 relaxation time in the weightbearing and posterior cartilage of the injured medial and lateral compartment (all $p<0.01$). Knee adduction moment impulse was the strongest factor for prolonged T2 relaxation time in all models (all $p\leq0.006$). Lower knee adduction moment impulse associated with prolonged T2 relaxation time in the injured medial compartment

(β = -0.659 to -0.841) and shorter T2 relaxation in the lateral compartment (β = 0.863 to 1.031).

Conclusion: At one month after ACL injury, altered knee joint adduction moment impulse, less knee flexion excursion, and higher daily PA were associated with poorer cartilage health. Statement of Clinical Significance: Gait biomechanics and daily PA may be modifiable targets to improve cartilage health acutely after ACL injury and slow progression to OA.

2.2 Introduction

Anterior cruciate ligament (ACL) injury is the most frequent intrarticular injury of the knee and occurs at an incidence of 68.6 per 100,000.[1,2] Young individuals participating in cutting and pivoting sports are at highest risk of ACL injury and ACL injury rates are on the rise.[2,3] The incidence of ACL injury increased by 74% in those less than 25 years old during the 15-year period between 2000 to 2015 and is projected to grow to 239% in females and 119% in males by 2025.[4] An unfortunate but common consequence of ACL injury is the development of post-traumatic knee osteoarthritis (PTOA) at an early age. ACL injury increases the risk of future OA by over eightfold within 11 years of injury.[5] Fifty percent of individuals with ACL injury will develop radiographic and symptomatic signs of PTOA within 10-20 years of ACL injury.[6-8] ACL injury increases the risk for PTOA in both the tibiofemoral and patellofemoral compartment.[7] The development of PTOA after ACL injury in turn increases risk for requiring a total knee arthroplasty (TKA) at a young age; the risk is 20 times greater during the third decade of life and 7.5 times greater during the fourth decade of life.[9] The rapidly increasing incidence of ACL injuries in young, active populations will likely result in a greater burden of PTOA in young adults in the years ahead. Mechanisms underlying the early and rapid development of PTOA after ACL injury are not well understood. Thus, interventions to prevent or delay cartilage breakdown after ACL injury do not exist.

Chondrocytes and the extracellular matrix of articular cartilage are responsive to repetitive, cyclic loading, in part due to its avascular structure. Dynamic joint loading results in stronger collagen and increased proteoglycan concentrations in cartilage.[10] Meanwhile, inadequate joint loading results in cartilage that is thinner, softer and more susceptible to

breakdown.[11,12] Abnormal walking patterns have been linked to compositional signs of articular cartilage breakdown after ACL injury, such as increasing T2 and T1rho relaxation time.[13-19] Although individuals have lower daily step counts and time spent in moderate and vigorous physical activity (PA) roughly 2.3 years (mean 27.8±17.5 months) after ACL reconstruction compared to uninjured matched controls,[20] knowledge about PA levels before ACLR are unknown. It is also unknown if daily magnitudes of PA early after ACL injury are associated with T2 relaxation time in the cartilage of the injured knee. An understanding of the association between measures of joint loading such as gait biomechanics and daily PA with articular cartilage structure are needed to inform modifiable strategies aiming to limit cartilage breakdown after ACL injury.

The purpose of this study was to determine if measures of knee joint loading (i.e. knee joint biomechanics during gait, PA levels) are associated with T2 relaxation time in the articular cartilage of the knee within 1 month of ACL injury. This time period was selected to understand early cartilage changes after ACL injury and how they relate to joint loading. We hypothesized that lower measures of knee joint loading would be associated with prolonged T2 relaxation time in the weightbearing regions of the femoral and tibial cartilage. A secondary aim was to determine if interlimb differences in gait biomechanics and T2 relaxation time are present within 1 month of ACL injury. We hypothesized that lower joint angles and moments would be present in the injured knee and that T2 relaxation time in the cartilage in the injured knee would be higher than the cartilage in the uninjured knee.

2.3 Methods

2.3.1 Participants

Participants between 15-35 years of age were enrolled within one month of ACL injury for this ongoing prospective study. Older individuals were excluded due to higher risk of baseline cartilage degeneration. Exclusion criteria included a previous knee injury or surgery to either knee, concomitant symptomatic grade III tear to other knee ligaments requiring surgical intervention, meniscus tear requiring meniscectomy (as determined using post-injury MRI by participant's orthopaedic surgeon), acute chondral lesions or degenerative cartilage changes identified on post-injury MRI, or open growth plate appearance requiring altered ACL reconstruction technique. Additional exclusion criteria included history of inflammatory disease, immune compromise, chronic use of nonsteroidal anti-inflammatory drugs, history of cortisone injection during the prior three months, current pregnancy, or contraindications to MRI. One participant was excluded secondary to motion artifact during MRI data acquisition, leaving 23 participants included in the current analysis. This study was approved by the Institutional Review Board at the University of Nebraska Medical Center. All participants provided written informed consent.

Participants reported age, sex, race, and pre-injury activity level in surveys within the REDCap electronic data capture tools hosted at the University of Nebraska Medical Center.[21,22] Height was measured using a portable stadiometer with shoes off.

2.3.2 Magnetic Resonance Imaging (MRI) Acquisition and T2 Relaxation Time

MR imaging was performed at the Lauritzen Outpatient Center at the University of Nebraska Medical Center. Participants sat in a non-weightbearing position for 30 minutes prior to the knee scan to unload the articular cartilage due to acute effects of loading on T2 relaxation time.[23] Each MRI scan began between 4:30-5:30 p.m. MR image data were acquired on a 3-Tesla Phillips Ingenia MRI scanner using a 16 channel transmit/receive knee coil (Phillips North America Corporation, Andover, MA, USA). The injured knee was scanned first followed by the uninjured knee. Participants were positioned in a supine position with the knee in 0° of knee flexion and neutral rotation. For T2 mapping, a multi-echo, multi-slice sequence was acquired with the parameters: TR= 6500 ms; TE= 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 ms; FOV: 120 mm x 120 mm; acquisition matrix=156x155; number of slices= 27; slice thickness= 3.0 mm; slice gap= 0.5 mm; pixel size= 0.3 mm x 0.3 mm;; number of excitations [NEX]= 1.

T2 maps were generated by fitting the multi-echo MRI data at each pixel to an exponential signal equation $S_i = S_0 \exp(-TE_i/T_2)$, using Levenberg-Marquardt nonlinear least squares algorithm. Here S_i is the signal at echo time TE_i, and S_0 is the signal at TE=0. Data corresponding to the first echo (TE=10 ms) was not used in the fitting procedure to minimize the errors due to stimulated echoes. The computer programs were written in Interactive Data Language (IDL; Harris Geospatial Solutions Inc, Broomfield, CO, USA).

Cartilage masks of the injured femoral, tibial and patellar articular cartilage were manually segmented using open sources ITK-SNAP software[24] on reference images corresponding to TE=40 ms from multi-echo data used for generating T2 maps. Thus, the segmented masks exactly match the T2 maps for T2 relaxation time extraction from each region of interest. Reference images of the injured knee were manually registered to the

reference images of the uninjured knee. A 12-degree of freedom affine registration was then applied to the manually transformed injured image. The combined transformation matrix was applied to the injured segmentation mask. Manual adjustments of the transformed segmentation mask were completed to anatomically match the cartilage in the uninjured reference scan as needed.

The sagittal images were subdivided into medial and lateral compartments for both the femur (MFC and LFC, respectively) and the tibia (MTC and LTC, respectively) according to the center of the intercondylar notch. The femoral and tibial cartilage masks in each tibiofemoral compartment (LFC, MFC, LTC, MTC) were divided into anterior, weightbearing, and posterior regions as defined by the location of the meniscus horns in the sagittal plane (Figure 2.1). Axial MR images were used to assist in identification of anterior and posterior meniscus horns. Anterior and posterior regions of the tibial cartilage contained very few pixels and thus were not included in this analysis. The patellar cartilage was not subdivided into smaller regions of interest. A board-certified, fellowship-trained musculoskeletal radiologist confirmed accurate location of segmentation masks on each slice as well as region of interest boundaries. Our lab has demonstrated reliable segmentation of both the femoral and tibial articular cartilage (intrarater intraclass correlation coefficients [ICC] [n=12]: femoral: 0.759; tibial: 0.775; interrater ICC [n=12]: femoral: 0.949; tibial: 0.930). The labeled cartilage masks were then applied to the corresponding T2 maps to extract mean T2 relaxation time within all 9 regions of interest using in-house computer software. Only pixels with T2 relaxation times between 10 ms and 90 ms were included in this analysis to remove any outliers due to fitting errors.[25]

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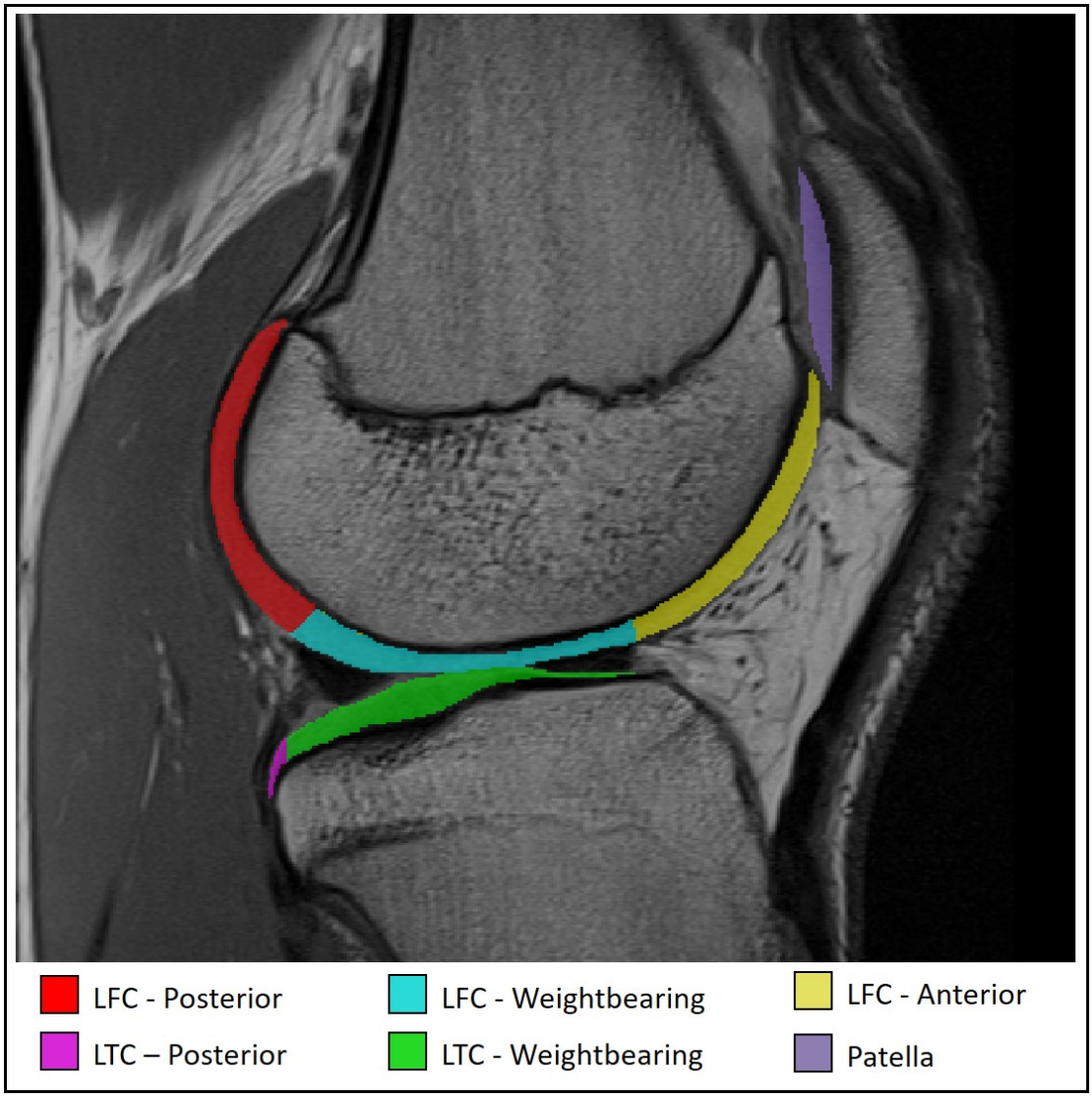

Figure 2.1: T2 Relaxation Time Regions of Interest. The femoral and tibial cartilage segmentation masks in each compartment (lateral compartment pictured above) were divided into anterior, weightbearing, and posterior regions as defined by the location of the meniscus horns in the sagittal plane. The anterior and posterior (not pictured) tibial cartilage was not used in analysis. The patellar cartilage comprised a single region.

2.3.3 Gait Biomechanics

Three-dimensional motion capture data were collected at the Clinical Movement Analysis Laboratory at the University of Nebraska Medical Center using an 8-camera Qualisys system (Qualysis AB, Sweden) sampled at 120 Hz and 2 embedded force plates (Bertec Corporation, Columbus, OH) sampled at 1080 Hz. Passive, 14-millimeter (mm) retroreflective markers were placed on the first and fifth metatarsal heads (over shoes), superior and inferior posterior heel (over shoes), medial and lateral malleoli, medial and lateral femoral epicondyles, anterior superior iliac spine (ASIS), superior edge of iliac crest, posterior superior iliac spine (PSIS), spinous process of T10 and C7, bilateral upper back placed inferior and medial to the inferior scapular angle, superior edge of the sternum, and acromion (Figure 2.2). Rigid shells each with four markers were placed at the lateral shanks and thighs and secured with elastic wraps and athletic tape.

Participants stood in anatomical position for a one-second static trial. Markers at the first metatarsal head, medial and lateral malleoli, medial and lateral femoral epicondyles, ASIS, and sternum were removed prior to gait trials. Participants completed eight gait trials with valid kinematic and kinetic data on each limb. Participants were instructed to walk at a self-selected, comfortable walking speed. Average gait speed was calculated along a 5.4 meter walkway during the first three gait trials and maintained within 5% for all remaining trials.

Marker data were labelled within Qualisys Track Manager software for all trials and then exported to Visual 3D software (C-Motion, Inc., Bethesda, MD) for custom data post-processing. Kinematic target data and kinetic ground reaction force data were low-pass filtered using a fourth-order bidirectional Butterworth filter with a cutoff frequency of 6 Hz. A cutoff frequency of 6 Hz was chosen after completing residual analysis of kinetic data as described by Winter.[26] Briefly, residuals were calculated for cutoff frequencies from 0.1 Hz to 49.9 Hz at increments of 0.1 Hz using all gait trials from the first ten participants in this study.

Residuals were normalized with respect to the maximum residual value. The linear section of high frequency residuals was defined as the collection of points where the values of the residuals' second discrete time derivative were below 0.0001. Linear regression was used to characterize this collection of points, and the cutoff frequency was set equal to the y-intercept of the linear regression line. The average cutoff frequency across both limbs was 4.5 ± 0.5 Hz. To account for two standard deviations of variance, a cutoff frequency of 6 Hz was chosen.

Participant height and kinematic data from the static trial was used to create a subject-specific model to determine segment lengths and joint centers. Virtual markers were created for markers on bony landmarks that were offset 9 millimeters toward the bone to account for half of the 14-mm marker and the 2 mm base.[27] The ankle joint center was defined as the mid-point of the virtual medial and lateral malleoli. The knee joint center was defined as the mid-point of the virtual medial and lateral femoral epicondyles. A Visual 3D composite pelvis was built from virtual ASIS and PSIS landmarks. The hip joint center was defined using estimates described by Bell and colleagues.[28,29] Knee joint moments were using an inverse dynamics approach.[26] Vertical ground reaction force (vGRF) data from the 1-second static trial was used to measure body mass. The beginning and end of stance phase during gait was determined using a 10 N threshold of the GRF. Variables of interest were averaged across five trials in each limb and included the knee flexion angle at initial contact, peak knee flexion angle during loading response, peak knee flexion moment during stance phase, and peak knee adduction moment during the first 50% of stance phase. All joint moments are reported as external moments. Knee excursion during loading response was defined by the difference in knee flexion angle from initial contact to its peak during loading response. In addition, the impulse of the external knee flexion and adduction moments over the entire stance phase were calculated using the trapezoidal rule. External knee joint moments were normalized to mass

(kilograms) and height (meters). Positive joint angles represent knee flexion. Positive joint moments represent knee flexion and adduction, respectively.

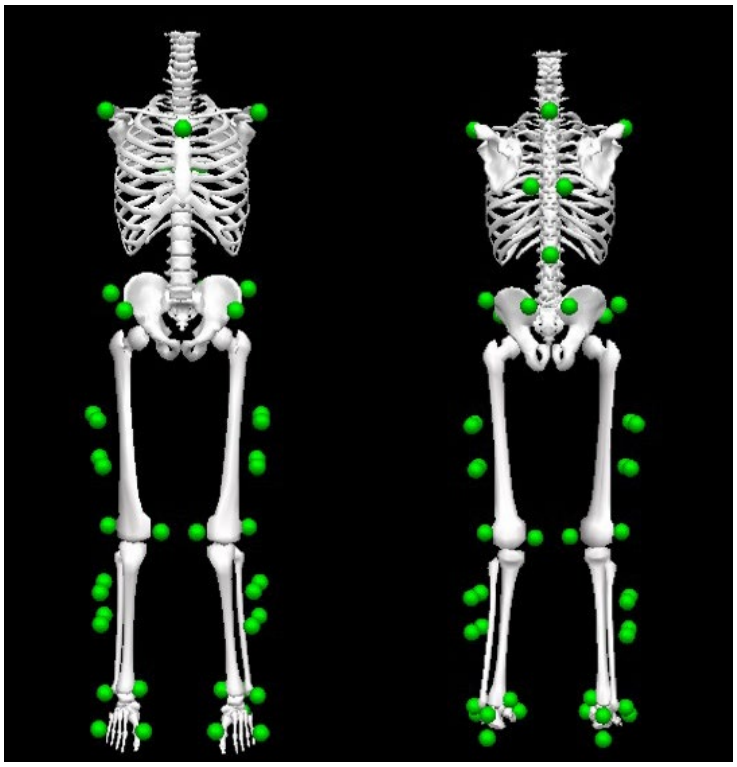

Figure 2.2: Biomechanics Marker Set. Individual markers (represented by green circles) were placed on bony landmarks of the trunk and lower extremities with rigid shells of markers placed at the thighs and shanks. Anterior view is on the left. Posterior view is on the right. Images made in Visual 3D software.

2.3.4 Physical Activity (PA)

PA was measured using an 3-axis Actigraph accelerometer (wGT3X-BT; Actigraph Corporation, Pensacola, FL) sampled at 100 Hz. This accelerometer is a reliable measure of step counts across varying gait speeds.[30] Participants were given an accelerometer on the day of MRI and biomechanics data acquisition and began wear the next day for seven consecutive days. Accelerometers were worn at the right iliac crest on an elastic belt in line with the axilla during all waking hours except when in water. A uniform handout was provided with instructions detailing appropriate wear and placement of accelerometer. Data were processed within Actilife 6 software (Actigraph Corporation, Pensacola, FL) using protocols established by the National Cancer Institute (NCI) for the National Health and Nutrition Examination Survey (NHANES).[31] Activity counts, which represent the weighted sum of the number of accelerations, were calculated for each 1-minute interval to identify wear periods and calculate PA levels. A valid week of data required four days with at least ten hours of wear to provide a reliable estimate of PA behavior.[31-33] Non-wear periods were defined as intervals of at least 90 minutes with activity counts equal to zero with no more than two minutes of activity counts between 1-99.[32] The variable of interest for this study was mean steps per day.

2.3.5 Statistical Analysis

Nominal data were described using counts and proportions. Continuous data were described using means, standard deviations, and 95% confidence intervals. Paired *t*-tests were used to determine if gait biomechanics and T2 relaxation time across tibiofemoral and patellofemoral cartilage regions of interests differed between the injured and uninjured knee.

Hierarchical multiple regression models were used to determine the association between knee joint loading predictors (daily step counts, knee flexion angle excursion, and knee adduction moment impulse) with the outcome of T2 relaxation time in each region of

interest. Interlimb differences (injured minus uninjured) in knee joint loading predictors and T2 relaxation time were used within all regression models. Daily step counts and knee flexion angle excursion were chosen as predictor variables because they represent global measures of joint loading that can be measured in clinical and real world settings. Interlimb differences in sagittal plane joint moments were not included because they demonstrate high collinearity with the interlimb difference in knee flexion angle excursion (peak knee flexion moment: $r = 0.751$; knee flexion moment impulse: $r = 0.675$). Knee adduction moment impulse was chosen because it represents the relative balance of joint loading in the frontal plane across the entire stance phase. Age, sex, and the presence of meniscus injury were entered as covariates in the first block, followed by the knee joint loading predictors in the second block. Concomitant meniscus injury was a covariate in this analysis because it increases the odds for development of knee OA compared to isolated ACL injury.[34] Medial meniscus injury was a covariate for medial tibiofemoral cartilage analyses. Lateral meniscus injury was a covariate for lateral tibiofemoral cartilage analyses. Injury to either or both menisci was a covariate for the patellar cartilage analysis. Independence of observations was tested using the Durbin-Watson statistic. Linearity of individual predictors was assessed using partial regression plots. Linearity of collective predictors and homoscedasticity of residuals was assessed using scatterplots of unstandardized predicted values versus studentized residuals. Multicollinearity was defined by a Pearson correlation value of <0.7 and a tolerance collinearity statistic of <0.1. Outliers were defined by a participant's standardized residual greater than ± 3 standard deviations. Normality of residuals were assessed using histograms and P-P plots. An *a-priori* p-value of 0.05 was considered statistically significant.

2.4 Results

Of the 23 participants included in this study, 18 were white (78.3%), 2 were Black or African American (8.7%), 2 were Hispanic, Latino, or Spanish (8.7%), and 1 was Asian (4.3%). Descriptive statistics for age, mass, height, and sex are presented in Table 2.1. All but one participant completed level 1 cutting and pivoting activities (e.g. soccer, basketball) prior to ACL injury.[35,36] Participants were enrolled and completed MRI and biomechanical testing at an average of 25 days after injury (Table 2.1). Approximately one-third of participants had a concomitant medial meniscus tear and nearly one-half had lateral meniscus injury (Table 2.1). Participants had an average of 5.7 ± 1.3 (95% confidence interval (CI): 5.1 to 6.2) valid days of accelerometer wear with an average of 875.5 ± 149.4 (95% CI: 810.9 to 940.2) daily minutes of wear. Participants completed an average of 6,337.6 ± 2375.9 (95% CI: 5310.2 to 7365.0) steps per day. Participants walked at an average 1.40 ± 0.21 (95% CI: 1.31 to 1.49) meters per second during biomechanics testing.

Table 2.1: ACL Cohort Participant Characteristics. Patient characteristics, concomitant meniscus injury, daily physical activity and gait speed during biomechanical analysis for all 23 participants. Mean (standard deviation) and 95% confidence intervals provided for continuous data. Counts (percentage) provided for categorical concomitant meniscus injury data. Abbreviations: SD, standard deviation; %, percentage; CI, confidence interval; yrs, years; kg, kilograms; m, meters.

Variable	Mean (SD) or Count (%)	95% CI
Age (yrs)	19.9 (5.2)	17.6 to 22.2
Mass (kg)	71.0 (9.8)	66.7 to 75.2
Height (m)	1.70 (0.09)	1.66 to 1.73
Sex (Female)	14 (60.9)	
Pre-Injury Activity Level (Level 1)[35,36]	22 (95.7)	
Time From ACL Injury (days)	25.2 (4.8)	23.1 to 27.2
Medial Meniscus Tear (Yes)	7 (30.4)	
Lateral Meniscus Tear (Yes)	11 (47.8)	
Medial Or Lateral Meniscus Tear (Yes)	13 (56.5)	

2.4.1 Gait Biomechanics and T2 Relaxation Time

Compared to the uninjured limb, the injured limb demonstrated an average of 3° more of peak knee flexion angle during loading response of gait (Table 2.2). However, this was accompanied by an average of 6° more knee flexion at initial contact resulting in smaller amounts of knee flexion excursion. A lower peak external knee adduction moment during the first 50% of stance phase was observed in the injured compared to the uninjured limb (Table 2.2). However, no interlimb differences were present for sagittal plane knee joint moments. There were no significant interlimb differences in T2 relaxation time within any articular cartilage regions of interest (Table 2.3).

Table 2.2: Gait Biomechanics in ACL Cohort. Knee flexion angle and sagittal and frontal plane joint moments during gait in each limb is presented for all 23 participants. Values in parentheses are standard deviations. The interlimb difference is presented with its 95% confidence interval. Boldface numbers indicate statistical significance (p-values <0.05).
Abbreviations: CI, confidence interval; p, p-value; KFA, knee flexion angle; IC, initial contact; º, degrees; KFM, knee flexion moment; KAM, knee adduction moment.

	Injured	Uninjured	Difference	95% CI	p
KFA at IC (º)	6.4 (5.0)	-0.1 (4.2)	6.5	4.7 to 8.4	**<0.001**
Peak KFA (º)	21.5 (5.3)	18.8 (6.7)	2.7	0.5 to 5.0	**0.021**
Knee Excursion (º)	15.1 (3.2)	18.9 (4.2)	-3.8	-5.6 to -2.1	**<0.001**
Peak KFM (N·m/kg·m)	0.38 (0.13)	0.41 (0.18)	-0.02	-0.09 to 0.04	0.486
KFM Impulse (N·m·s/kg·m)	0.059 (0.018)	0.058 (0.018)	0.001	-0.008 to 0.010	0.808
Peak KAM (N·m/kg·m)	0.22 (0.09)	0.28 (0.10)	-0.06	-0.11 to -0.01	**0.014**
KAM Impulse (N·m·s/kg·m)	0.069 (0.038)	0.093 (0.036)	-0.023	-0.047 to 0.000	0.051

Table 2.3: T2 Relaxation Time in ACL Cohort. Mean T2 relaxation time (milliseconds) in the cartilage of each region of interest is presented for all 23 participants. Values in parentheses are standard deviations. The interlimb difference is presented with its 95% confidence interval. Abbreviations: CI, confidence interval; p, p-value; LFC, lateral femoral condyle; LTC, lateral tibial condyle; MFC, medial femoral condyle; MTC, medial tibial condyle.

	Injured	Uninjured	Difference	95% CI	p
LFC – Anterior	47.3 (2.6)	47.4 (2.5)	0.1	-1.4 to 1.3	0.948
LFC – Weightbearing	47.0 (3.4)	47.7 (4.4)	-0.7	-2.5 to 1.2	0.462
LFC – Posterior	42.9 (4.7)	43.1 (5.4)	-0.2	-2.7 to 2.4	0.900
LTC – Weightbearing	41.6 (3.4)	42.6 (4.9)	-1.0	-3.6 to 1.4	0.382
MFC – Anterior	46.7 (3.6)	46.7 (3.7)	0.0	-2.1 to 2.1	0.989
MFC – Weightbearing	46.9 (4.2)	47.5 (3.6)	-0.6	-2.9 to 1.7	0.586
MFC – Posterior	42.5 (6.0)	41.0 (4.7)	1.5	-2.0 to 4.9	0.385
MTC – Weightbearing	42.8 (4.4)	42.8 (3.8)	0.0	-2.8 to 2.9	0.978
Patella	39.2 (2.5)	39.7 (3.3)	-0.5	-1.6 to 0.6	0.367

2.4.2 Lateral Tibiofemoral Cartilage

Daily step counts and interlimb differences in knee flexion angle excursion and knee adduction moment impulse accounted for an additional 46.0%, 58.2%, and 71.5% of the variability in interlimb differences in T2 relaxation time in the weightbearing femoral, posterior femoral, and weightbearing tibial cartilage of the lateral compartment, respectively, after controlling for age, sex, and concomitant lateral meniscus injury (Table 2.4). In the weightbearing cartilage of the lateral femoral condyle, knee adduction moment impulse was the only significant factor of T2 relaxation time (β: 0.863, p: 0.001). Knee adduction moment impulse also most strongly associated with T2 relaxation time in the posterior cartilage of the lateral femoral condyle (β: 0.980, $p<0.001$) and the weightbearing cartilage of the tibia (β: 1.031, $p<0.001$). The association of interlimb differences between the knee adduction moment impulse and T2 relaxation time in the lateral compartment was always positive, such that asymmetrically lower knee adduction moment impulse in the injured knee was associated with asymmetrically shorter T2 relaxation time in the injured knee, while asymmetrically greater knee adduction moment impulse in the injured knee was associated with asymmetrically longer T2 relaxation time in the injured knee. In the posterior cartilage of the lateral femoral condyle, greater steps per day (β: 0.699, p: 0.004), less knee flexion excursion (β: -0.692, p: 0.003), female sex (β: -0.461, p: 0.008) and younger age (β: -0.375, p: 0.018) were associated with prolonged T2 relaxation in the injured knee. In the weightbearing cartilage of the lateral tibial condyle, less knee flexion excursion (β: -0.426, p: 0.012), greater steps per day (β: 0.365, p: 0.037) and younger age (β: -0.300, p: 0.015) associated with prolonged T2 relaxation in the injured knee.

Table 2.4: Linear Regression Models in Lateral Compartment. Results of linear regression models with daily physical activity (step counts), interlimb difference in knee flexion angle excursion, and interlimb difference in knee adduction moment impulse as independent variables and interlimb differences in T2 relaxation times in the lateral tibiofemoral regions of interest as the outcome of interest, after adjusting for age, sex, and concomitant meniscus injury. P-values represent statistical significance of the R square change and Beta coefficient, respectively. Boldface numbers represent statistically significant predictor variables in regression models that had statistically significant changes in R square. Abbreviations: p, p-value; LFC, lateral femoral condyle; LTC, lateral tibial condyle; MFC, medial femoral condyle; MTC, medial tibial condyle; PA, physical activity; KFA, knee flexion angle; KAM, knee adduction moment.

Cartilage Region	R Square	R Square Change	p	Factor	Unstandard-ized B	β	p
LFC – Anterior	0.305	0.257	0.159				
				Age	0.040	0.067	0.765
				Sex	-0.910	-0.145	0.548
				Meniscus Injury	-1.784	-0.292	0.197
				Daily PA	0.000	0.250	0.449
				KFA Excursion	-0.176	-0.226	0.465
				KAM Impulse	36.145	0.624	0.034
LFC – WB	0.581	**0.460**	**0.007**				
				Age	-0.156	-0.193	0.277
				Sex	-2.737	-0.321	0.099
				Meniscus Injury	0.960	0.115	0.502
				Daily PA	0.001	0.469	0.079
				KFA Excursion	-0.413	-0.390	0.117
				KAM Impulse	67.990	**0.863**	**0.001**
LFC – Posterior	0.710	**0.582**	**<0.001**				
				Age	-0.418	**-0.375**	**0.018**
				Sex	-5.394	**-0.461**	**0.008**
				Meniscus Injury	-1.517	-0.133	0.357
				Daily PA	0.002	**0.699**	**0.004**
				KFA Excursion	-1.009	**-0.692**	**0.003**
				KAM Impulse	106.138	**0.980**	**<0.001**

LTC – WB	Knee	**0.715**	**<0.001**				
				Age	-0.330	**-0.300**	**0.015**
				Sex	-1.593	-0.138	0.262
				Meniscus Injury	-0.079	-0.007	0.950
				Daily PA	0.001	**0.365**	**0.037**
				KFA Excursion	-0.614	**-0.426**	**0.012**
				KAM Impulse	110.349	**1.031**	**<0.001**

2.4.3 Medial Tibiofemoral Cartilage

Daily step counts and interlimb differences in knee flexion angle excursion and knee adduction moment impulse accounted for an additional 55.8%, 45.9%, and 61.3% of the variability in interlimb differences in T2 relaxation time in the weightbearing femoral, posterior femoral, and weightbearing tibial cartilage of the medial compartment, respectively, after controlling for age, sex, and concomitant medial meniscus injury (Table 2.5). Knee adduction moment impulse was the strongest factor of T2 relaxation time in the weightbearing cartilage of the medial femoral condyle (β: -0.819, $p<0.001$) and medial tibial condyle, (β: -0.841, $p<0.001$). Unlike the lateral compartment, older age associated with prolonged T2 relaxation time in these two regions of the medial compartment (weightbearing medial femoral condyle: β: 0.351, p: 0.024; weightbearing medial tibial condyle: β: 0.301, p: 0.042). Knee adduction moment impulse was the only significant factor of T2 relaxation time in the posterior cartilage of the medial femoral condyle (β: -0.659, p: 0.006). The association of interlimb differences between the knee adduction moment impulse and T2 relaxation time in the medial compartment was always negative (opposite of the lateral compartment), such that asymmetrically lower knee adduction moment impulse in the injured knee was associated with asymmetrically longer T2 relaxation time in the injured knee, while asymmetrically greater knee adduction moment impulse in the injured knee was associated with asymmetrically shorter T2 relaxation time in the injured knee. The opposite relationships between the knee adduction moment impulse and T2 relaxation time in the medial compared to lateral compartment is illustrated in Figure 2.3.

Table 2.5: Linear Regression Models in Medial Compartment. Results of linear regression models with daily physical activity (step counts), interlimb difference in knee flexion angle excursion, and interlimb difference in knee adduction moment impulse as independent variables and interlimb differences in T2 relaxation times in the medial tibiofemoral regions of interest as the outcome of interest, after adjusting for age, sex, and concomitant meniscus injury. P-values represent statistical significance of the R square change and Beta coefficient, respectively. Boldface numbers represent statistically significant predictor variables in regression models that had statistically significant changes in R square. Abbreviations: p, p-value; LFC, lateral femoral condyle; LTC, lateral tibial condyle; MFC, medial femoral condyle; MTC, medial tibial condyle; PA, physical activity; KFA, knee flexion angle; KAM, knee adduction moment.

Cartilage Region	R Square	R Square Change	p	Factor	Unstandard-ized B	β	p
MFC – Anterior	0.474	0.320	0.050				
				Age	0.105	0.112	0.569
				Sex	-4.688	-0.477	0.036
				Meniscus Injury	0.486	0.047	0.814
				Daily PA	0.001	0.395	0.179
				KFA Excursion	-0.277	-0.227	0.418
				KAM Impulse	-29.882	-0.329	0.169
MFC – WB	0.719	**0.558**	**<0.001**				
				Age	0.348	**0.351**	**0.024**
				Sex	-1.810	-0.173	0.273
				Meniscus Injury	0.696	0.063	0.665
				Daily PA	0.000	-0.122	0.560
				KFA Excursion	0.228	0.176	0.391
				KAM Impulse	-79.030	**-0.819**	**<0.001**
MFC – Posterior	0.574	**0.459**	**0.007**				
				Age	0.380	0.250	0.169
				Sex	-3.292	-0.206	0.290
				Meniscus Injury	0.661	0.039	0.827
				Daily PA	0.000	0.073	0.778
				KFA Excursion	0.107	0.054	0.829
				KAM Impulse	-97.348	**-0.659**	**0.006**

MTC – WB	0.738	**0.613**	**<0.001**				
				Age	0.375	**0.301**	**0.042**
				Sex	-2.141	-0.163	0.284
				Meniscus Injury	1.004	0.072	0.607
				Daily PA	0.000	-0.094	0.643
				KFA Excursion	0.213	0.131	0.506
				KAM Impulse	-101.890	**-0.841**	**<0.001**

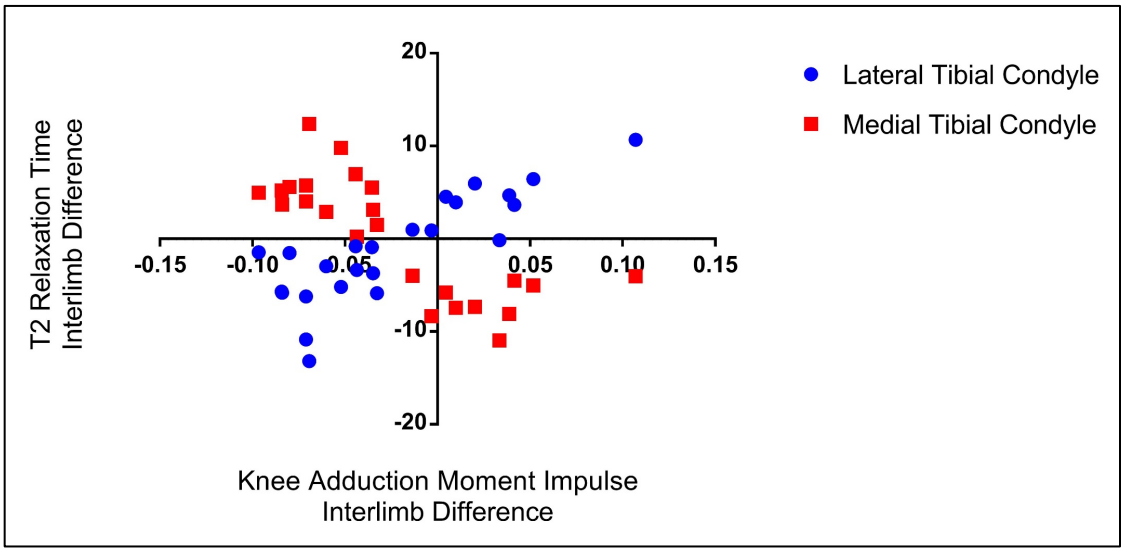

Figure 2.3: Knee Adduction Moment Impulse And T2 Relaxation Time In Lateral Compared To Medial Comparment. Interlimb differences in knee adduction moment impulse (N·m·s/kg·m) were positively associated with interlimb differences T2 relaxation time (milliseconds) in the lateral femoral and tibial cartilage but negatively associated with T2 interlimb differences in the medial femoral and tibial cartilage. Data for the lateral and medial tibial condyles are presented here. Lower knee adduction moment impulse in the injured limb (i.e. lesser medial loading vs. greater lateral loading) was associated with prolonged (i.e. worse) T2 relaxation time in the medial tibial condyle and shorter T2 relaxation time in the lateral tibial condyle. Greater knee adduction moment impulse in the injured limb (i.e. greater medial loading vs. lesser lateral loading) was associated with shorter T2 relaxation time in the medial tibial condyle and prolonged (i.e. worse) T2 relaxation time in the lateral tibial condyle.

2.4.4 Patellar Cartilage

Knee joint loading factors did not associate with T2 relaxation time in patellar cartilage. Daily step counts, knee flexion angle excursion, and knee flexion and adduction moment impulses were not associated with T2 relaxation time in in the patellar cartilage after controlling for age, sex, and medial or lateral meniscus injury (R square: 0.261, R square change: 0.063, p: 0.716). Patellar cartilage findings were consistent with the absence of association with knee joint loading factors in the anterior lateral and medial femoral cartilage (Tables 4 and 5).

2.5 Discussion

The purpose of this study was to determine if measures of knee joint loading (i.e. PA levels, knee joint biomechanics during gait) are associated with T2 relaxation time in the articular cartilage of the knee within 1 month of ACL injury. Our findings partially support our hypothesis that lower measures of knee joint loading would be associated with prolonged T2 relaxation time in the weightbearing regions of the femoral and tibial cartilage in the injured knee. Lower knee adduction moment impulse in the injured knee associated with prolonged T2 relaxation time in the medial femoral and tibial cartilage but shorter T2 relaxation time in the lateral femoral and tibial cartilage. Lower amounts of knee flexion angle excursion from initial contact through loading response in the injured knee associated with prolonged T2 relaxation time in the lateral posterior femoral and weightbearing tibial cartilage. Our PA findings did not support our hypothesis; higher daily step counts associated with prolonged T2 relaxation time in the injured posterior femoral and weightbearing tibial cartilage. Our secondary aim was to determine if interlimb differences in gait biomechanics and cartilage T2 relaxation time are present within 1 month of ACL injury. Participants

walked with greater knee flexion angles at initial contact, less knee flexion angle excursion during loading response, and lower external knee adduction moment in the injured compared to uninjured knee. There were no differences in T2 relaxation time within any articular cartilage regions of interest in the femur, tibia, or patella.

The findings of this study demonstrate the acute interaction between knee joint loading patterns and the structural response of articular cartilage after ACL injury. Participants in this study walked with a 21% lower peak knee adduction moment during loading response (p: 0.014) and a 26% lower knee adduction moment impulse over stance phase (p: 0.051) compared to the uninjured knee within 1 month of ACL injury. The knee adduction moment is a mechanical estimate of the relative joint loading between the medial compared to lateral compartment of the knee and has been widely studied within knee OA populations.[37] A lower knee adduction moment indicates a relatively lower amount of loading in the medial compartment and relatively higher amount of loading in the lateral compartment. Within this study, the knee adduction moment impulse demonstrated a differential relationship with T2 relaxation time in the medial compared to lateral weightbearing and posterior regions of both femoral and tibial cartilage (Figure 2.3). Participants who walked with lower knee adduction moment impulse (relatively lower medial loading, greater lateral loading) demonstrated T2 relaxation time that was prolonged in the medial compartment but shorter in the lateral compartment when compared to the contralateral uninjured limb. The relationship was opposite in those that walked with higher knee adduction moment impulse (relatively greater medial loading, lower lateral loading). In these participants, T2 relaxation time was prolonged in the lateral compartment and shorter in the medial compartment compared to the contralateral uninjured knee. Prolonged T2 relaxation time indicates increased water content and disorganization of the collage matrix and is a sensitive marker for symptomatic and

structural progression of knee OA.[38-42] However, changes in quantitative MRI markers of articular cartilage, including T2 relaxation time, may also reflect temporary changes in response to initial ACL injury and subsequent healing as well as altered movement mechanics.[43-45] development. Our cohort taken together did not show significantly different T2 relaxation time within any region of interest across the femoral, tibial and patellar cartilage (Table 2.3). The prolonged T2 relaxation time in some, but not all, individuals in this study may have been influenced from variations in severity of injury. For example, bone marrow edema patterns that are common after ACL injury are associated with increased cartilage loss up to three years later.[46] Other evidence suggests that quantitative MRI markers such as ultrashort echo time (UTE)-T2* mapping may be more sensitive to the health status of deep cartilage correlated to injury severity.[44] This work developed by Chu and colleagues demonstrated increases of UTE-T2* time by 45% in the deep cartilage of the medial femoral condyle compared to the uninjured knee before ACL reconstruction that resolved by 2 years after ACL reconstruction in patients with intact menisci. Longitudinal study of acute changes in T2 relaxation time is needed to determine if alterations are transient or predictive of future cartilage degeneration and if rehabilitative strategies to change knee joint loading can modify these pathways.

Our findings that knee adduction moment impulse associates with prolonged T2 relaxation time in the femoral and tibial cartilage are consistent with some previous studies correlating joint unloading to negative qMRI markers early after ACL injury and reconstruction. Lower ground reaction force and knee adduction moment during walking in the ACL-injured limb has been associated with greater T1ρ relaxation time in ACL-injured femoral and tibial cartilage compared to the uninjured knee as well as deleterious increases in biochemical markers of cartilage breakdown at 6 months after ACL reconstruction.[18,47] Lower

knee adduction moment and medial compartment contact forces before and 6 months after ACL reconstruction are also associated with radiographic knee OA 5 years later.[48] However, other studies have reported that higher or increasing parameters of knee joint loading are associated with worsening markers of cartilage health. Kumar et al. reported that increases in knee adduction moment from before to 6 months after ACL reconstruction were associated with increases in medial T1ρ and T2 relaxation time over the same period.[13] Teng et al. reported that a higher knee flexion moment and knee flexion angle before ACL reconstruction were associated with increased medial T1ρ and T2 relaxation time at 6 months, and that these biomechanical parameters at 6 months were related to increased medial T1ρ and T2 relaxation time at 1 and 2 years.[14] Titchenal et al. reported that greater peak knee adduction moments correlated with higher UTE-T2* values in the medial femoral and tibial cartilage.[17] Three factors may explain discrepancies between our findings and previous observations of deleterious qMRI markers of cartilage health with both lower joint loading (i.e. underloading) and elevated joint loading (i.e. overloading). First, in studies where increases in joint loading are a correlate of worsening qMRI markers, it is unknown whether the initial underloading, the progressive increase in loading, or both factors influence the qMRI change. Second, knee joint angles and moment change over time after ACL injury. A knee stiffening and unloading strategy early after ACL injury and reconstruction progresses to more symmetrical gait patterns 1 to 2 years later.[49,50] In middle-aged adults with knee OA, greater knee adduction and flexion moments are related to cartilage thinning in the medial compartment five years later.[51] It is possible that both lower and higher magnitudes of knee joint loading are harmful to articular cartilage depending on circumstances such as time from injury. Third, comparisons of only the injured limb without normalization to the contralateral limb may negative individual variation in both walking patterns and cartilage markers. For example, an increased

knee flexion angle may initially appear to represent a more normal gait pattern, but if it coincides with limited knee flexion excursion as demonstrated in the current study it may instead indicate an abnormal loading strategy. Comparison of injured limb biomechanics to the contralateral limb after ACL injury provides greater context of the loading environment than isolated injured limb analyses.

Participants in this study walked with 3° greater peak knee flexion angle and symmetrical peak knee flexion moments in the ACL-injured knee compared to the uninjured knee, but with 6° less of knee flexion excursion during loading response. The association between less knee joint excursion and prolonged T2 relaxation time in the cartilage of the posterior lateral femoral condyle and weightbearing region of the lateral tibial condyle supports previous evidence suggesting that changes in cartilage contact points not accustomed to these changing loads is a factor in the initiation of PTOA.[52-55] The greater peak knee flexion angle and symmetric knee flexion moment in the current cohort within 1 month of injury represents a stiffened knee gait pattern that results from beginning and progressing through stance phase with a more flexed knee compared to the uninjured limb. Although knee flexion angle and joint moments are commonly reduced during the first post-operative year and are used as a recovery target of normal gait patterns,[49,50] initial elevations in sagittal plane biomechanics acutely after ACL injury may not be optimal and instead represent a strategy to minimize normal joint loading within some regions of the knee.

Knee adduction moment and knee flexion angle excursion were not related to T2 relaxation time alterations in the anterior femoral cartilage of the trochlea or the patellar cartilage. These findings are consistent with work from Capin and colleagues reporting weak to absent associations with walking mechanics and T2 relaxation differences in the trochlear cartilage 6 months after ACL reconstruction.[15] Instead, slower walking speed was a stronger

correlate of prolonged T2 relaxation time in the trochlea. However, Culvenor et al. reported that individuals with MRI-defined patellofemoral cartilage lesions 1 to 2 years after ACL reconstruction hopped with smaller knee flexion angles and moments that those without patellofemoral OA[56]. Indeed, the odds for developing patellofemoral OA are similar to that of tibiofemoral OA after ACL injury, with 50% showing symptomatic and radiographic OA signs by 10 years.[57] Sagittal plane joint angles and moments may not be sensitive to the loading environment of the patellofemoral joint early after ACL injury. Instead, models of patellofemoral joint contact stress that incorporate joint moments, estimated muscle forces, and patellofemoral joint contact area may be required to sufficiently characterize patellofemoral joint loading after ACL injury within the context of PTOA development.

Participants walked an average of 6,337.6 ± 2375.9 steps per day (range: 2377 – 11,960 daily steps) at 1 month after ACL injury. To our knowledge, this is the first study to objectively measure PA levels prior to ACL reconstruction. A higher number of steps per day was associated with prolonged T2 relaxation time in the cartilage of the posterior lateral femoral condyle and weightbearing region of the lateral tibial condyle. Although there is mixed evidence about the role of PA and OA development, data from the Osteoarthritis Initiative (OAI) suggests that participation by middle- and older-aged adults in moderate and vigorous PA does not alter risk for development or progression of symptomatic or radiographic knee OA compared to lower PA levels.[58,59] However, extreme levels of low PA (i.e. non-weightbearing activity) results in cartilage thinning and atrophy.[11,60] Although the optimal level of PA after ACL injury to cyclically load the articular cartilage and maintain structural health is unknown, it likely resides somewhere between extremely low and high PA levels. The findings of this study suggest that common PA targets for healthy adolescents and adults (e.g. 10,000 steps per day) may be detrimental to cartilage within the first month of

ACL injury. This observation may be explained by a greater number of daily steps exacerbating underlying aberrant walking patterns (e.g. altered knee adduction moment) that magnify their contribution to early cartilage breakdown.

The cross-sectional design of the current study prohibits causal conclusions between knee joint loading patterns and altered cartilage T2 relaxation time. Therefore, we are unable to determine: a) if knee joint unloading patterns led to prolonged T2 relaxation time, b) if the severity of ACL injury influenced increases T2 relaxation time that resulted in altered walking patterns or c) if changes in knee joint loading patterns and T2 relaxation time occurred independently of each other. However, the strong and consistent relationships between knee flexion excursion, knee adduction moment, and daily PA levels provides further support that walking patterns that are amenable to change play an important role in PTOA pathogenesis. Longitudinal study of articular cartilage and walking patterns is needed to determine if the loading patterns demonstrated in this study are detrimental or protective mechanisms of long-term cartilage health.

Additional limitations exist in this study. Accelerometry-based PA levels were measured for the one-week period following MRI data acquisition and may differ from PA behaviors before MRI. Lower extremity alignment was not measured in this study but is associated with compositional articular cartilage changes after ACL reconstruction.[17] Slower walking speed is associated with quantitative MRI markers of articular cartilage 6 to 12 months after ACL reconstruction.[15,16] Walking speed was not adjusted for within the current regression models, but its influence was likely mitigated by controlling walking speed for each participant within 5% across limbs and by normalizing gait biomechanics between limbs. The age of the current cohort was relatively young (19.9 ± 5.2 years). Although the incidence of ACL injury is highest among adolescents and young adults,[2,3] our findings may differ in older

populations and limit comparison to other studies that have investigated walking patterns and quantitative MRI markers of cartilage health in populations with older and more variable age.[13-15,19,44] In this study, both age and sex were significant covariates of T2 relaxation time in the cartilage of the posterior lateral femoral condyle and bilateral tibial condyles indicating biological and non-modifiable factors likely also influence PTOA development after ACL injury.

2.6 Conclusion

Altered knee joint adduction moment, less knee flexion excursion, and greater daily step counts are associated with prolonged T2 relaxation time within 1 month of ACL injury. Gait biomechanics and daily PA may be modifiable targets to alter cartilage degeneration pathways acutely after ACL injury.

2.7 Acknowledgements

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Appendix A

INSTITUTIONAL REVIEW BOARD APPROVAL – HEALTHY COHORT

University of Nebraska Medical Center

NEBRASKA'S HEALTH SCIENCE CENTER

Office of Regulatory Affairs (ORA)
Institutional Review Board (IRB)

May 18, 2017

Elizabeth Wellsandt, DPT, PhD
Physical Therapy Education
UNMC-4420

IRB # 213-17-EP

TITLE OF PROPOSAL: Role of Cumulative Knee Joint Loading in Healthy Articular Cartilage

DATE OF EXPEDITED REVIEW: *04/12/2017*

DATE OF FINAL APPROVAL AND RELEASE: 05/18/2017 **VALID UNTIL:** 04/12/2018

CLASSIFICATION OF RISK: Minimal

SUBPART D CATEGORY OF REVIEW: *404*

EXPEDITED CATEGORY OF REVIEW: 45 CFR 46.110; 21 CFR 56.110, Category *4 and 7*

The IRB has completed its review of the above-titled protocol. The IRB has determined you are in compliance with HHS Regulations (45 CFR 46), applicable FDA Regulations (21 CFR 50, 56) and the Organization's HRPP policies. Furthermore, the IRB is satisfied you have provided adequate safeguards for protecting the rights and welfare of the subjects to be involved in this study. This letter constitutes official notification of final approval and release of your project by the IRB. You are authorized to implement this study as of the above date of final approval.

Please be advised that only the IRB approved and stamped consent form(s)/information sheet(s) can be used to make copies to enroll subjects. Also, at the time of consent all subjects/legally authorized representatives (LARs)/parent(s) must be given a copy of *The Rights of Research Subjects* and "What Do I Need to Know" forms.

The IRB wishes to remind you that the PI is ultimately responsible for ensuring that this research is conducted in full compliance with the protocol, applicable Federal Regulations, and Organizational policies.

Finally, under the provisions of this institution's Federal Wide Assurance (FWA00002939), the PI is directly responsible for submitting to the IRB any proposed change in the research or the consent form(s)/information sheet(s). In addition, any adverse events, unanticipated problems involving risk to the subject or others, noncompliance, and complaints must be promptly reported to the IRB in accordance with HRPP policies.

This project is subject to periodic review and surveillance by the IRB and, as part of the Board's surveillance, the IRB may request periodic progress reports. For projects which continue beyond one year, it is the responsibility of the PI to initiate a request to the IRB for continuing review and update of the research project.

On behalf of the IRB,

Signed on: 2017-05-18 15:48:00.000

Sue Logsdon, MS, CIP
IRB/IBC/SROC Administrator III
Office of Regulatory Affairs

Academic and Research Services Building 3000 / 987830 Nebraska Medical Center / Omaha, NE 68198-7830
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Appendix B

HUMAN SUBJECTS INFORMED CONSENT – HEALTHY COHORT

University of Nebraska Medical Center

IRB PROTOCOL # 213-17-EP **Page 1 of 8**

ADULT CONSENT - CLINICAL BIOMEDICAL

Title of this Research Study
Role of Cumulative Knee Joint Loading in Healthy Articular Cartilage

Invitation
You are invited to take part in this research study. You have a copy of the following, which is meant to help you decide whether or not to take part:

- Informed consent form
- "What Do I need to Know Before Being in a Research Study?"
- The Rights of Research Subjects

Why are you being asked to be in this research study?
You are being asked to be in this study because you are between the ages of 19-30 and have not had any previous leg injuries or surgeries. If you are pregnant, you may not be in this study.

What is the reason for doing this research study?
Knee osteoarthritis is a breakdown of the cartilage that leads to pain and decreased function. Abnormal patterns of movement and physical activity levels have been linked to the development of knee osteoarthritis. This research is trying to see how movement patterns and physical activity levels affect the knee's cartilage in individuals who do not have knee osteoarthritis.

What will be done during this research study?
Your involvement in this research study will consist of the following procedures completed over 8 days:

Magnetic Resonance Imaging (MRI) (Day 1):
Magnetic resonance imaging (MRI) will take place in the Center for Brain, Biology and Behavior MRI Facility at the University of Nebraska-Lincoln. This study uses structural MRI of the knee. Structure MRI shows the structure of the knee. The MRI scanning session will take 1 hour to complete once you are in the scanner.

You will be encouraged to hold as still as possible and communicate any discomfort to the investigators any time before or during a scan. You will have a squeeze bulb that will help to communicate with us while you are in the machine. You will also wear ear protection to minimize the loud noises made by the machine.

The MRI scan is being done in a research facility, not in a hospital setting, and therefore is not to be used for diagnostic purposes. The MRI scan will be coded with a unique identifying number assigned to you and sent to the Radiology Center at Nebraska Medicine to be interpreted. If the radiologist reviewing the MRI detects anything that appears abnormal, the results will be returned to you along with a digital copy of the MRI, and you will be encouraged to follow-up with your primary doctor for confirmation testing. If the MRI appears normal you will not be provided with the results.

Movement Analysis Testing (Day 1):
Movement analysis testing will be completed in the Nebraska Athletic Performance Laboratory at the University of Nebraska-Lincoln. Markers will be affixed to your skin and sneakers on both legs and your back using adhesive skin tape. Shells with markers on them will be placed on your back, pelvis, thighs and calves and will be held in place with elastic wraps. These markers will allow the motion cameras to track the position of your body.

You will be asked to perform several walking and squatting trials in our laboratory. These trials will give us information about the way your back, hips, knee and ankles move while you move. You will be asked to perform 8 trials of walking and squatting at comfortable, self-selected speeds, although additional trials may be required to obtain enough data. While you are walking, a computer will record the 3 dimensional motions of your back, hips, knees, and ankles. The entire movement analysis session will last approximately 30 minutes.

Strength Testing (Day 1):
Strength testing will be completed in the Nebraska Athletic Performance Laboratory at the University of Nebraska-Lincoln. The test will measure the strength of the quadriceps muscle on the front of your thigh. You will be seated in a dynamometer, a device that resists your kicking motion, and measures how much force you muscle can exert. Testing will require a series of practice and recorded kicking motions. Trials will be repeated (up to a maximum of 4) on each leg. Strength testing will last approximately 15 minutes.

Questionnaires (Day 1):
You will be asked to complete a test packet which includes questions about your past and current functional status and your perceived functional capabilities. Questionnaires will last approximately 15 minutes.

Physical Activity Monitoring (Days 2-8):

Physical activity monitoring will be completed by you wearing a physical activity monitor (accelerometer) worn at your hip that records your movement throughout the day. This research device is similar to products that can be worn on your wrist or apps on your cell phone that measure the number of steps you take each day. You will be asked to wear the device during all waking hours for 7 consecutive days beginning the day after all other testing in this study. You will need to remove the physical activity monitor during bathing, swimming or other activities that expose the device to water to avoid damaging the physical activity monitor. You will be asked to return the physical activity monitor to the principal investigator in a pre-postaged, pre-addressed envelope provided to you.

What are the possible risks of being in this research study?
There is a potential loss of your confidentiality.

Magnetic Resonance Imaging (MRI):
Cautions: Tattoos could cause warming, redness or burns around the tattooed body part. You will be informed to not make skin contact to the sides of the tunnel when in the MRI machine. In other words, you should stay as still as possible in the machine, and not touch any other part of the machine. If your skin makes direct contact to the sides of the machine, it could potentially cause mild to severe burns. Certain medications could also enhance side effects such as dizziness, light-headedness, or nausea.

At this time, as a policy of the CB3 MRI Facility, women who are pregnant or trying to become pregnant are excluded from participation in research projects involving a MRI scan. Although there are no known negative effects on pregnant women or fetuses, very little is known of the possibility of any negative effects. This study may involve risks that we cannot predict.

Likely risks when following all security measures: None.

Less likely risks: During the MRI scan, potential discomforts may include: feeling cold, feeling warm, anxiety, body discomfort/stiffness, or a metallic taste. Lying still for a prolonged time may prove uncomfortable. Some individuals may have a claustrophobic response, which is a fear of confined spaces, and some may experience stiffness from lying still. The MRI machine makes loud banging noises while taking measurements, so ear protection will be used to reduce the noise.

You will be in communication with the MRI technologist throughout the MRI scan. If you experience any of these or other discomforts, you will be instructed to notify the

MRI technologist immediately. You will be given a squeeze bulb to contact the MRI technologist, which may be used at any time before or during scanning.

Rare risks: An additional risk, though highly unlikely, is the possibility that metal objects could be pulled into the magnetic center of the MRI machine and hit you. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. It is important to know that no metal can be brought into the MRI Room at any time. Once you are in the MRI machine, the door to the MRI Room will be closed so that no one from the outside accidentally goes near the MRI machine.

Movement Analysis Testing:
You may experience a loss of balance during testing; however, your other leg is free to touch down to provide support and prevent loss of balance. You may experience discomfort from the removal of tape holding markers in place.

Strength Testing:
The strength testing can be associated with local muscle soreness and fatigue. Following the testing, your muscles may feel as if you have exercised vigorously.

Questionnaires (Day 1):
No known risks exist.

Physical Activity Monitoring (Days 2-8):
No known risks exist.

It is possible that other rare side effects could occur which are not described in this consent form. It is also possible that you could have a side effect that has not occurred before.

What are the possible benefits to you?
You are not expected not get any benefit from being in this research study.

What are the possible benefits to other people?
The results of this study may help us understand how human movement affects the knee's cartilage in healthy individuals.

What are the alternatives to being in this research study?
Instead of being in this research study, you can choose not to participate.

What will being in this research study cost you?
There is no cost to you to be in this research study.

Will you be paid for being in this research study?
You will be paid a Visa gift card with a maximum value of $50. Compensation will be provided at a rate of $12.50 per hour. Testing is expected to require 2 hours and will be compensated at a maximum of $25.00. If you are traveling from outside of Lincoln, travel will be compensated at a maximum of $25.00. Payments will be prorated on an hourly basis, accruing as the study progresses and not being contingent upon you completing the entire study. Prorated payment will be made regardless of whether withdrawal was voluntary (you decided to withdraw from the study) or involuntary (based on withdrawal criteria of the research protocol).

Who is paying for this research?
This research is being paid for by grant funds from the Center for Brain, Behavior and Biology at the University of Nebraska-Lincoln. The University of Nebraska Medical Center receives money from the Center for Brain, Behavior and Biology to conduct this study.

What should you do if you are injured or have a medical problem during this research study?
Your welfare is the main concern of every member of the research team. If you are injured or have a medical problem or some other kind of problem as a direct result of being in this study, you should immediately contact one of the people listed at the end of this consent form.

How will information about you be protected?
You have rights regarding the protection and privacy of your medical information collected before and during this research. This medical information is called "protected health information" (PHI). PHI used in this study may include your medical record number, address, birth date, medical history, the results of physical exams, blood tests, x-rays as well as the results of other diagnostic medical or research procedures. Only the minimum amount of PHI will be collected for this research. Your research and medical records will be maintained in a secure manner.

Who will have access to information about you?
By signing this consent form, you are allowing the research team to have access to your PHI. The research team includes the investigators listed on this consent form and other personnel involved in this specific study at UNMC and the Nebraska Medical Center.

Your PHI will be used only for the purpose(s) described in the section "What is the reason for doing this research study?"

Your PHI will be shared, as necessary, with the Institutional Review Board (IRB) and with any person or agency required by law. You are also allowing the research team to share your PHI with other people or groups listed below. All of these persons or groups listed below are obligated to protect your PHI.

1. Researchers at the Center for Brain, Biology and Behavior at the University of Nebraska-Lincoln involved in this study.
2. Researchers at the Nebraska Athletic Performance Laboratory at the University of Nebraska-Lincoln involved in this study.

Your PHI may also be shared with the Center for Brain, Biology, and Behavior at the University of Nebraska-Lincoln which sponsors this research and provides funds to UNMC to conduct this research.

You are authorizing us to use and disclose your PHI for as long as the research study is being conducted.

You may cancel your authorization for further collection of PHI for use in this research at any time by contacting the principal investigator in writing. However, the PHI which is included in the research data obtained to date may still be used. If you cancel this authorization, you will no longer be able to participate in this research.

How will results of the research be made available to you during and after the study is finished?

In most cases, the results of the research can be made available to you when the study is completed, and all the results are analyzed by the investigator or the sponsor of the research. The information from this study may be published in scientific journals or presented at scientific meetings, but your identity will be kept strictly confidential.

If you want the results of the study, contact the Principal Investigator at the phone number given at the end of this form or by writing to the Principal Investigator at the following address:
Division of Physical Therapy Education
984420 Nebraska Medical Center
Omaha, NE 68198-4420

What will happen if you decide not to be in this research study?

You can decide not to be in this research study. Deciding not to be in this research study will not affect your relationship with the investigator, the University of Nebraska Medical Center or the Nebraska Medical Center. You will not lose any benefits to which you are entitled.

What will happen if you decide to stop participating once you start?
You can stop participating in this research (withdraw) at any time by contacting the Principal Investigator or any of the research staff. Deciding to withdraw will otherwise not affect your relationship with the investigator or this institution. You will not lose any benefits to which you are entitled.

You may be taken off the study if you don't follow instructions of the investigator or the research team.

Any research data obtained to date may still be used in the research.

Will you be given any important information during the study?
You will be informed promptly if the research team gets any new information during this research study that may affect whether you would want to continue being in the study.

What should you do if you have any questions about the study?
You have been given a copy of "*What Do I Need to Know Before Being in a Research Study?*" If you have any questions at any time about this study, you should contact the Principal Investigator or any of the study personnel listed on this consent form or any other documents that you have been given.

What are your rights as a research participant?
You have rights as a research subject. These rights have been explained in this consent form and in The Rights of Research Subjects that you have been given. If you have any questions concerning your rights, or want to discuss problems, concerns, obtain information or offer input, or make a complaint about the research, you can contact any of the following:

- The investigator or other study personnel
- Institutional Review Board (IRB)
    - Telephone: (402) 559-6463.
    - Email: IRBORA@unmc.edu
    - Mail: UNMC Institutional Review Board, 987830 Nebraska Medical Center, Omaha, NE 68198-7830
- Research Subject Advocate

- Telephone: (402) 559-6941
- Email: unmcrsa@unmc.edu

Documentation of informed consent

You are freely making a decision whether to be in this research study. Signing this form means that:

- You have read and understood this consent form.
- You have had the consent form explained to you.
- You have been given a copy of The Rights of Research Subjects
- You have had your questions answered.
- You have decided to be in the research study.
- If you have any questions during the study, you have been directed to talk to one of the investigators listed below on this consent form.
- You will be given a signed and dated copy of this consent form to keep.

Signature of Subject _____
Date _____

My signature certifies that all the elements of informed consent described on this consent form have been explained fully to the subject. In my judgment, the subject possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate.

Signature of Person obtaining consent _____
Date _____

Authorized Study Personnel
Principal
* Wellsandt, Elizabeth (Liz)
phone: 402-559-4309
degree: DPT, (402) 559-4309PhD

Appendix C

INSTITUTIONAL REVIEW BOARD APPROVAL – ACL COHORT

University of Nebraska Medical Center

NEBRASKA'S HEALTH SCIENCE CENTER

Office of Regulatory Affairs (ORA)
Institutional Review Board (IRB)

September 14, 2017

Elizabeth A. Wellsandt, PT, DPT, Ph.D.
Physical Therapy Education
UNMC - 4420

IRB # 416-17-FB

TITLE OF PROTOCOL: Role of Cumulative Knee Joint Loading in ACL-Injured Articular Cartilage

DATE OF FULL BOARD REVIEW: September 7, 2017

DATE OF FINAL APPROVAL AND RELEASE: September 14, 2017

VALID UNTIL: September 7, 2018

SUBPART D CATEGORY OF REVIEW: 45 CFR 46.404/21 CFR 50.51

CLASSIFICATION OF RISK: Minimal

The UNMC IRB has completed its review of the above-titled research protocol. The IRB has determined you are in compliance with HHS Regulations (45 CFR 46), applicable FDA Regulations (21 CFR 50, 56) and the Organization's HRPP policies. Furthermore, the IRB is satisfied you have provided adequate safeguards for protecting the rights and welfare of the subjects to be involved in this study. This letter constitutes official notification of final approval and release of your project by the IRB. You are authorized to implement this study as of the above date of final approval. The following items were reviewed and approved by the IRB:

- Revised Recruitment Flyer
- Subject Instructions for Actigraph Use
- REDCap Survey
- Adult Consent Form IRB Version1
- Parental Consent Form IRB Version 1
- Youth Information Sheet IRB Version1

Please be advised that only the IRB approved and stamped consent forms/youth information sheet can be used to make copies to enroll subjects. Also, at the time of consent all subjects/parents must be given a copy of *The Rights of Research Subjects* and "What Do I Need to Know" forms.

The IRB wishes to remind you that the principal investigator (PI) is ultimately responsible for ensuring that this research is conducted in full compliance with the protocol, applicable Federal Regulations, and Organizational policies.

Finally, under the provisions of this institution's Federal Wide Assurance (FWA00002939), the PI is directly responsible for submitting to the IRB any proposed change in the research or the consent forms/information sheet. In addition, any adverse events, unanticipated problems involving risk to the subject or others, noncompliance, and complaints must be promptly reported to the IRB in accordance with HRPP policies.

This project is subject to periodic review and surveillance by the IRB and, as part of the Board's surveillance, the IRB may request periodic progress reports. For projects which continue beyond one year, it is the responsibility of the PI to initiate a request to the IRB for continuing review and update of the research project.

On behalf of the IRB,

Academic and Research Services Building 3000 / 987830 Nebraska Medical Center / Omaha, NE 68198-7830
402-559-6463 / FAX: 402-559-3300 / Email: irbora@unmc.edu / http://www.unmc.edu/irb

University of Nebraska Medical Center
NEBRASKA'S HEALTH SCIENCE CENTER

Office of Regulatory Affairs (ORA)
Institutional Review Board (IRB)

Signed on: 2017-09-14 15:19:00.000

Kevin J. Epperson, CIP
IRB Administrator III
Office of Regulatory Affairs

cc: Bruce G. Gordon M.D.
IRB Executive Chair

Academic and Research Services Building 3000 / 987830 Nebraska Medical Center / Omaha, NE 68198-7830
402-559-6463 / FAX: 402-559-3300 / Email: irbora@unmc.edu / http://www.unmc.edu/irb

Appendix D

HUMAN SUBJECTS INFORMED CONSENT – ACL COHORT

Nebraska Medicine
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PT NAME:
MR#:

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ADULT CONSENT - CLINICAL BIOMEDICAL

Title of this Research Study
Role of Cumulative Knee Joint Loading in ACL-Injured Articular Cartilage

Invitation
You are invited to take part in this research study. You have a copy of the following, which is meant to help you decide whether or not to take part:

- Informed consent form
- "What Do I need to Know Before Being in a Research Study?"
- The Rights of Research Subjects

Why are you being asked to be in this research study?
You are being asked to be in this research study because you have experienced an acute (within 1 month) anterior cruciate ligament (ACL) injury, are between the ages of 19-35 years, and have not had any previous leg injuries or surgeries. If you are pregnant, you may not be in this study.

What is the reason for doing this research study?
Knee osteoarthritis is a breakdown of the cartilage that leads to pain and decreased function. Abnormal patterns of movement and physical activity levels have been linked to the development of knee osteoarthritis. This research is trying to see how movement patterns and physical activity levels affect the knee's cartilage in individuals with an ACL injury.

What will be done during this research study?
Your involvement in this research study will consist of the following procedures completed over 5 testing sessions (baseline; 2 months after ACL surgery; 4 months after ACL surgery; 6 months after ACL surgery; 18 months after ACL surgery). The five testing sessions will take a total of approximately 15 hours to complete. You will have the option of also completing return-to-sport testing at 9 months after ACL surgery. Return-to-sport testing takes approximately 2 hours to complete.

Magnetic Resonance Imaging (MRI) (baseline, 6 months and 18 months after ACL surgery):
Magnetic resonance imaging (MRI) will take place at the University of Nebraska Medical Center (UNMC). This study uses structural MRI of the knee. Structure MRI shows the structure of the knee. The MRI scanning session will take 1 hour to complete once you are in the scanner.

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You will be encouraged to hold as still as possible and communicate any discomfort to the investigators any time before or during a scan. You will have a squeeze bulb that will help to communicate with us while you are in the machine. You will also wear ear protection to minimize the loud noises made by the machine.

The MRI is done for research purposes, and not to look for any specific abnormalities. The MRI are not the same as you might get to diagnose a medical condition. However, occasionally, the MRI will find something unexpected which the research was not looking for. This is called an incidental finding. Incidental findings may be nothing to worry about, or they may be significant or even life-threatening.

If one of the researchers sees something on your MRI which he/she is concerned about, he/she may review the scan/test with an expert. The expert review will be supplied if needed with no cost to you If the researcher and/or the expert thinks the finding may be of importance to you the researcher will tell you. You can refuse to get this information. If you agree he/she will also tell your doctor.

There may be benefits to learning such results (such as early detection and treatment of a medical condition), but there are also risks. These include anxiety over a finding which may not be real or may not require treatment. You and/or your insurance company may be billed for follow-up to the incidental finding to see if the abnormality is real or a medical problem.

Blood and Knee Fluid Draws (baseline, ACL surgery, and 6 months):
7 mL of blood will be drawn from your arm by laboratory services at UNMC at baseline and 6 months after ACL surgery. Your physician will collect a sample of up to 20 mL of fluid from your knee during ACL surgery. These fluids will be tested for biomarkers including COMP, MMP-3, NTX, and IL-6 after you complete testing at 6 months after ACL surgery.These tests are performed for research purposes only. If you wish to receive the results of these tests, you must make this request to study personnel in writing.

Movement Analysis Testing (all testing sessions):
Movement analysis testing will be completed in the Clinical Movement Analysis Lab at UNMC. Markers and small devices called accelerometers will be affixed to your skin and sneakers on both legs and your back using adhesive skin tape. Shells with markers on them will be placed on your back, pelvis, thighs and calves and will be held in place with wraps. These markers will allow the motion cameras to track the position of your body.

You will be asked to perform several walking, squatting, jumping, and hopping trials in our laboratory. These trials will give us information about the way your back, hips, knee and ankles move while you move. While you are moving, a computer will record the 3 dimensional motions of your back, hips, knees, and ankles. The entire movement analysis session will last approximately 1 hour.

Measures of Knee Function (all testing sessions):
Measures of knee function will be assessed in the Clinical Movement Analysis Lab at UNMC. We will measure the range of motion in your knees as you bend and straighten them and also if you have any swelling in your knees. We will measure the strength of the quadriceps muscle on the front of your thigh. You will be seated in a dynamometer, a device that resists your kicking motion, and measures how much force you muscle can exert. Testing will require a series of practice and recorded kicking motions. Trials will be repeated (up to a maximum of 5) on each leg. We will also measure how your knee performs while you stand up as fast as you can from a chair in 30 seconds, walk as fast as you can over a distance of 40 meters, and ascend/descend a flight of stairs as fast as you can. At 9 months and 18 months after ACL surgery, will will measure how far you can hop on each leg during 4 different hop tests. Testing measures of knee function will last approximately 45 minutes.

Questionnaires (all testing sessions):
You will be asked to complete a test packet which includes questions about your past and current functional status and your perceived functional capabilities. Questionnaires will last approximately 15 minutes.

Physical Activity Monitoring (all testing sessions):
Physical activity monitoring will be completed by you wearing a physical activity monitor (accelerometer) worn at your hip that records your movement throughout the day. This is a FDA cleared and marketed device. This research device is similar to products that can be worn on your wrist or apps on your cell phone that measure the number of steps you take each day. You will be asked to wear the device during all waking hours for 7 consecutive days beginning after all other testing in this study. You will need to remove the physical activity monitor during bathing, swimming or other activities that expose the device to water to avoid damaging the physical activity monitor. You will be asked to return the physical activity monitor to the principal investigator in a pre-postaged, pre-addressed envelope provided to you.

What are the possible risks of being in this research study?
There is a potential loss of your confidentiality.

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Magnetic Resonance Imaging (MRI):
Cautions: Tattoos could cause warming, redness or burns around the tattooed body part. You will be informed to not make skin contact to the sides of the tunnel when in the MRI machine. In other words, you should stay as still as possible in the machine, and not touch any other part of the machine. If your skin makes direct contact to the sides of the machine, it could potentially cause mild to severe burns. Certain medications could also enhance side effects such as dizziness, light-headedness, or nausea.

Women who are pregnant are excluded from participation in research projects involving a MRI scan. Although there are no known negative effects on pregnant women or fetuses, very little is known of the possibility of any negative effects. This study may involve risks that we cannot predict.

Likely risks when following all security measures: None.

Less likely risks: During the MRI scan, potential discomforts may include: feeling cold, feeling warm, anxiety, body discomfort/stiffness, or a metallic taste. Lying still for a prolonged time may prove uncomfortable. Some individuals may have a claustrophobic response, which is a fear of confined spaces, and some may experience stiffness from lying still. The MRI machine makes loud banging noises while taking measurements, so ear protection will be used to reduce the noise.

You will be in communication with the MRI technologist throughout the MRI scan. If you experience any of these or other discomforts, you will be instructed to notify the MRI technologist immediately. You will be given a squeeze bulb to contact the MRI technologist, which may be used at any time before or during scanning.

Rare risks: An additional risk, though highly unlikely, is the possibility that metal objects could be pulled into the magnetic center of the MRI machine and hit you. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. It is important to know that no metal can be brought into the MRI Room at any time. Once you are in the MRI machine, the door to the MRI Room will be closed so that no one from the outside accidentally goes near the MRI machine.

Blood and Knee Fluid Draws:
There is a risk of bruising and infection with the blood collection and synovial fluid draw. There is a risk of fainting following blood collection. The personnel completing

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these draws are highly skilled and trained and will use processes to prevent bruising and the spread of germs.

Movement Analysis Testing and Hop Testing:
You may experience a loss of balance during testing; however, your other leg is free to touch down to provide support and prevent loss of balance. You may experience discomfort from the removal of tape holding markers in place.

Measures of Knee Function:
You may experience local muscle soreness and fatigue. Following the testing, your muscles may feel as if you have exercised vigorously.

Questionnaires:
No known risks.

Physical Activity Monitoring:
No known risks.

It is possible that other rare side effects could occur which are not described in this consent form. It is also possible that you could have a side effect that has not occurred before.

What are the possible benefits to you?
You will learn information on the current status of your knee during testing, such as how strong your muscles are and how you are moving. If you consent, this information will be shared with members of your ACL medical team, such as your doctor and physical therapist, to help guide your treatment and return-to-sport decisions.You may not get any benefit from being in this research study.

What are the possible benefits to other people?
The results of this study may help us understand how human movement affects the knee's cartilage in individuals with ACL injury.

What are the alternatives to being in this research study?
Instead of being in this research study, you can choose not to participate. If you do not participate, you may still be able to complete some of the return-to-sport testing at UNMC.

What will being in this research study cost you?
There is no cost to you to be in this research study.

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Will you be paid for being in this research study?
You will be paid $50 for baseline, 6 month and 18 month testing and $25 for 2 month and 4 month testing. Each testing session is expected to require 2-4 hours. Payments will be prorated on an hourly basis, accruing as the study progresses and not being contingent upon you completing the entire study. Prorated payment will be made regardless of whether withdrawal was voluntary (you decided to withdraw from the study) or involuntary (based on withdrawal criteria of the research protocol).

To receive payment you must provide your social security number, name and address in order to comply with Internal Revenue Service (IRS) reporting requirements. When payment is reported to the IRS, we will not say what the payment is for, only that you have been paid. If you do not wish to provide this information, you can still participate in the study; however, you will not be paid.

By participating in this study, you will also be eligible for return-to-sport testing at no cost. This testing is optional and is typically completed at about 9 months after ACL reconstruction. Testing includes strength, hop, and biomechanics testing and is used to help your medical team decide when to allow you to go back to sport. This information will be used for research purposes.

Who is paying for this research?
This research is being paid for by grant funds from the National Institutes of Health (NIH) and the Rheumatology Research Foundation. The Institution receives money from the NIH and the Rheumatology Research Foundation to conduct this study.

What should you do if you are injured or have a medical problem during this research study?
Your welfare is the main concern of every member of the research team. If you are injured or have a medical problem or some other kind of problem as a direct result of being in this study, you should immediately contact one of the people listed at the end of this consent form.

How will information about you be protected?
You have rights regarding the protection and privacy of your medical information collected before and during this research. This medical information is called "protected health information" (PHI). PHI used in this study may include your medical record number, address, birth date, medical history, the results of physical exams, blood tests, x-rays as well as the results of other diagnostic medical or research procedures. Only the minimum amount of PHI will be collected for this research. Your research and medical records will be maintained in a secure manner.

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PT NAME:
MR#:

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Who will have access to information about you?
This research is covered by a Certificate of Confidentiality from the National Institutes of Health. A Certificate of Confidentiality means that the researcher in most cases cannot reveal identifiable information about you to others without your permission. He or she can report things like potential child abuse or intent to hurt self or others. He or she can report contagious diseases, and can share information with agencies paying for the research or with the Food and Drug Administration. He or she can also share the information with other scientific researchers, as allowed by federal regulations protecting research subjects. A Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research.

By signing this consent form, you are allowing the research team to have access to your PHI. The research team includes the investigators listed on this consent form and other personnel involved in this specific study at UNMC and Nebraska Medicine.

Your PHI will be used only for the purpose(s) described in the section "What is the reason for doing this research study?"

Your PHI will be shared, as necessary, with the Institutional Review Board (IRB) and with any person or agency required by law. You are also allowing the research team to share your PHI with other people or groups listed below. All of these persons or groups listed below are obligated to protect your PHI.

- Researchers at the University of Nebraska Medical Center

Your PHI may also be shared with UNMC who sponsors this research and provides funds to conduct this research.

You are authorizing us to use and disclose your PHI for as long as the research study is being conducted.

You may cancel your authorization for further collection of PHI for use in this research at any time by contacting the principal investigator in writing. However, the PHI which is included in the research data obtained to date may still be used. If you cancel this authorization, you will no longer be able to participate in this research.

How will results of the research be made available to you during and after the study is finished?
In most cases, the results of the research can be made available to you when the study is completed, and all the results are analyzed by the investigator or the sponsor

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of the research. The information from this study may be published in scientific journals or presented at scientific meetings, but your identity will be kept strictly confidential.

If you want the results of the study, contact the Principal Investigator at the phone number given at the end of this form or by writing to the Principal Investigator at the following address:
Division of Physical Therapy Education
984420 Nebraska Medical Center
Omaha, NE 68198-4420

What will happen if you decide not to be in this research study?
You can decide not to be in this research study. Deciding not to be in this research study will not affect your relationship with the investigator, the University of Nebraska Medical Center or Nebraska Medicine. You will not lose any benefits to which you are entitled.

What will happen if you decide to stop participating once you start?
You can stop participating in this research (withdraw) at any time by contacting the Principal Investigator or any of the research staff. Deciding to withdraw will otherwise not affect your relationship with the investigator or UNMC/Nebraska Medicine. You will not lose any benefits to which you are entitled.

You may be taken off the study if you don't follow instructions of the investigator or the research team. Any research data obtained to date may still be used in the research. If you become pregnant during the course of the study, you will be withdrawn from the study. Any data collected prior to you becoming pregnant will be kept and analyzed within the study.

Will you be given any important information during the study?
You will be informed promptly if the research team gets any new information during this research study that may affect whether you would want to continue being in the study.

What should you do if you have any questions about the study?
You have been given a copy of "What Do I Need to Know Before Being in a Research Study?" If you have any questions at any time about this study, you should contact the Principal Investigator or any of the study personnel listed on this consent form or any other documents that you have been given.

Nebraska Medicine
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PT NAME:
MR#:

CONSENT FORM

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What are your rights as a research participant?
You have rights as a research subject. These rights have been explained in this consent form and in The Rights of Research Subjects that you have been given. If you have any questions concerning your rights, or want to discuss problems, concerns, obtain information or offer input, or make a complaint about the research, you can contact any of the following:

- The investigator or other study personnel
- Institutional Review Board (IRB)
  - Telephone: (402) 559-6463
  - Email: IRBORA@unmc.edu
  - Mail: UNMC Institutional Review Board, 987830 Nebraska Medical Center, Omaha, NE 68198-7830
- Research Subject Advocate
  - Telephone: (402) 559-6941
  - Email: unmcrsa@unmc.edu

Documentation of informed consent
You are freely making a decision whether to be in this research study. Signing this form means that:

- You have read and understood this consent form.
- You have had the consent form explained to you.
- You have been given a copy of The Rights of Research Subjects
- You have had your questions answered.
- You have decided to be in the research study.
- If you have any questions during the study, you have been directed to talk to one of the investigators listed below on this consent form.
- You will be given a signed and dated copy of this consent form to keep.

Signature of Subject _____
Date _____

My signature certifies that all the elements of informed consent described on this consent form have been explained fully to the subject. In my judgment, the subject possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate.

Signature of Person obtaining consent _____
Date _____

Nebraska Medicine
SERIOUS MEDICINE. EXTRAORDINARY CARE.

PT NAME:
MR#:

CONSENT FORM

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Authorized Study Personnel
Principal
* Wellsandt, Elizabeth (Liz)
phone: 402-559-4309
alt #: 402-559-4309
degree: PT, DPT, PhD

Participating Personnel
Chien, Jung
phone: 402-559-5052
alt #: 402-554-3225
degree: PhD

* Jorgensen, Alyx
phone: 402-559-7870
degree: BS

Lead Coordinator
* Barber, Robert
phone: 402-836-9165
alt #: 402-554-3225
degree: MS, ATC

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