ASSOCIATIONS BETWEEN VIBRATORY PERCEPTION THRESHOLD, QUADRICEPS/HAMSTRINGS CO-ACTIVATION RATIO, AND CHANGES IN FEMORAL ARTICULAR CARTILAGE

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ABSTRACT

Emily Kathryn Guadagno: Associations Between Vibratory Perception Threshold, Quadriceps/Hamstrings Co-Activation Ratio, and Changes in Femoral Articular Cartilage
(Under the direction of J. Troy Blackburn)

Introduction: The hallmark sign of knee osteoarthritis (OA) is cartilage damage. Patients with knee OA present impulse loading, co-activation of knee musculature, and somatosensory deficits have been. These characteristics could be precursors rather than disease outcomes. Purpose: to evaluate associations between vibratory sensation (VPT), quadriceps and hamstring co-activation, and percent change of cartilage cross-sectional area (CSA) in healthy population. Methods: VPT was measured, participants performed walking trial where co-activation was measured, and pre/post ultrasound cartilage images were collected. Pearson product (r) determined associations between VPT, co-activaiton indices, and change in CSA. Results: 31 participants (age=22(2.5)years, height=1.71(0.15)m weight=74.9(11.8)kg) completed this study. Significant associations between medial and composite co-activation index and change in CSA (r=0.031, p=0.009), between lateral malleolus VPT and co-activation indices laterally during PRE-phase of gait, and composite during LA (r=-0.443,p=0.014 and r=0.415, p=0.023) were found. Discussion: Greater co-activation during gait may be associated with changes in knee cartilage.

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LIST OF ABBREVIATIONS

OA	Osteoarthritis
VPT	Vibratory Perception Threshold
EMG	Electromyography
US	Ultrasound
CSA	Cross-sectional Area
IRB	International Review Board
SMRL	Sports Medicine Research Lab
IPAQ	International Physical Activity Questionnaire
HPC	Human Performance Lab
FSR	Force Sensitive Resister
SPSS	Statistical Package for the Social Sciences
BMI	Body Mass Index
PRE	Preparatory Phase
HS	Heel Strike Phase
LA	Load Acceptance Phase
KAM	Knee Abduction Moment

CHAPTER I: INTRODUCTION

Approximately 14 million individuals in the US have symptomatic knee osteoarthritis (OA), with advanced OA comprising over half of those cases.[1] The disorder involves chronic breakdown of cartilage within a joint, which is associated with risk factors that include joint injury, obesity, and repetitive joint stress.[2] Because no disease-modifying treatments are available, treatments for symptomatic knee OA focus on symptom relief and functional restoration, including physical therapy, medications, joint injections, and total knee replacement.[3] Knee OA costs the US healthcare system $165 billion annually.[4-6] Even with interventions, a large population of individuals with knee OA decrease their activity level or stop activity completely due to associated pain. Despite the financial and physical burden suffered by individuals with knee OA, the underlying causes of this disease are poorly understood.

Excessive and abnormal joint loading are important factors leading to an imbalance between the degenerative process and subsequent repair of articular cartilage.[7] The significance of loading rate to the overall development and progression of knee OA has previously been demonstrated in animal studies.[8,9] These studies showed the subsequent processes of healing and bone remodeling increase the stiffness of the subchondral bone, which decreases its ability to dissipate forces. As a result, the overlying cartilage is exposed to greater stress during everyday tasks such as walking.[8,9] Muscle forces also contribute to abnormal joint loading. Reduced range of knee flexion and heightened muscular co-contraction during the loading phase of gait are mechanical hallmarks of those with knee OA, and together they represent what is considered the "stiffened knee response."[2] Schmitt and Rudolph found that increased muscular co-contraction and

a subject's sense of knee instability elevates joint load, which could explain cartilage deformation as a precursor to knee OA.[10-12]

Previous studies have demonstrated that proprioceptive acuity in the lower extremities is diminished with normal aging, and that proprioceptive deficits are exacerbated in individuals with knee OA independent of aging.[13-15] Barrett et al. found that patients with osteoarthritis had poorer joint position sense than similarly aged individuals with no joint disease by comparing accuracy in a joint repositioning task.[16] Vibratory perception threshold has been correlated with measures of proprioception and is a fast, inexpensive, clinically feasible measure of somatosensory function. Shakoor et al. found significant deficits in vibratory sensation in the lower extremity in subjects with OA of the knee compared with age-matched healthy subjects.[17] Vibratory sense represents a quantifiable and reliable sensory measure that may serve as a valid marker of the role of sensory deficits in OA.[17]

The stiffened knee response previously reported in individuals with knee OA during gait is typically accompanied by increased co-contraction of the quadriceps and hamstrings musculature, which increases joint contact pressures.[18] Deficits in proprioception could be the cause of ineffective muscle activation resulting in elevated impulsive loading. These deficits may cause a sense of instability resulting in increased muscular co-contraction as a way to stabilize the joint, but at the expense of increasing compressive stresses across the joint. Individuals with knee OA demonstrate greater co-contraction of these muscle groups compared to healthy individuals in an effort to improve the lacking stability of the knee, potentially causing further cartilage damage.[19]

With ultrasound (US) imaging, researchers and clinicians are able to quantify acute changes in femoral cartilage following dynamic tasks such as walking.[20,21] It is also less expensive and more easily accessible than other common imaging modalities such as MRI and radiography.

Harkey et al. evaluated femoral cartilage thickness before and after loading during walking and running at a self-selected speed as well as following a control condition, and observed a 6.7% decrease in cartilage thickness after the walking condition measured within 5 minutes of movement, and found significant decreases in compartment thickness and cross-sectional area (CSA) of femoral cartilage post-walking.[20]

Somatosensory deficits and increased co-contraction have been observed in subjects with knee OA.[17-19] However, there is a gap in the literature evaluating these characteristics in healthy individuals and their effect on cartilage structure. It is essential to assess healthy individuals in order to decipher if sensory deficits and/or co-contraction influence cartilage measures during functional tasks. This information would be crucial for deciphering whether these phenomena are knee OA sequelae or pre-existing characteristics. Therefore, the purpose of this study was to evaluate the influence of somatosensory function (VPT) and quadriceps and hamstring co-activation on percent change of cartilage CSA assessed via ultrasound imaging following walking. We evaluated the association between somatosensory function, measured via VPT, and co-contraction ratio of quadriceps and hamstrings musculature during walking, measured via EMG.

The Specific Aims of this study include the following:

1. To determine the relationship between VPT and changes in femoral cartilage CSA after walking. We hypothesized that higher VPT (i.e. poorer somatosensory function) would be associated with a decrease in cartilage cross-sectional area following walking (i.e. larger decreases in femoral cartilage thickness).
2. To determine the relationship between quadriceps and hamstring co-activation during gait and changes in femoral cartilage CSA after walking. We hypothesized that higher co-

3. activation ratios would be associated with decrease in cartilage cross-sectional area.
4. To determine if there is a significant relationship between VPT and quadriceps and hamstrings co-activation ratio during gait. We hypothesized that greater VPT would be associated with greater co-activation ratios.

CHAPTER II: REVIEW OF THE LITERATURE

Introduction

Approximately 14 million individuals in the US have symptomatic knee osteoarthritis (OA), with advanced OA comprising over half of those cases.[1] This disorder involves chronic breakdown of cartilage within a joint, and considerable research has investigated biomechanical characteristics that predispose individuals to knee OA. Over half of all persons with symptomatic knee OA are younger than 65 years of age.[1] Patients with knee OA often suffer from debilitating pain, balance deficits, and challenges with simple activities of daily living such as walking up and down stairs or standing from a seated position.[1,3]

Because no disease-modifying treatments are available, treatments for symptomatic knee OA focus on symptom relief and functional restoration, including physical therapy, medications, joint injections, and total knee replacement.[3] Knee OA costs the US healthcare system $165 billion annually.[4-6] Even with these interventions, a large population of individuals with knee OA decrease their activity level or stop activity completely due to the pain associated with it. Many patients must take time off of work during symptom flare ups associated with knee OA, resulting in a loss of necessary income. Despite the financial and physical burden suffered by individuals with knee OA, the underlying causes of this disease are poorly understood. Our study will consider the roles of muscular co-contraction and vibration sense as pathophysiological factors of cartilage breakdown.

Pathophysiology of Knee OA

Loading rate/Impulsive loading

Excessive and abnormal joint loading are important factors leading to an imbalance between the degenerative process and subsequent repair of articular cartilage.[7] The significance of loading rate to the overall development and progression of knee OA has previously been demonstrated in animal studies. Studies by Ewers et al. and Radin et al. investigating the effects of repetitive loading have demonstrated that microfractures are present in the trabecular bone of rabbits when subjected to repetitive loading and that greater cartilage fissuring results from the same magnitude impact loads applied at higher loading rates.[8,9] The subsequent process of healing and bone remodeling increases the stiffness of the subchondral bone, which decreases its ability to act as a dissipater of peak force.[8,9] As a result, the overlying cartilage is exposed to greater stress. Abnormal loading presents a possible contributor to the mechanical pathogenesis of knee OA.

Compressive Force

As muscle forces contribute to joint loading, muscle activation in knee OA is also likely to influence disease course.[22,23] Coordination of the knee flexor and extensor muscles is a determinant of knee loading. Schmitt and Rudolph found that individuals with heightened co-contraction between the quadriceps and hamstring musculature reported the sensation of knee instability.[10] Research shows that increased muscular co-contraction elevates joint load, which could explain cartilage deformation as a precursor to knee OA.[11,12]

Somatosensory Deficits

Proprioception is the perception of body segment position. Previous studies have demonstrated that proprioceptive acuity in the lower extremities is diminished with normal aging, and that proprioceptive deficits are exacerbated in individuals with knee OA independent of

aging.[13-15] Barrett et al. found that patients with osteoarthritis showed poorer joint position sense than those of a similar age with no joint disease by comparing accuracy in a joint repositioning task.[16] Pai et al. and Hurley et al. found similar results of significantly decreased joint displacement detection threshold in OA patients compared to elderly controls and found that this measure was associated with worse disease-specific functional status.[13,14] It has been hypothesized that this reduced sense of position may be pathophysiologically related to the onset or progression of knee OA.[2] The reason behind this hypothesis is that individuals who do not have adequate movement or position sense may be unable to appropriately protect the joints from impulsive loading during ambulation.

Sensory information is a critical component of neuromuscular control of the limb, and somatosensory alterations could therefore negatively affect loading of the joints while walking. Riskowski et al. reported a correlation between proprioception and rate of loading in healthy subjects, indicating that poorer proprioceptive acuity was associated with higher loading rates.[24] Shakoor et al. found that dynamic knee joint loading was directly associated with proprioception measurements, also indicating that the worse the proprioception, the higher the knee load during gait.[25] Collins et al. applied a knee sleeve to subjects with knee OA in an attempt to improve somatosensory function and the sensation of joint stability in the arthritic limb, and found significant decreases in loading rates during walking.[26] This was accompanied by a decrease in quadriceps/hamstring co-activation, suggesting a link between co-activation, somatosensory function, and loading rate. If these somatosensory deficits contribute to greater joint loading, it is important to consider their possible role as predisposing factors to the onset of knee OA.

Somatosensory Function and Vibration Sense

Somatosensory function has been measured in previous studies as balance, joint position

sense (ability to reproduce a specific flexion angle at the knee), and kinesthesia (ability to detect subtle flexion or extension at the knee).[13,14,16] Because these methods require movement of a diseased and potentially unstable joint in individuals with knee OA, they may be affected by disease severity and pain, independent of any true lower extremity sensory deficits.[15] Results from these methods may be further confounded by the fact that they require a fair degree of comprehension, concentration ability, and memory, and may be influenced by patient's reaction time.[15] The reliability of these procedures in most instances is not ideal and are not clinically feasible, as they require laboratory-specific motion capture equipment.[27] Additionally the different methods of measuring somatosensory function are not highly correlated.[28] It is intuitive to use a measure that is clinician friendly and safe for a person with knee OA that may be non-weight bearing.

One such clinically feasible assessment is vibratory perception threshold (VPT). The sensation of vibration is transmitted in parallel with proprioceptive acuity through the dorsal columns of the spinal cord. Both vibratory sense and proprioceptive acuity are commonly affected in the peripheral neuropathies.[27] VPT is a sensory measure that is commonly used to evaluate diabetic neuropathy and has been associated with neuropathic arthropathy.[12,28] Previous studies have found a strong positive correlation between proprioception and vibration, suggesting that they identify similar phenomena.[2,15] Considering their closely related anatomic associations, as well as their possible synergistic roles in the somatosensory system, deficits in these measures may represent interrelated alterations in sensory processing in OA.[16] However, in contrast to the poor precision of proprioceptive testing, the evaluation of VPT is simple and highly reproducible. Shakoor et al. found significant deficits in vibratory sensation at the lower extremity in subjects with OA of the knee compared with normal age-matched healthy subjects.[17] During this study, 5

sites of the lower extremity were tested and the average total testing time was approximately 10 minutes. Because it is inexpensive, portable, and the time requirement is shorter than that required to measure proprioception, VPT is a clinically feasible measure. Multiple studies also found high reliability and reproducibility with the procedure.[30,31] Thus, vibratory sense represents a quantifiable and reliable sensory measure that may serve as a valid marker of the role of sensory deficits in OA.

Heightened Co-activation

Alterations in the mechanical environment of the knee joint due to the breakdown of cartilage can adversely affect load distribution, resulting in abnormal wear within the joint and further breakdown of cartilage.[8] Reduced range of knee flexion and heightened muscular co-contraction during the loading phase of gait are mechanical hallmarks of those with knee OA, and together they represent what is known as the 'stiffened knee response.'[10,12,18] This stiffening of the knee is typically accompanied by increased co-contraction of the quadriceps and hamstrings musculature, which increase joint contact pressures.[32] Deficits in proprioception could be the cause of ineffective muscle activation resulting in elevated impulsive loading. These deficits may cause a sense of instability resulting in increased muscular co-contraction as a way to stabilize the joint, but at the expense of increasing compressive stresses across the joint. Individuals with knee OA demonstrate greater co-contraction of these muscle groups compared to healthy individuals in an effort to improve the lacking stability of the knee, potentially causing further cartilage damage.[26]

In individuals with knee OA, co-contraction of knee muscles is heightened during walking as quantified by greater amplitude, longer duration, and greater co-contraction indices than disease free individuals.[2,33,34,35] Hodges et al. evaluated the relationship between knee muscle activation and changes in knee joint cartilage and found that greater duration of medial muscle co-contraction

and greater duration of medial relative to lateral co-contraction was associated with greater annual percent loss of femoral cartilage volume.[36]

Interaction Between Somatosensory Deficits, Co-activation, and Impulsive Loading

Those with knee OA likely demonstrate greater co-contraction of the hamstrings and quadriceps muscle groups compared to healthy controls as a way to improve the stability of the knee.[7,32,33,34] However, this strategy increases joint contact pressures, which exacerbates pain and degradation of the joint.[2] Malalignment, weakness, and altered muscle activation patterns are a few biomechanical factors implicated in knee OA progression.[10] Patients with OA of the knee have also been shown to have lower extremity sensory deficits, measured as poor joint position sense, abnormal kinesthesia, poor balance, and reduced vibratory sense.[16]

Alterations in the mechanical environment of the knee joint due to the breakdown of cartilage can adversely affect load distribution, resulting in abnormal wear within the joint and further breakdown of cartilage.[12] Collins et al. found that employing means to improve proprioception in patients with knee OA via neoprene sleeve around the knee resulted in less co-contraction of the hamstrings and quadriceps musculature and reduced loads in the knee joint.[26] This study will evaluate muscular co-contraction in a healthy population and its association with somatosensory function and changes in cartilage cross-sectional area caused by walking. In order to understand knee OA, it is important to evaluate how cartilage breakdown occurs, and to discern predictive factors from compensatory mechanisms.

Cartilage Imaging

Progressive degradation of articular cartilage leading to a decrease in cartilage thickness is one of the hallmark characteristics of OA.[33,34] Joint space narrowing visualized with radiography is considered a surrogate of cartilage thickness decline, and evidence of joint space narrowing is

often used in the diagnosis of OA.[38] Joint space narrowing can be influenced by changes in the meniscus and/or position of the knee during imaging. Cartilage cannot be viewed directly via radiography, therefore it cannot provide signs of early OA. Though it is considered the gold standard for imaging-based diagnosis of OA because of its low cost and ease of access, it does not provide clinicians with pathophysiological information.

Magnetic resonance imaging (MRI) has become a key tool for OA research because of its ability to visualize characteristics of structures not imaged by radiography.[38-41] With MRI, the joint can be evaluated as a whole organ; multiple tissue changes can be monitored simultaneously over several time points; pathologic changes of pre-radiographic OA can be detected at an earlier stage of the disease; and physiologic changes within joint tissues (eg, cartilage and menisci) can be assessed before morphologic changes become apparent.[42] In the clinical setting, this is not feasible because of the high cost per examination.

Ultrasonography (US) imaging enables real-time, multi-planar imaging at low cost. It offers reliable assessment of OA associated features including inflammatory and structural abnormalities without contrast administration or exposure to radiation.[43] US has recently emerged as a valid tool for quantitatively assessing femoral cartilage changes by demonstrating high agreement when compared to cross-sectional cadaver measurements and MRI.[44,45] Harkey et al. found significant medial femoral cartilage deformation in thickness and cross-sectional area after both running and drop landing interventions via US.[46] US is also portable and able to be moved from lab to lab, making it conducive to measuring changes in cartilage in the clinical setting. Harkey et al. also evaluated femoral cartilage thickness before and after loading during walking and running at a self-selected speed as well as following a control condition and observed a 6.7% decrease in cartilage thickness after the walking condition and was able to measure this within 5

minutes of movement, showing that US may be utilized to monitor changes in cartilage thickness and CSA following acute loading.[20]

Rationale for Study

Knee OA is a prevalent disease, with approximately 14 million Americans suffering from symptoms caused by it.[1] Somatosensory deficits and exacerbated co-contraction have been identified in subjects with knee OA.[18,19] However, these studies are retrospective in nature, including individuals who have been previously diagnosed. Thus, it is unknown if these characteristics contribute to knee OA development or if they are complications or compensatory mechanisms resulting from with the disease. It is essential to evaluate these characteristics in healthy individuals to decipher if somatosensory deficits and/or co-contraction contribute to greater cartilage loading.

This study will be the first to evaluate the effects of proprioception on co-activation and changes in cartilage cross-sectional area. The purpose of this study is to evaluate the influence of somatosensory function (VPT) on quadriceps and hamstring co-activation and changes in cartilage structure assessed via US following walking. Changes in cartilage cross-sectional area have been observed in healthy individuals after loading tasks, including walking. We will evaluate the associations between VPT, co-contraction ratio of quadriceps and hamstrings musculature, and cartilage cross-sectional area induced by walking. We expect to see a decrease in cartilage cross-sectional area in participants with poor somatosensory function (high VPT), and/or high co-contraction ratio between the quadriceps and hamstrings. If a correlation is found between these variables, this study will provide further insight regarding the pathophysiology of knee OA development. This study will then open avenues for further research into ways to improve these deficits and abnormal loading in healthy people in hopes of preventing knee OA.

CHAPTER III: METHODS

Experimental Design

In this observational study, femoral articular cartilage thickness was measured prior to and following treadmill walking in healthy, physically active individuals. Vibratory perception threshold (VPT) was assessed prior to treadmill walking, and quadriceps/hamstrings co-activation was assessed during treadmill walking. Statistical analyses were conducted to evaluate the influence of VPT and co-activation on the change in cartilage cross-sectional area caused by treadmill walking.

Participants

Data was collected during the control session of a study which analyzed the effects of acute weight gain on changes in femoral cartilage cross-sectional area after a walking task. Participants reported for a screening, control, and intervention session. The screening session involved participant education on procedures of the study and signing an International Review Board, IRB, approved informed consent. During the control session, cartilage area was measured prior to and following walking 5,000 steps. The same procedure was carried out during the intervention session, but participants wore a weighted vest containing 15% of their body weight while walking. A moderate effect ($d = 0.568$) between cartilage cross-sectional area changes in the weighted and non-weighted condition was predicted based on pilot testing and means and standard deviations from previously published work.[17] Power analysis indicated 27 participants were necessary to detect two-tailed statistical significance with an alpha level of 0.05 and 80% power. Thirty-two

healthy volunteers [males = 16, females = 16, age = 22 (2.5) years, height 1.71 (0.15) m, mass 74.9 (11.8) kg] were recruited to ensure that 27 participants completed all sessions and account for a 15% dropout rate. All participants were required to be between a body mass index (BMI) of 18.5 and 24.5, have no history of acute or chronic lower extremity musculoskeletal injury, or lower extremity surgery, and have no current joint pain.

Procedures

Session I

Upon arrival to the Sports Medicine Research Laboratory (SMRL), participants were required to sign an IRB approved informed consent form (Appendix 1). We recruited a convenience sample of healthy individuals between the ages of 18 and 30 years who self-reported participating in physical activity for at least 20 minutes 3 days per week, assessed via the International Physical Activity Questionnaire (IPAQ) (Appendix 2). The participants were screened to ensure they met the inclusion criteria through a data collection sheet where they reported history of acute and chronic lower extremity musculoskeletal injury (Appendix 3). If they passed, 5 trials of "normal" walking speed were obtained. Instructions were given to participants to "walk as if you're walking on the sidewalk of a road" as they walked through 2 infrared timing gaits spaced 1 meter apart. Walking speed was then averaged and calculated to miles per hour to use during the second session on the treadmill.

Vibratory Perception Threshold

VPT was evaluated using a biothesiometer (Bio-Medical Instrument Co., Newbury, OH) (Figure 3). This device consists of a solid applicator tip that vibrates at a constant frequency of 120 Hz and a manual dial that is used to adjust vibration intensity. Participants were positioned side-lying and the biothesiometer was applied uniformly to four bony prominences of the lower

extremity (medial and lateral epicondyles, medial and lateral malleoli) with the weight of the device as the only source of pressure (Figure 4). VPT was also assessed at the dorsal surface of the head of the first metatarsal with the ankle in a neutral position and the foot resting on the floor. Prior to testing, the participant was given a demonstration of the effect of the biothesiometer on the hand to familiarize him/her with the vibratory sensation. With the biothesiometer placed at the testing site and the voltage/intensity set to 0, the intensity was increased at a rate of 1 V/s, and the participant was instructed to verbally indicate when he/she first sensed the vibration. The corresponding voltage was then recorded as the VPT. Three trials were conducted at each testing site and averaged for statistical analysis. Each testing site was indicated with a marker to increase reliability. A higher VPT measure represents poorer somatosensory function.

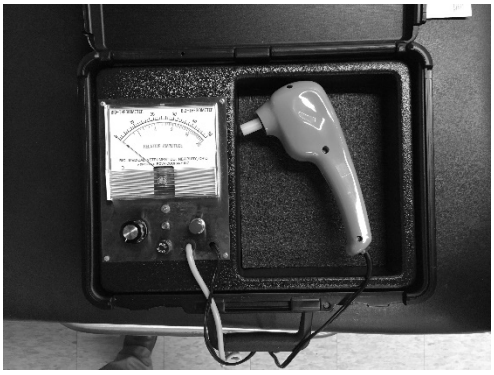


Figure 1. VPT was evaluated using a biothesiometer (Bio-Medical Instrument Co., Newbury, OH). This device consists of a solid applicator tip that vibrates at a constant frequency of 120 Hz and a manual dial that is used to adjust vibration intensity.

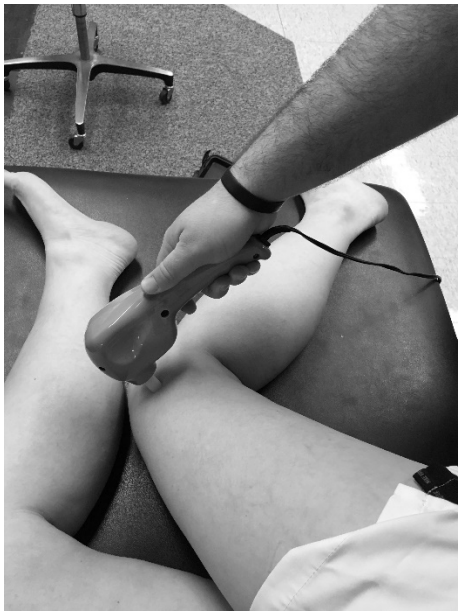


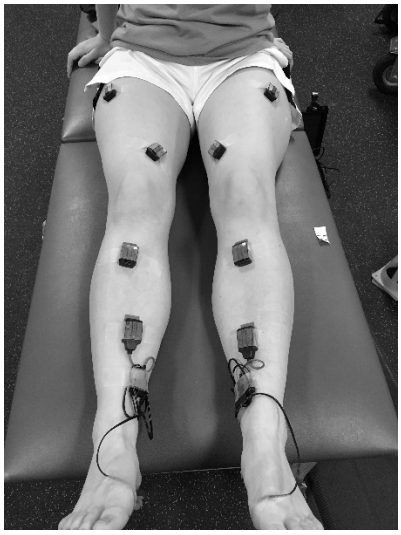
Figure 4. Participants were positioned side-lying and the biothesiometer was applied uniformly to four bony prominences of the lower extremity (medial and lateral epicondyles, medial and lateral malleoli) with the weight of the device as the only source of pressure.

Session II

Upon arrival to the Human Performance Center (HPC) at the University of North Carolina-Chapel Hill for data collection, electromyography (EMG) electrodes were placed over the quadriceps and hamstring muscles, VPT was measured, then participant rested on a padded table in a long-sit position with the knees in full extension for 45 minutes to unload the cartilage, permit fluid rebound, and minimize effects of preceding activity.[20,46,47] Ultrasound images of the femoral cartilage were then obtained prior to and following treadmill walking during which EMG data were sampled. Data were sampled from both the right and left legs, but for the purpose of this study only data from the dominant limb were used for statistical analyses. The dominant leg was defined as the leg a participant would choose to kick a ball with. EMG Electrode Placement

Preamplified surface EMG electrodes (Trigno, DelSys Inc., Natick, MA) were placed bilaterally over the vastus lateralis, vastus medialis, biceps femoris, and medial hamstrings to

evaluate the electrical activity of these muscles during treadmill walking (Figures 1 and 2). The investigator identified the area of greatest muscle bulk for each muscle, and these areas were then shaved, lightly abraded, and cleaned with isopropyl alcohol. The electrodes were secured to the skin with adhesive collars and tape approximately parallel to the direction of action potential propagation, and electrode placements were verified via manual muscle testing and observing the signal on an oscilloscope.



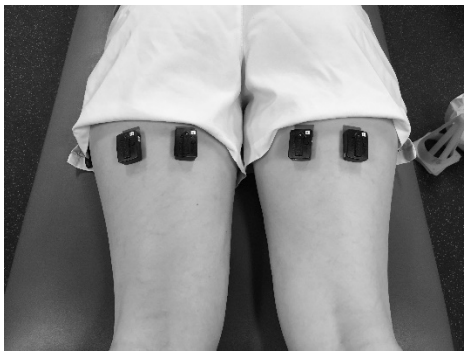
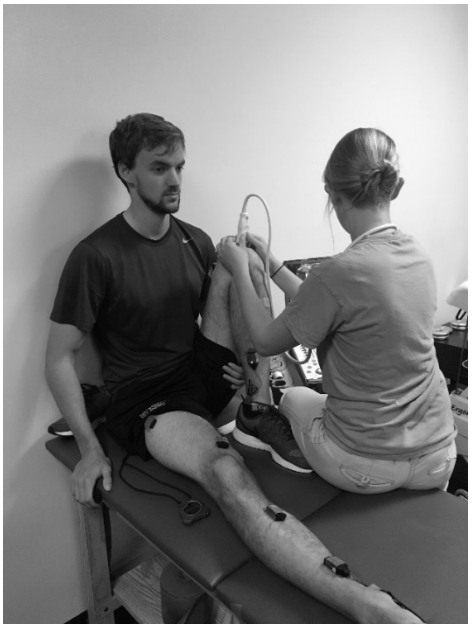


Figure 3 and 4. EMG Electrode placement over the area of greatest muscle bulk approximately parallel to the muscle fibers for the quadriceps and hamstrings musculature.

Ultrasonographic Assessment of the Femoral Articular Cartilage

After the participant was fitted with EMG electrodes, VPT was measured, and he/she had been sitting for 45 minutes, ultrasound images were obtained from both knees in a counterbalanced order. The knee being imaged was positioned in 140° of flexion using a manual goniometer while the participant was seated with his/her back against a wall. A tape measure was secured to the table and the distance between the wall and the posterior calcaneus was recorded to ensure accurate repositioning for post-test assessments. A LOGIQe US system (General Electric Co., Fairfield, CT) with a 12MHz linear probe was used to image the femoral cartilage. The probe was placed

transversely in line with the medial and lateral femoral condyles above the superior edge of the patella and rotated to maximize reflection of the ultrasound beam off the articular cartilage surface (Figures 5 and 6). A transparency grid was placed over the US screen divided in increments of 2mm by 70mm to aid in reproducibility of the probe placement. With the intercondylar notch centered on the grid, the positioning of the lateral and medial condyles on the grid was recorded and replicated in subsequent assessments. Three images were recorded for each participant.



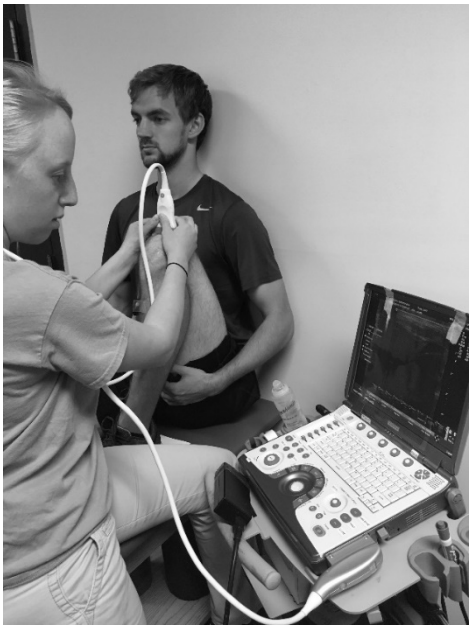


Figure 5 and 6. A 12MHz linear probe was used to image the femoral cartilage. The probe was placed transversely in line with the medial and lateral femoral condyles above the superior edge of the patella and rotated to maximize reflection off the articular cartilage surface.

Treadmill Walking Protocol

After the US images were obtained, an investigator transported the participant from the treatment table to the treadmill via wheelchair. The participants then walked at a self-selected pace for 5,000 stepson a treadmill (4Front, WOODWAY, Waukesha, WI) at the previously determined self-selected speed.

Force Sensitive Resistor (FSR) Sensors (Trigno, DelSys Inc., Natick, MA) placed on the plantar surfaces of the calcaneus and head of the first metatarsal (Figure 7 and 8) were used to identify steps and phases of the gait cycle. After 5 minutes of the participant walking at a self-selected speed, FSR sensor and EMG data were sampled for 1 minute. Custom LabVIEW code was then used to calculate the number of steps per minute and estimate the time required to attain 5,000 steps. Total stance phase, the weight bearing phase of gait, was defined as the interval from heel strike to toe-off from the FSR sensors. This information was used as a timestamp for interpreting the muscular activation recorded via EMG.

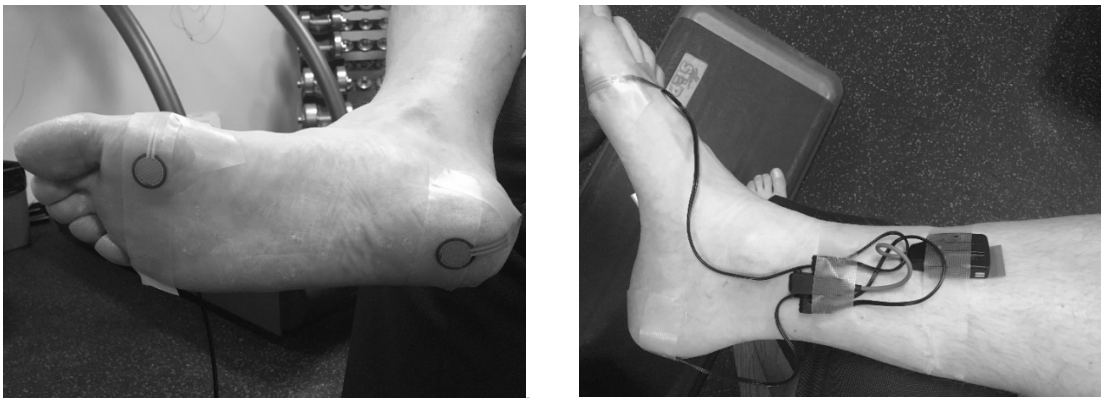


Figure 7 and 8. One channel of the FSR sensor was placed beneath the calcaneous, while the other was placed under the first distal phalanxes. These recorded activity via contact during the participant's heel strike and toe-off phases of gait.

Post-Walking Ultrasonographic Assessment of the Femoral Articular Cartilage

Ultrasound images of the femoral cartilage were again obtained immediately following the walking protocol. Using the tape measure secured to the treatment table, the participant was placed in the same position as during the baseline US assessment, the US probe was repositioned using the transparency grid, and 3 images of the femoral cartilage were obtained via the same procedures. All post-loading US images were captured within 5 minutes following the walking protocol to minimize fluid rebound of the cartilage.

Ultrasonographic Image Analysis

US images were processed using ImageJ software (National Institutes of Health, Bethesda, MD). To determine femoral cartilage cross-sectional area, the femoral cartilage was segmented by identifying the entire visualized cartilage-bone interface and soft tissue-cartilage interface for the cartilage of the medial and lateral femur, measured in mm^2. This was completed in ImageJ by outlining the cartilage via segmentation. Values were obtained for each of the three images for each time point (pre and post walking) and averaged for statistical analysis.

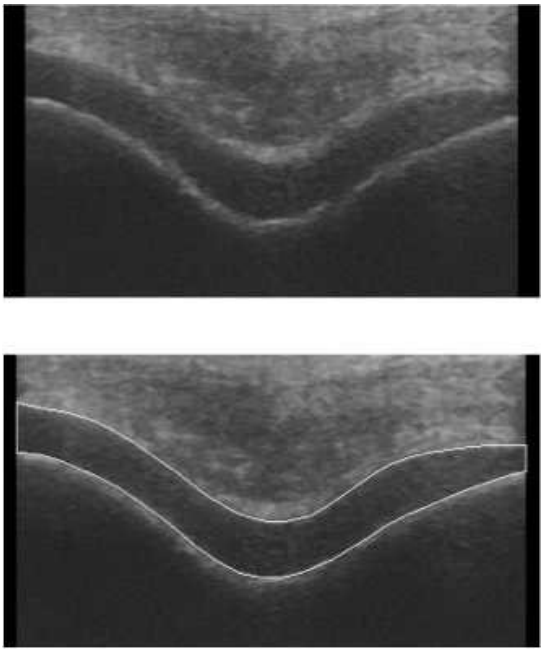


Figure 9. US Image Analysis.

Data Reduction

EMG and FSR sensor data were sampled for 1 minute after 5 minutes of walking, after the midpoint of each participant's walking protocol, and during the last minute of walking. EMG data were corrected for DC bias, bandpass (20-350 Hz) and notch (50.9-60.5 Hz) filtered (4^{th} order Butterworth), full wave rectified, and lowpass filtered at 10 Hz (8^{th} order Butterworth) to create a linear envelope.[26] Quadriceps/hamstrings co-contraction indices were calculated as described by

Schmitt and Rudolph.[10] The EMG linear envelope was normalized to the peak amplitude during each stance phase averaged across all stance phases within a given sampling interval (i.e. 1 minute intervals after 5 minutes, at the midpoint of the walking protocol, and the final minute), and the co-contraction index was evaluated via the following equation:

$$Co - contraction\ index = \frac{\sum_{i=0}^{n} \frac{EMGL_i}{EMGH_i} (EMGL_i + EMGLH_i)}{n}$$

EMGL and EMGH are the EMG activity of the least active and more active muscle between the two antagonists, respectively. The co-activation index was calculated over three distinct intervals: 1) preparatory (PRE) – the 100ms prior to heel strike, 2) the 200ms interval centered on heel strike (HS), and 3) weight acceptance (WA) – the 1st 50% of the stance phase. Co-Activation ratio was calculated between both the lateral musculature (vastus lateralis and long head of the biceps femoris), medial musculature (vastus medialis and medial hamstring muscles), and composite (both anterior to posterior muscles).

Statistical Analysis

Statistical analysis was performed using SPSS. Partial Pearson correlation coefficients were used to measure strength and direction of 3 relationships: 1) between participants' cartilage pre-post cross-sectional area change scores and the co-contraction index 2) between change in cartilage cross-sectional area change scores and VPT, and 3) between VPT and co-activation index. A partial Pearson correlation coefficient was used to account for differences in participant's walking speed, which has been shown to influence gait biomechanics and levels of cartilage breakdown.[48] In addition, multiple regression was performed considering co-contraction ratio and VPT measures as explanatory variables and change in cartilage CSA the dependent variable.

CHAPTER IV: RESULTS

Participants

Due to the stringency of the inclusion criteria on BMI, outcome measures were collected on 31 of the 32 initially enrolled subjects. The one subject whose measurements were not utilized did not have a qualifying BMI between 18.5 and 24.9, as subcutaneous adipose tissue can limit the effectiveness of the EMG amplitude signal.[49] Sixteen males and fifteen females completed the study. The participants' demographics can be found in Table 1.

Measure	**N = 31**
Age (years)	22 ± 2.5
Height (m)	1.71 ± 0.15
Mass (kg)	74.9 ± 11.8
Sex	16 males (51.6%), 15 females (48.4%)
Gait Speed (mph)	3.0 ± 0.3

Table 1. Subject Demographics (mean ± sd)

Correlations between Percent Change in Cartilage Cross-Sectional Area and Vibratory Perception Threshold

There was no significant association between percent change in cartilage cross-sectional area and vibratory perception threshold at the lateral femoral condyle ($p = 0.381$), medial femoral condyle ($p = 0.493$), lateral malleolus ($p = 0.952$), medial malleolus ($p = 0.850$), or head of the first metatarsal ($p = 0.330$) of the dominant limb. Descriptive statistics for these relationships are provided in Table 2.

	Lateral Femoral Condyle	Medial Femoral Condyle	Lateral Malleolus	Medial Malleolus	Metatarsal Head
% Change in Cartilage Area	-0.166 (0.381)	-0.130 (0.493)	0.011 (0.952)	-0.036 (0.850)	-0.184 (0.330)

Table 2. Correlations between Percent Change in Cartilage CSA and VPT (r correlation coefficient above p value in parentheses)

Correlations between Change in Cartilage Cross-Sectional Area and Co-Activation

Correlations between percent change in cartilage CSA and co-activation ratios are presented in Table 3. Greater medial ($r = 0.441$, $p = 0.015$) and composite ($r = 0.468$, $p = 0.009$) co-activation during the Heelstrike phase was associated with a greater change in cartilage CSA. Similarly, greater medial ($r = 0.511$, $p = 0.004$) and composite ($r = 0.392$, $p = 0.032$) co-activation during the Load Acceptance phase was associated with a greater change in cartilage CSA. The directions of these correlations were positive indicating that greater co-activation was associated with greater ***increases*** in cartilage area. The relationship between medial co-activation ratio during heel strike phase and changes in cartilage CSA is shown in a scatter plot labeled Figure 10. The relationships between composite co-activation ratio during Heelstrike phase and changes in cartilage CSA is shown in a scatter plot labeled Figure 11. The relationship between medial co-activation during Load Acceptance phase and change in cartilage CSA is shown in Figure 12. The relationship between composite co-activation during Load Acceptance phase and change in cartilage CSA is shown in Figure 13.

Co-activation Index	Percent Cartilage Change
Composite	
Preparatory	0.031 (0.872)
Heelstrike	**0.468** **(0.009)**
Load Acceptance	**0.392** **(0.032)**
Lateral	
Preparatory	0.184 (0.330)
Heelstrike	0.267 (0.154)
Load Acceptance	0.087 (0.649)
Medial	
Preparatory	-0.141 (0.457)
Heelstrike	**0.441** **(0.015)**
Load Acceptanc**e**	**0.511** **(0.004)**

Table 3. Correlations between Percent tFCSA Change and Co-activation (correlation coefficient r above p value in parentheses, significance bolded)

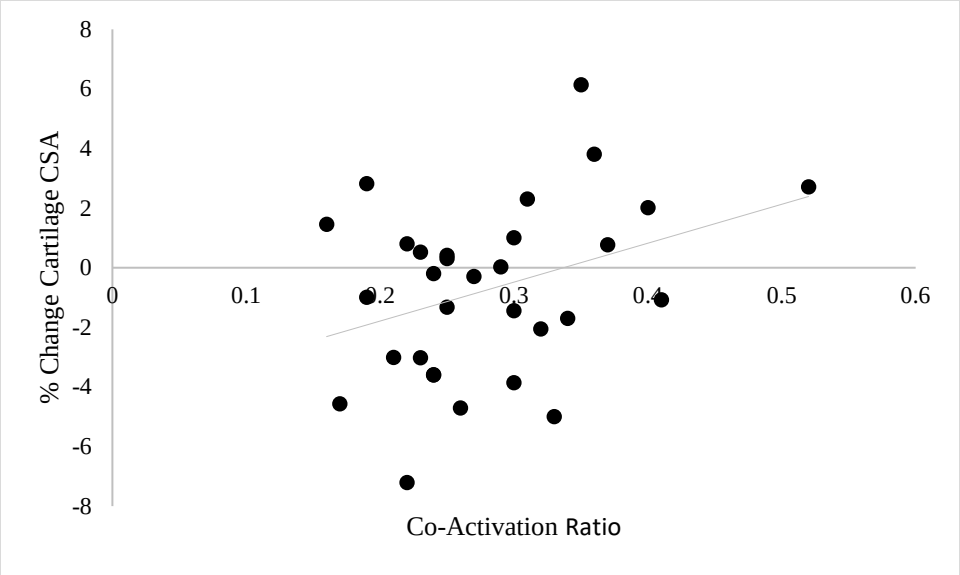


Figure 10. Percent Change in Cartilage CSA and Composite Co-Activation during HS

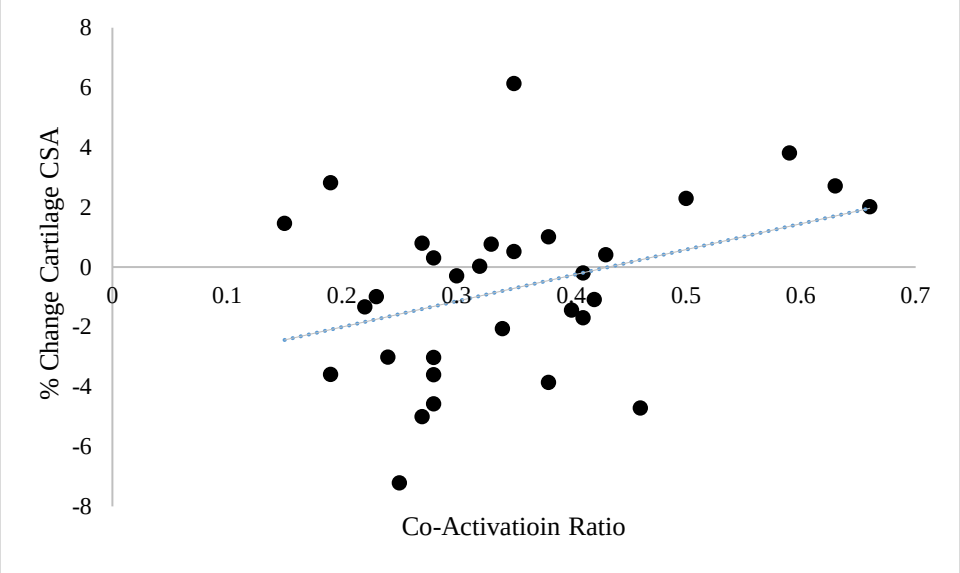


Figure 11. Percent Change in Cartilage CSA and Medial Co-Activation during HS

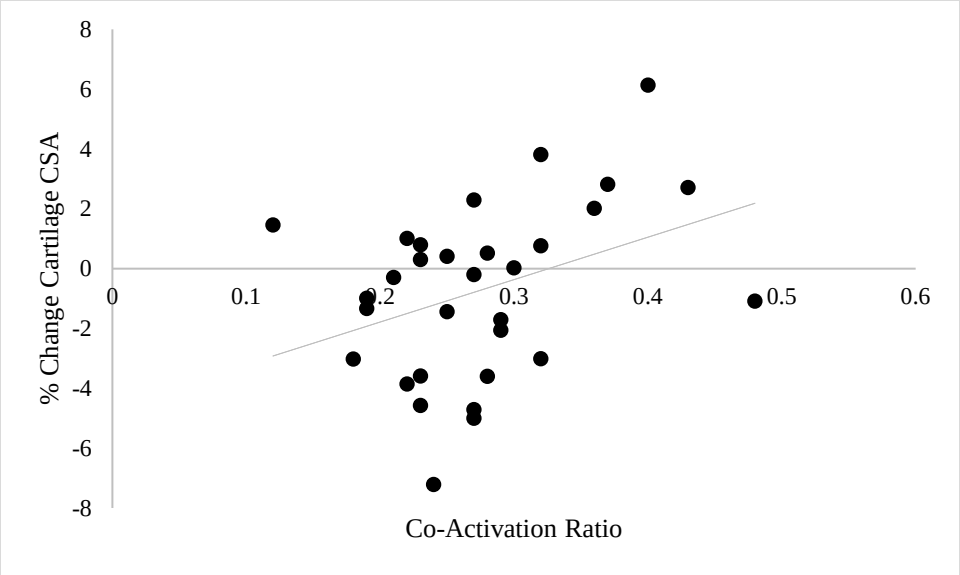


Figure 12. Percent Change in Cartilage CSA and Composite Co-Activation during LA

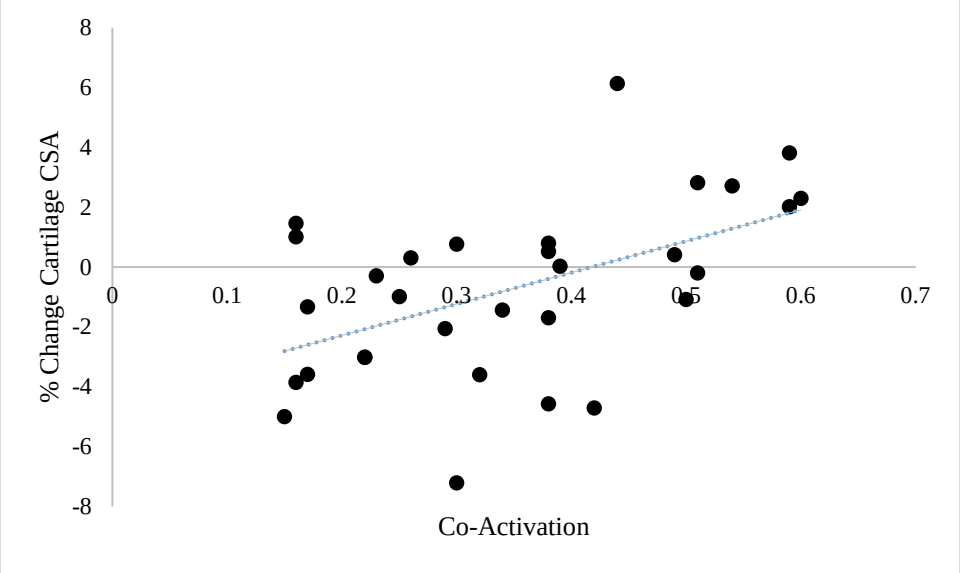


Figure 13. Percent Change in Cartilage CSA and Medial Co-Activation during LA

Correlations between Vibratory Perception Threshold and Co-Activation

Correlations between VPT and co-activation ratios are displayed in Table 4. A significant relationship was observed between lateral malleolus VPT and composite co-activation ratio during the preparatory phase ($r = -0.443$, $p = 0.014$). The negative direction of this indicates that lower VPT measures were associated with higher co-activation of the quadriceps and hamstrings during

the preparatory phase of gait. Another significant correlations was observed between lateral co-activation during the load acceptance phase and lateral malleolus VPT ($r = 0.415$, $p = 0.023$). The positive direction of this correlation indicates that greater VPT at the lateral malleolus was associated with greater co-activation. The association between lateral malleolus VPT measures and composite co-activation ratio during preparatory phase of gait is further defined by the scatter plot in Figure 14, and the association between lateral malleolus VPT measures and lateral co-activation during the load acceptance phase of gait is further defined by the scatter plot in Figure 15.

Co-activation Index	Lateral Femoral Condyle	Medial Femoral Condyle	Lateral Malleolus	Medial Malleolus	Metatarsal Head
Composite					
Preparatory	-0.246 (0.190)	-0.049 (0.799)	**-0.443 (0.014)**	-0.337 (0.069)	-0.284 (0.128)
Heelstrike	0.066 (0.731)	0.076 (0.689)	-0.033 (0.861)	-0.078 (0.682)	-0.103 (0.587)
Load Acceptance	0.174 (0.358)	0.264 (0.158)	0.304 (0.103)	.117 (0.537)	0.032 (0.868)
Lateral					
Preparatory	-0.076 (0.688)	0.116 (0.543)	-0.241 (0.199)	-0.142 (0.453)	0.029 (0.878)
Heelstrike	0.123 (0.519)	0.261 (0.164)	0.102 (0.591)	-0.075 (0.693)	-0.131 (0.492)
Load Acceptance	0.170 (0.368)	.323 (.082)	**0.415 (0.023)**	0.106 (0.577)	-0.099 (0.604)
Medial					
Preparatory	-0.103 (0.587)	0.008 (0.965)	-0.157 (0.407)	-0.065 (0.734)	-0.192 (0.309)
Heelstrike	0.026 (0.890)	-0.065 (0.734)	-0.046 (0.810)	0.097 (0.610)	0.149 (0.432)
Load Acceptance	.102 (0.591)	-0.025 (0.895)	0.139 (0.465)	0.220 (0.244)	.351 (0.057)

Table 4. Correlations between Co-Activation and VPT (correlation coefficient r above p value in parentheses, significance bolded)

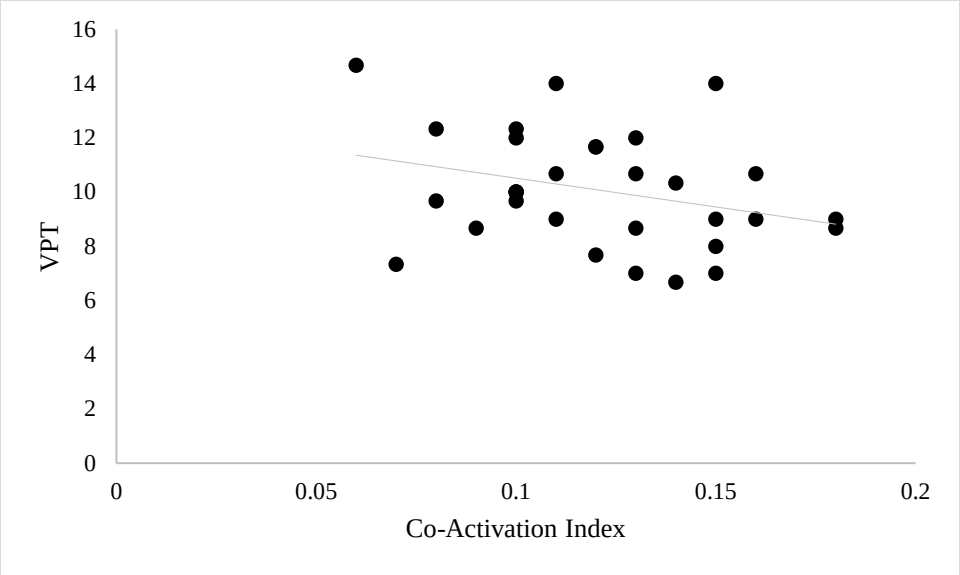


Figure 14. Lateral Malleolus VPT and Composite Co-Activation during Preparatory Phase

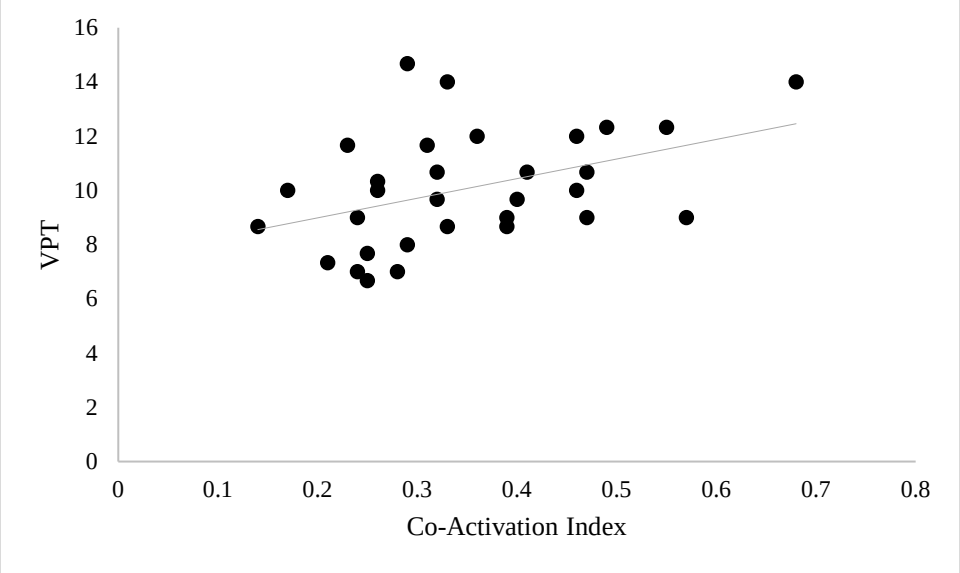


Figure 15. Lateral Malleolus VPT and Lateral Co-Activation during Load Acceptance

CHAPTER V: DISCUSSION

The purpose of this study was to examine associations between somatosensory function, lower extremity muscular co-activation, and changes in cartilage pre and post-walking. The first of three research hypotheses stated that greater VPT, or poorer vibration sense, would associate with a greater percent change in cartilage CSA. The second hypothesis stated that greater co-activation of the hamstrings and quadriceps would associate with a greater percent change in cartilage CSA. The third hypothesis stated that greater VPT would associate with greater co-activation of the hamstrings and quadriceps.

Vibratory Perception Threshold and Percent Change in Cartilage CSA

The results of this study showed no association between vibratory perception threshold and percent change in cartilage CSA. These results did not support the original hypothesis that participants with greater VPT would display greater cartilage deformation.

The sensation of vibration is transmitted in parallel with proprioceptive acuity through the dorsal columns of the spinal cord. Both vibratory sense and proprioceptive acuity are commonly affected by peripheral neuropathies.[27] VPT is a sensory measure that is commonly used to evaluate diabetic neuropathy and has been associated with neuropathic arthropathy.[12,28] Previous studies have found a strong positive correlation between proprioception and vibration, suggesting that they identify similar phenomena.[2,15] Considering their closely related anatomic associations, as well as their possible synergistic roles in the somatosensory system, deficits in these measures may represent interrelated alterations in sensory processing in individuals with OA.[16] Shakoor et al. found significant deficits in vibratory sensation in the lower extremity in subjects with knee OA

compared to age-matched healthy subjects, and that significantly higher VPT was present in subjects with moderate-to-severe severity of knee OA compared to those with mild radiographic knee OA.[17] In another study by Shakoor et al. also observed a direct association between peak external knee adduction moment, an indicator of medial compartment knee joint loading, and VPT in individuals with knee OA.[25] This finding linked the structural consequences associated with somatosensory deficits in patients with knee OA. Based on findings in individuals with knee OA, it was hypothesized that worse sensation in healthy individuals would correlate with greater cartilage deformation.

Although insignificant, the mean VPT values obtained from our sample were similar to those reported by Shakoor et al. in healthy individuals (Table 5).[17] The relatively small population of 31 healthy participants may not have had enough variation within the cohort to prove or disprove this hypothesis. While the same number of subjects were utilized in both studies, Shakoor et al. found large variability between locations and subjects, with standard deviations as high as 7.0 and 9.1 volts.[17] This could be explained by due to their age matched controls, which were significantly older than the population of this study (51.8+10.8 years). Shakoor's study also had no BMI restrictions on healthy individuals (29.8+5.6kg/cm^2), only exclusion criteria of the presence of diabetes for less than 10 years as well as no hip or knee replacement or neuropathy, in contrast to our healthy, active participants. This highlights the large distribution of the measurement process of obtaining VPT measures. Evaluation of VPT may be simple and highly reproducible, but it's validity as a marker of sensory deficits in healthy subjects must be validated for future studies.

	Shakoor et al. (n = 31)	Current Study (n = 31)
Lateral Femoral Condyle	18.9 ± 9.1	15.4 ± 4.2
Medial Femoral Condyle	15.9 ± 7.0	16.5 ± 4.5
Lateral Malleolus	10.4 ± 3.2	10.1 ± 2.1
Medial Malleolus	12.3 ± 5.2	10.8 ±2.9
Metatarsal Head	6.4 ± 3.3	6.0 ± 1.7

Table 5 Healthy Population VPT (mean ± sd)

Co-Activation and Percent Change in Cartilage CSA

The results of this study showed an association between co-activation and cartilage deformation. However, the direction of this correlation was opposite our hypothesis, in that greater co-activation was associated with greater ***increases*** in cartilage CSA. These significant findings were consistent along multiple phases of gait including composite and medial co-activation measures during the heel strike and load acceptance phases. Of the 31 participants in this study, seventeen individuals displayed a decrease in cartilage cross-sectional area, and the remaining fourteen displayed an increase in cross-sectional area. Our hypothesis was inspired by previous studies that observed significant cartilage deformation, or decrease in cartilage thickness, post-walking or running in healthy individuals.[20,47,50] Hodges et al. observed a positive association between the duration of medial quadriceps and hamstrings co-contraction and medial cartilage loss.[36] Collins et al. reported decreases in co-activation associated with decreased knee loading rates in subjects with knee OA.[26] The stiffened knee response previously reported in individuals with knee OA during gait is typically accompanied by increased co-contraction of the quadriceps and hamstrings musculature, which increases joint contact pressures.[18] Based on the stiffened knee

response, this study attempted to support the theory that greater co-contraction of the quadriceps and hamstrings musculature leads to greater joint contact pressures, and ultimately deforms cartilage excessively.[2]

These conflicting results may be explained by the methodology of knee cartilage area measurement and analysis. This study measured cartilage cross-sectional area rather than thickness measures, as well as the ultrasound imaging method, which provides a limited view of the cartilage. Kinematics were also excluded from this study, which would provide further details about knee joint loading and associated cartilage changes. The results may also be an effect of the blinding used to analyze the US images.

Cartilage Thickness vs Cross-Sectional Area

Past MRI studies have measured decreases specifically in medial cartilage thickness, Niehoff et al. observed a 2.6% decline after over-ground running and Boocock et al. observed a 5.3% decrease post-running.[47,50] Hodges et al. found that greater duration of medial muscle co-contraction and greater duration of medial relative to lateral co-contraction was associated with greater annual percent loss of femoral cartilage volume via MRI.[36] In order to utilize ultrasound measures, this study was based off of the methodology presented by Harkey et al., who observed a 6.7% decrease in medial compartment cartilage thickness after walking via ultrasound.[20] In another study by Harkey (2018), femoral cartilage CSA measures were validated when he observed a significant time and condition (time increments of US measures post-jump landing) for decreases in medial and lateral cross-sectional area.[46] However, we evaluated the area of the entire section of cartilage that was visible in the field of view rather than medial and lateral cartilage CSA or thickness separately, which may have been more sensitive to changes in the cartilage post-loading tasks.

Location of Cartilage Assessed

Previous studies have found significant changes in cartilage deformation using US.[20,45,51] However, US only utilizes a small portion of the cartilage as a whole. Cartilage is viscoelastic in nature, and fluid flow throughout the cartilage to attenuate load is pertinent. Niehoff et al. observed pronounced cartilage deformation after running and drop landing task on the lateral tibia alone when looking at patellar, tibial, and femoral cartilage measures via MRI.[47]
Coleman et al. observed diurnal changes in tibiofemoral and patellofemoral cartilage and cumulative strain via MRI, including increases in thickness in some areas of the cartilage simultaneous with decreases in others.[52] Our ultrasound images permitted assessment of a section of the femoral cartilage that was 4cm wide in the frontal plane x 1cm wide in the transverse plane. As such, the methods of this study only utilized a single 1cm width about the cartilage, the increase in cartilage CSA observed in this study may not have been the most significant change throughout the cartilage post-walking trial when associated with muscular co-activation.

Increase in cartilage following walking that we observed may be attributable to fluid rebound in the cartilage surrounding the specific location being loaded. Differences in muscular co-contraction between during gait alter load distribution throughout the lower extremity. Previous studies have linked co-activation of the quadriceps/hamstrings during walking gait and increased knee joint loads via kinematics in participants with knee OA.[10,53,54] Hubley-Kozey et al. found a greater co-activation ratio in a severe knee OA group compared with an asymptomatic group.[54] Selistre et al. included subjects specifically with medial compartment knee OA, and found their external knee adduction moment, which is strongly associated with medial and lateral load ration and disease progression.[53,55,56] The US methodology of only looking at distal femoral cartilage US with the participant in 140º knee flexion would miss this location-specific change within the knee

joint as a whole. Wretenberg et al. utilized MRI to define tibiofemoral contact points at 0º, 30º, and 60º of knee flexion and found that with increases in angle, contact point moved anteriorly along the tibial and posterior along femoral cartilage.[57] If this is the case, our ultrasound method may have assessed an area of the cartilage that was anterior or posterior to the aspect of the contact point where concentrated cartilage loading from muscular co-activation occurred. It is pertinent to look specifically at kinematics such as knee joint angle in addition to muscular co-activation patterns during gait to further understanding about the location of cartilage being loaded versus the location shown in US imaging.

Blinding

In this study, a single individual obtained and analyzed all cartilage US images. This individual was blinded to subject and condition (pre- vs post-test). In previous studies by Harkey et al., where significant decreases in cartilage area were reported, the analyzer was not blinded.[20,46] Felson et al. analyzed MRI images of wrist cartilage effected by rheumatoid arthritis and found that accuracy (based on the relationship between sensitivity and specificity) was slightly greater when films were read with known chronology.[58] The authors concluded that knowing the chronological sequence leads to an increase in detecting clinically relevant changes in patients without serious overestimation of non-relevant differences, and that 'analyzing a clinical trial should be done preferably by reading films in chronological order'.[58] While our rationale was to reduce reader bias toward finding expected decreases in cartilage post-walking, it can be argued that risk of false positives was greater due to decreased accuracy and increase in noise of the images, overall decreasing power and sensitivity. In a policy paper on best practices in Medical Imaging Techniques for trials, the FDA and PhRMA worked together to identify multiple different acceptable scenarios for image presentation including blinded to sequence or known chronology

displays.[59] They stated "the type of presentation often depends on the therapeutic area, the imaging technology being deployed, and the reasons for the review" of images.[59] Therefore, further research on the best blinding technique for femoral articular cartilage US of cross-sectional area is necessary to set a gold standard.

Vibratory Perception Threshold and Co-Activation

VPT at the lateral malleolus was associated with composite co-activation during the preparatory phase. VPT at the lateral malleolus was also associated with lateral co-activation during the load acceptance phase of gait, but this association presented in an opposite direction. The results of this study observed greater VPT at the lateral malleolus was associated with increased composite co-activation during the PRE-phase of gait, but decreased lateral co-activation during LA. The conflicting nature of these associations are displayed in Figure 14 and Figure 15. A portion of this finding supports our hypothesis in that individuals with greater VPT, a sign of worse vibration sense, would display greater co-activation. Shakoor et al. observed a correlation between higher VPT at the 1st metatarsal head and greater knee joint load, which was attributed to this site being most sensitive and closely related anatomic site to appreciate the sensation of the foot touching the ground during ambulation.[17] The associations observed in this study may be spurious findings due to the conflicting significance with composite muscular co-activation in one phase of gait, and lateral muscular co-activation in another. The limited number of significant associations across the 5 bony landmarks that were tested prompts further research within healthy populations and VPT.

Limitations

The current study has some limitations that should be considered. First, regarding ultrasound image acquisition and analysis, we only assessed cartilage area. Cartilage thickness

may have given us more information about the nature of cartilage pre and post walking, as well as measurements at different knee flexion angles in order to view multiple contact areas of the femoral articular cartilage. This study's methods also blinded US image analyzer to condition. Also, we did not address participants' kinematics during gait in order to identify those who walked with a 'stiffened knee response' similar to individuals diagnosed with knee OA.

The second set of limitations revolve around VPT assessment. This study measured VPT during a separate screening session rather than during the same session when cartilage deformation was assessed. Shakoor et al., measured VPT on the same day as the walking task and data collection sessions. It is possible that the delay between VPT measurement and the loading task as well as US images influenced the results.[17] This study also measured VPT in a non-weight bearing position. While a non-weight bearing position is ideal for patients with knee OA because it removes the risk of pain and further injury, it is not a functional measurement. This study attempted to correlate somatosensory function with co-activation while walking. Walking consists of double leg stance and single leg swing phase, therefore a weight bearing somatosensory measure may have shown significant correlations with co-activation while walking.

The third limitation to be addressed is the walking task itself. This study required participants walk 5,000 steps in order to simulate daily walking throughout the day for a healthy individual. A more challenging task such as applying a weighted vest during walking could have produced more variability between participants in co-activation. The additional weight could highlight the need for activation of musculature to stabilize about the knee joint while walking. In this case, increased variability within the sample would further explain the relationship between somatosensory function and co-activation when compared with cartilage measures.

Conclusion

In summary, this study provides information about potential associations between somatosensory function, muscular co-activation, and changes in femoral articular cartilage measures. The results showed no association between VPT and percent change in cartilage CSA. It also observed an association between greater VPT at the lateral malleolus and increased composite co-activation during the PRE-phase of gait, but decreased lateral co-activation during LA. The most prominent finding of this study showed a positive association between VPT measures, and co-activation indices. These results conflict with previous studies that found a decrease in cartilage thickness and CSA via US post-walking, and studies that correlated increases in co-activation with decreases in cartilage measures.[20,36,46] Measuring cartilage CSA rather than thickness, the limited view and methodology of US, or blinding for US analysis utilized here may explain these findings. Participants included in this study were screened for previous history of hip, knee, or ankle pain and injury, as well as required to be physically active for at least thirty minutes three times per week. This strict inclusion criteria may have limited the amount of variability to find significant differences within the sample. Future studies will be important for better defining a gold-standard methodology linking these measurements to predisposing factors to knee OA.

APPENDIX 1: IRB APPROVED CONSENT

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants

Consent Form Version Date: 06/28/2017
IRB Study # 17-0274
Title of Study: Effect of Simulated Weight Gain on Articular Cartilage Deformation and Neuromuscular Function Following Walking
Principal Investigator: Cassie Perrella
Principal Investigator Department: Exercise and Sport Science
Principal Investigator Phone number: 904-671-3312
Principal Investigator Email Address: cassiep@live.unc.edu
Co-Investigators: Emily Guadagno, Brian Pietrosimone, Troy Blackburn, William Prentice, Erik Wikstrom, Brett Pexa, Hope Davis, Chris Johnston

Study Contact Telephone Number: 904-671-3312
Study Contact Email: cassiep@live.unc.edu

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.
You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
The purpose of this research study is to determine how walking with and without weight affects knee and ankle cartilage health in healthy individuals.

You are being asked to be in the study because you are a healthy, physically active person between the ages of 18-35 years.

Are there any reasons you should not be in this study?
You should not be in this study if you:

1) Do not participate in at least 30 minutes of physical activity at least three times per week.
2) Have a history of the following general orthopedic conditions
    a. Congenital or degenerative joint condition
    b. Orthopedic implant
    c. Current joint pain (quantified as less than 2 on a 10 cm visual analog scale
    d. Cartilage injury of any joint
    e. Lower extremity fracture

f. Upper extremity fracture

3) Have a history of ligamentous or cartilage injury to the knee or hip
4) Have a BMI that exceeds 30 kg/m^2

How many people will take part in this study?
There will be approximately 32 people in this research study.

How long will your part in this study last?
If you agree to participate, you will complete a quick screening session (<30 minutes) in the Sports Medicine Research Laboratory. For data collection, you be asked to report to the Sports Medicine Research Laboratory for two testing sessions separated by approximately 7 days. Each testing session will last approximately 2.5 hours.

What will happen if you take part in the study?
If you agree to take part in this study, you will begin by completing an initial screening session. During this screening session, you will begin by completing some electronic forms to measure your physical activity. Then we will have you walk in our biomechanics area to determine your height, weight, quadriceps strength, and average walking speed, which will be used in future data collection sessions.

For each data collection session, you will report to the Sports Medicine Research Laboratory. The following procedures will occur identically between the three sessions, except the physical activity that occurs during each session will be different.

1. Vibratory Perception Threshold- While lying on your side, we will place the tip of a biothesiometer on different points on your lower body. The biothesiometer will emit small vibrations and you will be asked to tell us when you begin to feel the vibratory sensation.
2. Electromyography- You will also be asked to contract different leg muscles so that we can identify where to place electrodes. Once identified, small areas of skin will be shaved, lightly abraded, and cleaned and the electrodes will be secured to your skin with tape. You will be asked to complete the walking protocol with the electrodes secured to your skin.
3. Rest – You will sit on a treatment table for 1 hour. This allows for your knee cartilage to "unload".
4. Ultrasound Assessment – You will then be instructed to maximally bend your knee and ankle, and we will use an ultrasound machine to take a picture of the inside of your knee and ankle to measure your cartilage.
5. Walking-
    a. Non-weighted – You will be asked to walk at a self-selected speed on a treadmill for 5,000 steps. This will take approximately 45 minutes, depending on how fast you walk.
    b. Weighted – You will be fitted with a weighted vest consisting of 20% of your total body weight. You will then be asked to walk at a self-selected speed on a treadmill for 5,000 steps. This will take approximately 45 minutes, depending on how fast you walk.
6. Post Test– You will immediately return back to the treatment table and we will take an identical ultrasound assessment measures.

What are the possible benefits from being in this study?
Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

What are the possible risks or discomforts involved from being in this study?
The physical activity conditions you will perform (i.e. Walking) carry the minimal potential for muscle or joint injury. Since you are physically active, these risks are not different from the risks you experience with normal physical activity/exercise.

There may be uncommon or previously unknown risks. You should report any problems to the researcher.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will information about you be protected?
Any information obtained in connection with this research study that can be linked to you will remain confidential. You will be identified only by a subject identification number. A code list that associates your name and information with a specific subject identification number will be kept under key-card access on a password-protected computer in the Sports Medicine Research Laboratory. Only the research team will have access to this information.

Participants will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The UNC-Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. You do not give up any of your legal rights by signing this form.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will not receive anything for being in this study.

Will it cost you anything to be in this study?
It will not cost you anything to be in this study. A parking pass can be provided for you, if needed, to attend each data collection session.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research participant?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to

obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Participant's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

Signature of Research Participant	Date
Printed Name of Research Participant	
Signature of Research Team Member Obtaining Consent	Date
Printed Name of Research Team Member Obtaining Consent	

APPENDIX 2: SCREENING QUESTIONNAIRE

Screening Form

Subject #: _____ **Sex**: Male Female **Age**: _____ y/o

Height: _____cm **Weight**: _____ kg _____ lbs **BMI**: _____ kg/m^2

Are you between the age 18 and 35?	Yes	No
Do you participate in at least 30 minutes of physical activity at least 3 times per week?	Yes	No
Are you unable to walk without the use of an external device?	Yes	No

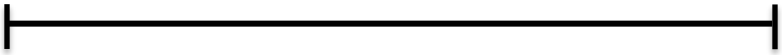
QUESTIONNAIRE

Congenital or degenerative joint condition	Yes	No
Orthopedic Implant	Yes	No
Cartilage injury of any joint	Yes	No
Lower extremity fracture within the last 12 months	Yes	No
Upper extremity fracture within the last 12 months	Yes	No
Ligamentous injury of the knee	Yes	No
Ligamentous injury of the hip	Yes	No
Ligamentous injury of the ankle	Yes	No
Concussion within the last 12 months	Yes	No

Please select yes or no regarding your situation:

Do you have a history of any of the following conditions?

Rate your current level of joint pain:



0 (No pain) 10 (Worst)

APPENDIX 3: IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

REFERENCES

1. Deshpande B, Katz J, Solomon D et al. Number of Persons With Symptomatic Knee Osteoarthritis in the US: Impact of Race and Ethnicity, Age, Sex, and Obesity. *Arthritis Care Res (Hoboken)*. 2016;68(12):1743-1750.

2. Collins J, Whittle M. Impulsive forces during walking and their clinical implications. *Clinical Biomechanics*. 1989;4(3):179-187.

3. Losina E, Weinstein A, Reichmann W et al. Lifetime Risk and Age at Diagnosis of Symptomatic Knee Osteoarthritis in the US. *Arthritis Care Res (Hoboken)*. 2013;65(5):703-711.

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

5. Dillon C.F., Rasch E.K., Gu Q., and Hirsch R. Prevalence of knee osteoarthritis in the United States: arthritis data from the Third National Health and Nutrition Examination Survey 1991-94. J Rheumatol 2006; 33(11): 2271-9.

6. United States Census Bureau 2013; http://quickfacts.census.gov/qfd/states/00000.html.

7. Maly M. Abnormal and cumulative loading in knee osteoarthritis. *Curr Opin Rheumatol*. 2008;20(5):547-552.

8. Ewers B, Jayaraman V, Banglmaier R, Haut R. Rate of blunt impact loading affects changes in retropatellar cartilage and underlying bone in the rabbit patella. *J Biomech*. 2002;35(6):747-755.

9. Radin, E., Parker, H., Pugh, J., Steinberg, R., Paul, I., & Rose, R. (1973). Response of joints to impact loading — III. *Journal Of Biomechanics*, *6*(1), 51-57.

10. SchmitT L, Rudolph K. Influences on knee movement strategies during walking in persons with medial knee osteoarthritis. *Arthritis & Rheumatism*. 2007;57(6):1018-1026.

11. Hortobágyi T, Westerkamp L, Beam S et al. Altered hamstring-quadriceps muscle balance in patients with knee osteoarthritis. Clinical Biomechanics. 2005;20(1):97-104.

12. Childs J, Sparto P, Fitzgerald G, Bizzini M, Irrgang J. Alterations in lower extremity movement and muscle activation patterns in individuals with knee osteoarthritis. *Clinical Biomechanics*. 2004;19(1):44-49.

13. Hurley M, Scott D, Rees J, Newham D. Sensorimotor changes and functional performance in patients with knee osteoarthritis. *Ann Rheum Dis*. 1997;56(11):641-648.

14. Pai Y, Rymer W, Chang R, Sharma L. Effect of age and osteoarthritis on knee proprioception. *Arthritis & Rheumatism*. 1997;40(12):2260-2265.

15. Lephart, S.M. Proprioception and neuromuscular control in joint stability. Champaign (IL): Human Kinetics; 2000.

16. Barrett D, Cobb A, Bentley G. Joint proprioception in normal, osteoarthritic and replaced knees. *J Bone Joint Surg Br*. 1991;73-B(1):53-56.

17. Shakoor N, Lee K, Fogg L et al. The relationship of vibratory perception to dynamic joint loading, radiographic severity, and pain in knee osteoarthritis. *Arthritis & Rheumatism*. 2011;64(1):181-186.

18. Pierrynowski M. Co-activation differences in lower limb muscles between asymptomatic controls and those with varying degrees of knee osteoarthritis during walking. *Yearbook of Sports Medicine*. 2010;2010:76-77.

19. Al-Khlaifat L, Herrington L, Hammond A, Tyson S, Jones R. The effectiveness of an exercise programme on knee loading, muscle co-contraction, and pain in patients with medial knee osteoarthritis: A pilot study. *Knee*. 2016;23(1):63-69.

20. Harkey M, Blackburn J, Davis H, Sierra-Arévalo L, Nissman D, Pietrosimone B. Ultrasonographic assessment of medial femoral cartilage deformation acutely following walking and running. *Osteoarthr Cartil*. 2017;25(6):907-913.

21. R.J. Schmitz, H.-M.M. Wang, D.R. Polprasert, R.A. Kraft, B.G. Pietrosimone, Evaluation of knee cartilage thickness: a comparison between ultrasound and magnetic resonance imaging methods, The Knee. 24 (2) (2017) 217–223.

22. Lewek M, Rudolph K, Snyder-Mackler L. Control of frontal plane knee laxity during gait in patients with medial compartment knee osteoarthritis. *Osteoarthritis and Cartilage*. 2004;12(9):745-751.

23. Bennell K, Hunt M, Wrigley T, Lim B, Hinman R. Role of Muscle in the Genesis and Management of Knee Osteoarthritis. *Rheumatic Disease Clinics of North America*. 2008;34(3):731-754.

24. Riskowski J, Mikesky A, Bahamonde R, Burr D. Design and Validation of a Knee Brace With Feedback to Reduce the Rate of Loading. *J Biomech Eng*. 2009;131(8):084503

25. Shakoor N, Lee K, Fogg L et al. The relationship of vibratory perception to dynamic joint loading, radiographic severity, and pain in knee osteoarthritis. *Arthritis & Rheumatism*. 2011;64(1):181-186.

26. Collins A, Blackburn J, Olcott C, Yu B, Weinhold P. The impact of stochastic resonance electrical stimulation and knee sleeve on impulsive loading and muscle co-contraction during gait in knee osteoarthritis. *Clinical Biomechanics*. 2011;26(8):853-858.

27. Gilman, S. (2002). Joint position sense and vibration sense: anatomical organisation and assessment. *Journal Of Neurology, Neurosurgery & Psychiatry*, *73*(5), 473-477.

28. Mendoza J. Foundes A. Clinical neuroanatomy. New York: Springer Science and Business Media; 2008.

30. Davis E, Jones T, Walsh P, Byrne G. The Use of Biothesiometry to Detect Neuropathy in Children and Adolescents With IDDM. *Diabetes Care*. 1997;20(9):1448-1453.

31. Frenette B, Mergler D, Ferraris J. Measurement precision of a portable instrument to assess vibrotactile perception thresh- old. Eur J Appl Physiol Occup Physiol 1990;61:386–91.

32. Ramsey D, Snyder-Mackler L, Lewek M, Newcomb W, Rudolph K. Effect of anatomic realignment on muscle function during gait in patients with medial compartment knee osteoarthritis. *Arthritis & Rheumatism*. 2007;57(3):389-397.

33. Andriacchi T, Mündermann A. The role of ambulatory mechanics in the initiation and progression of knee osteoarthritis. *Curr Opin Rheumatol*. 2006;18(5):514-518.

34. Schipplein OD, Andriacchi TP. Interaction between active and passive knee stabi- lizers during level walking. J Orthop Res 1991;9:113e9.

35. Lloyd D, Buchanan T. Strategies of muscular support of varus and valgus isometric loads at the human knee. *J Biomech*. 2001;34(10):1257-1267.

36. Hodges P, van den Hoorn W, Wrigley T et al. Increased duration of co-contraction of medial knee muscles is associated with greater progression of knee osteoarthritis. *Man Ther*. 2016;21:151-158.

37. Maly M. Linking Biomechanics to Mobility and Disability in People With Knee Osteoarthritis. *Exerc Sport Sci Rev*. 2009;37(1):36-42.

38. Eckstein F., Collins J.E., Nevitt M.C., et al. Brief Report: Cartilage Thickness Change as an Imaging Biomarker of Knee Osteoarthritis Progression: Data From the Foundation for the National Institutes of Health Osteoarthritis Biomarkers Consortium. Arthritis & rheumatology (Hoboken, N.J.). 2015;67(12):3184-3189.

39. Hunter D, Zhang Y, Tu X et al. Change in joint space width: Hyaline articular cartilage loss or alteration in meniscus?. *Arthritis & Rheumatism*. 2006;54(8):2488-2495.

40. Conaghan P, Hunter D, Maillefert J, Reichmann W, Losina E. Summary and recommendations of the OARSI FDA osteoarthritis Assessment of Structural Change Working Group. *Osteoarthr Cartil*. 2011;19(5):606-610.

41. Eckstein F, Wirth W, Nevitt M. Recent advances in osteoarthritis imaging—the Osteoarthritis Initiative. *Nature Reviews Rheumatology*. 2012;8(10):622-630.

42. Loeser R, Goldring S, Scanzello C, Goldring M. Osteoarthritis: A disease of the joint as an organ. *Arthritis & Rheumatism*. 2012;64(6):1697-1707.

43. Roemer F, Kwoh C, Hannon M et al. Risk factors for magnetic resonance imaging-detected patellofemoral and tibiofemoral cartilage loss during a six-month period: The Joints On Glucosamine study. *Arthritis & Rheumatism*. 2012;64(6):1888-1898.

44. Keen H, Conaghan P. Ultrasonography in Osteoarthritis. *Radiol Clin North Am*. 2009;47(4):581-594.

45. Naredo E, Acebes C, Moller I, Canillas F, de Miguel E. Ultrasound validity in the measurement of knee cartilage thickness. *Ann Rheum Dis*. 2008;68(8):1322-1327.

46. Harkey M, Blackburn T, Hackney A et al. Comprehensively Assessing the Acute Femoral Cartilage Response and Recovery After Walking and Drop-Landing: An Ultrasonographic Study. *Ultrasound Med Biol*. 2018;44(2):311-320.

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

48. Pietrosimone B, Troy Blackburn J, Harkey M et al. Walking Speed As a Potential Indicator of Cartilage Breakdown Following Anterior Cruciate Ligament Reconstruction. *Arthritis Care Res (Hoboken)*. 2016;68(6):793-800.

49. Kuiken T, Lowery M, Stoykov N. The Effect of Subcutaneous Fat on Myoelectric Signal Amplitude and Cross-Talk. *Prosthet Orthot Int*. 2003;27(1):48-54.

50. Boocock M, McNair P, Cicuttini F, Stuart A, Sinclair T. The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. *Osteoarthr Cartil*. 2009;17(7):883-890.

51. Akkaya S, Akkaya N, Ozcakar L et al. Ultrasonographic evaluation of the femoral cartilage thickness after unilateral arthroscopic partial meniscectomy. *Knee Surgery, Sports Traumatology, Arthroscopy*. 2012;21(5):1104-1110.

52. Coleman J, Widmyer M, Leddy H et al. Diurnal variations in articular cartilage thickness and strain in the human knee. *J Biomech*. 2013;46(3):541-547.

53. Selistre L, Mattiello S, Nakagawa T, Gonçalves G, Petrella M, Jones R. The relationship between external knee moments and muscle co-activation in subjects with medial knee osteoarthritis. *Journal of Electromyography and Kinesiology*. 2017;33:64-72.

54. Hubley-Kozey C, Hill N, Rutherford D, Dunbar M, Stanish W. Co-activation differences in lower limb muscles between asymptomatic controls and those with varying degrees of knee osteoarthritis during walking. *Clinical Biomechanics*. 2009;24(5):407-414.

55. Sharma L, Lou C, Felson DT, Dunlop DD, Kirwan-Mellis G, Hayes KW, et al. Laxity in healthy and osteoarthritic knees. Arthritis Rheum 1999;42:861e70.

56. Shelburne KB, Torry MR, Pandy MG. Contributions of muscles, ligaments, and the ground-reaction force to tibiofemoral joint loading during normal gait. J Orthop Res 2006;24:1983e90.

57. Wretenberg P, Ramsey D, Németh G. Tibiofemoral contact points relative to flexion angle measured with MRI. Clinical Biomechanics. 2002;17(6):477-485.

58. Felson D, Nevitt M. Blinding images to sequence in osteoarthritis: evidence from other diseases. *Osteoarthr Cartil*. 2009;17(3):281-283.

59. Ford RMD. Report of Task Force II: best practices in the use of medical imaging techniques in clinical trials: consensus from a public meeting, October 16e17, 2007.