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# **Title**

Isometric Hamstring:quadriceps Strength Ratio, Flexibility, And Gait Pattern As Predictors Of Knee Health

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# ISOMETRIC HAMSTRING:QUADRICEPS STRENGTH RATIO, FLEXIBILITY, AND GAIT PATTERN AS PREDICTORS OF KNEE HEALTH

By

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#### **ABSTRACT**

The knee joint, due to its frequent use, faces many movements and stresses that can cause its overall health to degrade, and subsequent pathologies to develop. I hypothesized that increased stress on the knee joint and imbalance in the supporting musculature would positively correlate with increased acoustic emission from the knee joint, a biomarker of inflammation and degraded cartilage in the joint. We tested this hypothesis by selecting a cohort of healthy, moderately active individuals aged 18-32 across a range of BMIs. We collected baseline knee acoustic measurements, measuring quadriceps and hamstring flexibility, measuring hamstring and quadriceps maximum voluntary isometric contraction, and analyzing participants' heel strike angle during self-selected walking gait. We found that heel strike angle did not correlate with increased acoustic emissions from the knee, but BMI had a negative correlation with hamstring:quadriceps strength ratio. We also found that left hamstring flexibility had a positive correlation with left heel strike angle. Finally, we found that right quadriceps flexibility had a positive correlation with right heel strike angle. Since the hamstring:quadriceps strength ratio is an important biomarker for knee health, especially in older populations, this finding may indicate early evidence of future knee health impairments in individuals with higher BMI. The changes in gait associated with muscle rigidity indicate that differential levels of upper leg muscle flexibility may translate to changes in the mechanics of everyday movements such as walking.

## **ACKNOWLEDGEMENTS**

I would like to thank my faculty mentor Dr. Erica Heinrich for her dedication, her support, and her guidance throughout this project. I feel incredibly fortunate that she took me into her lab and helped me develop both as a person and as a researcher. Without her, this project would not have been possible. I would also like Dr. Natalie Holt for generously lending her cameras, and Anthony Cobos, who guided me through using the cameras' software. Finally, I would like to thank my parents for their unconditional love and support throughout this project and throughout my college career.

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#### **INTRODUCTION**

The knee joint is a complex and significant weight-bearing joint used extensively throughout everyday life. Various aspects of this joint and the supporting musculature contribute to its overall health. One such factor is the hyaline cartilage (Nahian, 2022). Hyaline cartilage coats the condyles in the femur, tibia, fibula, and other synovial joints. It allows for bones in the joint to glide across each other smoothly, facilitating movement and absorbing impact. Cartilage is composed of both chondrocytes and the cartilaginous matrix, with very little nutrient supply from blood vessels. At equilibrium, chondrocytes maintain a balance between the production and the destruction of extracellular matrix components (Nahian, 2022). When an individual is injured, this balance shifts toward increased degradation, which may subsequently lead to osteoarthritis. These unique characteristics contribute to the slow healing nature of cartilage and to the development of knee pathologies. Most other body tissues do not respond to injury by increasing degradation and are therefore not subject to the same dysfunctions that affect the knee joint. Currently, there are gaps in our knowledge regarding how this equilibration changes and how knee pathologies subsequently progress.

Damage to the cartilage has several underlying causes, including but not limited to mechanical forces, genetic factors, and factors secondary to the individual's overall health. Osteoarthritis (OA), a debilitating and increasingly common disease, may result secondary to this cartilaginous damage. OA often affects the weight-bearing joints, such as the hips, knees, and ankles. It is accompanied by symptoms such as pain, limitations in range of motion, and muscular weakness (Martin, 1994). OA has long been regarded as a relatively simple pathology that arises due to chronic overuse. But new information indicates that OA has both primary (idiopathic) causes and secondary causes, such as trauma, underlying inflammatory conditions,

or crystalline disease (Martin, 1994). Additional factors related to metabolic and cardiovascular health have also been linked to osteoarthritis development, including chronic synovial inflammation, dyslipidemia, endothelial dysfunction, subchondral bone modification, and others (Coaccioli et al, 2022). Therefore, understanding the progression of knee health throughout an individual's life may provide enhanced preventative treatment options for knee pain and pathology.

One of the primary causes of OA is generally regarded to be excess force and stress on the knee joint. This may arise due to exercise, trauma, or other factors. Another significant contributor to this excess force may be less obvious: increased stress on the knee joint during daily walking. This increased stress may arise due to gait patterns or existing joint instability due to variation in supporting muscle strength (Baratta et al. 1988). While patients with OA (both symptomatic and asymptomatic) exhibit variations from normal gait, these are often due to pain and stiffness associated with the condition. Studies indicate that increased heel strike angle increases the force exerted on the knee joint during walking, placing increased stress on the knee joint (Levinger et al. 2008). Repetitive, increased stress is a key contributor to cartilaginous degradation and subchondral bone modification (Hurley, 1999).

Another well-documented and significant risk factor for osteoarthritis is obesity. Obesity increases stress on the knee joint due to the obese individual's heavier weight. Other etiological factors include adipokines and low-grade systemic inflammation which contribute to tissue inflammation and damage (Thijssen et al., 2015).

Furthermore, upper leg muscle imbalance plays a significant role in knee OA. Evidence suggests that weaker muscles are less able to stabilize the knee joint, therefore exacerbating degradation during routine locomotion and movement (Hurley, 1999). Multiple studies indicate

that while quadriceps weakness is frequently present in those with knee OA, it is not always secondary to pain. Weakness may therefore be a predictive or etiologic factor (Øiestad et al. 2015). The hamstring muscle also plays a significant part in knee joint stability (Blackburn et al., 2011). Higher levels of hamstring flexibility correlate with increased incidence of anterior cruciate ligament (ACL) tears, while hamstring tightness correlates with increased stability of the knee joint (Blackburn et al., 2011). When considering the significant role that the ACL has in maintaining overall knee joint stability, one can see how the hamstring would be a significant contributor to stability. This is further corroborated by existing literature. Antagonist (hamstring) muscles contribute significantly to knee joint stability during normal gait (Baratta et al. 1988). Hence, it is reasonable to suspect that characteristics of upper leg musculature are significant contributors to knee joint health. Differences in musculoskeletal stiffness between males and females may even contribute to differential injury rates between the sexes (Granata et al., 2002).

OA differs from rheumatoid arthritis in that it is not an autoimmune disease, but rather a degenerative one (Coaccioli et al, 2022). OA affects the hyaline cartilage most notably, but the damage spreads until the entire knee joint is afflicted. The tissues this pathology affect include the synovium, the subchondral bone, and the joint ligaments (**Figure 1**. Misalignments of soft tissue caused by strength imbalances may produce clicking or popping sounds within joints, due to the tissues rubbing against and over each other. This also contributes to declines in knee joint health. Hence, knee acoustic emissions have been used as biomarkers to quantify the state of the knee joint (Yiallourides et al., 2021). Increased peak and average knee acoustic emissions during flexion/extension suggest progressed pathology.

The goals of this research project are (1) to determine if hamstring:quadriceps strength ratio (HQ ratio) is correlated with biomarkers of knee health, including knee acoustic emissions,

and (2) to determine if gait patterns, quantified by heel strike angle, are associated with increased knee acoustic emissions. I hypothesize that a larger HQ ratio and an increased heel strike angle will both demonstrate a positive correlation with increased acoustic emissions from the knee. This study examines these relationships in a population of young adults to determine if these factors may lead to early signs of developing OA.



**Figure 1. Cellular mechanism of OA progression.** (Figure adapted from Coaccioli et al., 2022,

Created with BioRender.com).

### **METHODS**

# *Ethical statement:*

Experiments were approved by the UC Riverside Clinical IRB (HS 22-064). All work was conducted according to the *Declaration of Helsinki*, except registration in a database. Participants were informed about the benefits and risks of participating in the study prior to their participation. Participants were provided informed consent in their native language (English). *Study participants:*

27 moderately active, healthy university students (16 males, 11 females) aged 22 (3.85) were recruited in the winter of 2023 (**Table 1**). Exclusion criteria included: age greater than 35, history of major leg injury, surgery, or pain, elite athletes, history of neuromuscular impairment or disease, current knee or leg pain, and participants were required to be fluent in English to adequately follow experiment instructions. Prior to their appointment, participants were asked to shave the areas the microphone would be placed and wear shorts and form fitting clothing.



**Table 1. Participant demographics.** Data is represented as means (standard deviations). *Study design:*

Participants completed a knee evaluation questionnaire (2000 IKDC Subjective Knee Evaluation Form) and a lifestyle/demographics questionnaire prior to participation (Anderson et al., 2006). The IKDC form was scored by adding the participants' scores in each section and dividing by the highest possible score. We then measured basic physiological parameters including height, weight, and blood pressure. Blood pressure was collected using a manual sphygmomanometer. Following these procedures, participants completed a series of experimental maneuvers in the following order: Knee sound emission, Flexibility measures, Strength measures, Gait analysis.

## *Knee sound emission:*

2 cardiac microphones were placed on the inside and outside of the R tibiofemoral joint (MLT201, ADInstruments, Dunedin, FL, USA) and secured using cloth medical tape and a knee brace, as depicted in **Figure 2**. Hair removal was performed with a razor at the site of microphone placement if necessary to avoid interference with sound production. Audio emission signals were detected by the microphone and sent to a data interface (Powerlab 8/35, ADInstruments) which transmitted digital voltage measures to analog data which was collected using LabChart 8 software (ADInstruments). Subjects were instructed to perform 5 seated knee extensions, with at least one second of rest between each repetition. After this, subjects were instructed to perform 5 sit-to-stand repetitions, again resting for at least one second between each repetition. The same procedure was repeated for the left leg. To quantify audio emissions from the knee joint, peak and mean signal amplitudes were measured during each movement in LabChart 8.



**Figure 2. Experimental setup for the knee sound emission portion.** 

# *Flexibility measures:*

Hamstring flexibility was quantified using a popliteal angle test for both lower extremities. Quadriceps flexibility was measured using a modified Thomas test (Harvey, 1998). Briefly, subjects were instructed to lie on their back and bring one knee to their chest, grasping their shin with both hands. Participants were instructed to relax the opposite leg while keeping their lumbar spine in contact with the bench during the entire maneuver. Once participants

confirmed their leg was relaxed, popliteal joint angle was measured. Then, their knee was flexed until they reported discomfort and popliteal joint angle was re-measured. Angles were measured using a 12" goniometer (Ever Ready First Aid, Brooklyn, NY). A passive knee extension test was performed to measure hamstring flexibility (Gnat et al., 2010). The participant was placed on the bench with their hip at 90 degrees, pointing directly upward. Participants were then instructed to extend their knee as far as they voluntarily could, and popliteal joint angle was measured. We then further straightened the participant's knee until they reported discomfort and re-measured the popliteal joint angle.

#### *Strength measures:*

Maximal voluntary isometric contraction (MVIC) measures were determined for both the quadriceps and the hamstring. Surface electromyography (EMG) probes were placed on subjects' rectus femoris muscles, as this has been shown to be the most activated during quadriceps extension (Soderberg et al., 1983). Subjects were seated upright on an exercise bench with their knees placed at a 90-degree angle **(Figure 3A)**. The exercise bench was anchored in place to prevent movement. Subjects were then instructed to attempt to extend their knee for 3 seconds, timed using a stopwatch. Subjects' knees were reset to 90 degrees between trials and the left and right legs were tested separately. The hamstring MVIC test followed after both quadriceps were tested. EMG probes were placed on the semitendinosus muscle, as this has been shown to be the most activated during 90-degree MVIC of the hamstring (Onishi et al., 2002). Subjects were placed in the supine position, with one knee at 90 degrees and the opposite foot resting on the ground for support **(Figure 3B)**. Subjects were then instructed to maximally contract their hamstrings for 3 seconds, timed using a stopwatch. Subjects were allowed to relax their hamstring, bringing the knee to 180 degrees, to prevent injury and minimize unwanted fatigue.

During each contraction, peak force production was measured using a small scale that measured the maximum force output (Klau OCS-L Weighing Scale). MVIC measures were conducted in triplicate and averaged for each leg.



**Figure 3. (A) EMG placement and setup for quadriceps MVIC. (B) EMG placement and setup for hamstring MVIC.**

# *Gait analysis:*

Following the leg strength test, subjects removed their shoes and high contrast markers were placed on various aspects of the subjects' feet/legs/hips (**Figure 4**). Markers were placed on the greater trochanter of the femur, the lateral epicondyle of the femur, the lateral malleolus of the ankle, the calcaneus, the distal aspect of the 5th metatarsal, the first distal phalange, the distal aspect of the first metatarsal, and on the tibialis anterior tendon. Subjects were then instructed to walk at their preferred walking speed in a straight line on a solid ground surface. During this maneuver, high-speed video recordings of the hip joints and below were recorded (SC1 High Speed Video Camera, Edgertronic, San Jose, CA, USA). Subjects were instructed to fixate on a point at eye level straight in front of them and rest their arms on their shoulders. After subjects

walked through once, they were instructed to repeat the same procedure, but facing the opposite way so both the right and left leg could be visualized.



# **Figure 4. Point labeling conventions.**

Prior to each video recording, calibration was performed using a 1m x 1m x 0.3m calibration object, made of 3 acrylic sheets 15 cm apart **(Figure 5)**. Calibration points were arranged 10 cm apart in a grid on each acrylic sheet, for a total of 132 points per sheet. 3-D tracking of each point was performed by uploading each video to dltdv8 software in MATLAB (MathWorks, Natick, MA) and manually tracking each point.



#### **Figure 5. Calibration object from the lateral camera view.**

To calculate heel-strike angles, ImageJ (LOCI, University of Washington, WA) was used. The video frame in which the heel first contacted the floor was chosen for analysis. Heel strike angle was calculated by using the angle function and measuring from a perfect level, determined by the y-value in the image used, as well as points R/L4 and R/L5.

#### *Statistical analyses:*

All statistical analyses were performed in R Studio (R version 4.2.2). To determine if knee joint audio emissions were correlated with our variables of interest (heel strike angle and HQ ratio), we first checked the normality of each variable's distribution using a Shapiro-Wilks tests for normality and visualization via Q-Q plots. If the assumption of normality was met, Pearson correlations were performed using the stat\_cor function in the *ggpubr* package in R to examine linear relationships between these variables. We also conducted a large-scale correlation analysis of all variables of interest using the *rcorr* function in the *Hmisc* package in R. To adjust for the impact of potential covariates on our outcome of interest, we performed general linear model analyses with age, sex, and BMI as covariates using the lm function in R.

## **RESULTS**

Healthy university students (N=16 men, 11 women) between 18 and 32 years of age (22  $\pm$ 3.8 years) were recruited for this study. To determine baseline knee health characteristics, participants completed the IKDC Subjective Knee Evaluation Form. **Figure 6** shows a histogram indicating the distribution of the IKDC Subjective Knee Evaluation Form scores. **Figure 7** shows a Pearson correlation matrix for all the variables of interest. **Figure 8** shows a negative correlation between bilateral hamstring:quadriceps strength ratio and BMI on the right leg with a similar trend on the left leg. The left leg was less likely to be the dominant limb, with n=20 participants reporting that they were right-leg dominant. **Figure 9** demonstrates a slight negative trend between peak/average mic amplitude data and BMI.



**Figure 6. Distribution of IKDC Knee Health scores.** X-axis shows the range of scores, y-axis

shows the number of participants whose scores fall within that range of scores.



**Figure 7. Pearson correlation matrix for all variables of interest.** Dot size indicates

significance level (p value). Dot color represents correlation coefficient as indicated by the rightside legend.



**Figure 8. Negative correlation observed between BMI and HQ ratio.** R and p values for a Pearson correlation analysis are provided. Each point represents data from a single participant, with one measurement plotted per individual.



**Figure 9. Results for average acoustic emission from the knee joint and HQ strength ratio.**  Plots represent data collected during either seated knee extension (B, D, F, H) or during movement from a seated to standing position (A, C, E, G). Data is recorded from two microphone positions located on the medial joint line (M1) or the lateral joint line (M2). R and p values from a Pearson correlation analysis are provided in blue text. Each data point represents a measure from a single participant, with one measurement plotted per individual.



**Figure 10. Representative results for average acoustic emission from the knee joint and heel strike angles.** Histogram showing the distribution of heel strike angles across participants **(A)**. Representative data from 1 microphone position compared to heel strike angles from left foot **(B)** and right foot **(C)**. R and p values from a Pearson correlation analysis are provided in blue text. Each data point represents a measure from a single participant, with one measurement plotted per individual.



**Figure 11**. **Representative results for quadriceps/hamstring flexibility and heel strike angles.**

Plots represent data collected during either the modified Thomas test (A, B, E, F) or during the passive knee extension test (C, D, G, H). For hamstring flexibility measures, larger angles represent increased flexibility. For quadricep flexibility measures, larger angles represent decreased flexibility. R and p values from a Pearson correlation analysis are provided in blue text. Each data point represents a measure from a single participant, with one measurement plotted per individual. **(I)** Diagram of modified Thomas test. The red semicircle indicates the angle measured. **(J)** Diagram of popliteal angle test. The red semicircle indicates the angle measured.

To determine if BMI and HQ ratio were related, Pearson correlation coefficients were calculated for both the left and right HQ ratio. Correlation coefficients in **Figure 8** indicate that BMI has a negative relationship with HQ ratio. To determine if HQ strength ratio and knee acoustic emission were related, we performed a Pearson correlation analysis comparing heel strike angle with the mean and peak amplitude of acoustic emissions from the microphones placed on both the medial and lateral joint line. There may be a relationship between decreased HQ strength ratio and reduced audio emissions from the knee **(Figure 9H)**. To determine if heel strike angle and knee acoustic emissions were related, we performed a Pearson correlation analysis between the two variables. Heel strike angle may have a weak relationship with mean audio emission amplitude from the knee **(Figure 10)**. We performed Pearson correlation analyses to determine if quadriceps and hamstring flexibility had any relationship with heel strike angle. We compared both hamstring flexibility and quadriceps flexibility (assisted and unassisted) to heel strike angle. Left hamstring flexibility (both assisted and unassisted) has a strong positive correlation with left heel strike angle **(Figure 11C, D)**. Right assisted quadriceps flexibility has a strong positive correlation with right heel strike angle **(Figure 11E)**. Unassisted right quadriceps flexibility showed a weaker correlation with right heel strike angle **(Figure 11F)**.

#### **DISCUSSION**

The goal of this study was (1) to determine if hamstring:quadriceps strength ratio relates to biomarkers of knee health, including knee acoustic emissions, and (2) to determine if gait patterns, quantified by heel strike angle, are associated with increased knee acoustic emissions. To accomplish this goal, we collected knee acoustic emissions, upper leg muscle flexibility measures, and normal walking gait data from moderately active, healthy young adults across a range of BMIs. IKDC scores in this cohort were all above 87, with 16 participants ranking their score as 100. This indicates that our cohort had good knee health scores on average. Our results demonstrate a negative relationship between HQ ratio and BMI for the right leg (R=-0.38, p<0.05). However, no relationship was identified between knee joint acoustic emission amplitude and BMI, and no relationship was identified between heel strike angle and acoustic emission amplitude in this cohort. We did not find any links between this measure of knee joint health and HQ ratio or gait pattern in this cohort of young adults. We found a positive relationship between left hamstring flexibility (both assisted and unassisted) and left heel strike angle  $(R=0.5, p<0.01)$ . We also found a positive correlation between assisted right quadriceps flexibility and right heel strike angle  $(R=-0.46, p<0.02)$ . The negative relationship observed between HQ ratio and BMI implies that individuals with a higher BMI may be at greater risk for eventual knee dysfunction due to decreased knee joint stability. The relationship observed between hamstring/quadriceps flexibility and heel strike angles indicates that differential flexibility levels may affect normal gait.

Our findings corroborate much of the existing literature regarding the relationship between obesity and increased risk of eventual OA (Pottie et al. 2006). Other studies examine both mechanical factors (increased stress on the knee joint due to heavier body weight) and

inflammatory factors (chronic synovial inflammation and adipokines) that put obese individuals at higher risk for this pathology. However, such a correlation between the two variables was unexpected in the cohort studied here, especially when considering that the study employed healthy, moderately active young adults. This finding portrays the importance of maintaining a healthy lifestyle throughout the course of one's life. While individuals with higher BMI may be able to eventually lose weight, their time spent at higher BMIs may accelerate the degradation of their knee joint. Individuals with higher BMIs already face increased stress on the knee joint, and this study indicates that the effects of this stress may be exacerbated by the imbalance in the QH ratio.

The mechanism behind the negative correlation between BMI and HQ ratio remains unclear. The quadriceps muscle is more involved than the hamstring muscle in walking (Hurley, 1999). Since walking is the most common form of human locomotion, we hypothesize that individuals with higher BMI may perform comparatively more of this exercise versus exercises that target the hamstring more directly. This observed pattern may also be a question of differential rates of atrophy between the two muscle groups. Currently, there is little data that examines how BMI and HQ ratio are associated in healthy, young adults. As such, future work will investigate the trend observed.

In the cohort studied, there was a lack of a relationship between age and knee acoustic emissions. Other work indicates that peak physiological performance is maintained until approximately age 35, which corroborates our study's findings (Tanaka et al. 2008). It would therefore be reasonable to conclude that age-related declines in knee health begin after this time frame. This is further corroborated when considering incidence of knee osteoarthritis as a function of age. As a population ages, knee OA incidence increases (Prieto-Alhambra et al.,

2014). Finally, a relatively narrow age range was purposely selected to minimize the effect of age in our study, which is already known to be a significant contributor to declines in knee health.

While we expected to see a relationship between heel strike angle and knee joint acoustic emissions, there was no significant correlation between these two variables. We did observe a slight negative between heel strike angle and many of the measures of knee acoustic emission. We therefore cannot conclude that increased heel strike angle is associated with declines in knee health in this cohort. However, heel strike angle is just one aspect of the myriad of gait components. The lack of a correlation between these two variables does not mean that abnormal walking patterns do not place excess stress on the knee joint. Future research can investigate other aspects of self-selected walking gait, such as walking speed, stance/swing phase parameters, and knee varus/valgus.

Dominant leg preference may contribute to the trends visualized between flexibility and heel strike angle. Most participants  $(n=20)$  were R leg dominant. However, the relationship between upper leg muscle flexibility and gait is likely complex and multi-joint. Furthermore, there is little data examining how muscle flexibility affects gait in healthy individuals. These two factors make it difficult to draw conclusions with the available data. Additionally, although muscle rigidity itself may not be pathologic, the collected data indicates an association with different gait patterns in this cohort. It is reasonable to suspect therefore that chronic upper leg muscle stiffness may lead to lasting effects on the way individuals walk or run. If these changes in gait cause chronic increased stress on the knee joint, knee pathologies may arise secondary to upper leg muscle rigidity. Future analyses will use other aspects of self-selected walking gait to observe patterns in the relationship between upper leg muscle flexibility and gait.

Of note: there was a weak correlation between HQ ratio and acoustic emissions from the knee joint. While evidence indicates that imbalanced upper leg musculature leads to decreased stability of the knee joint, the knee joints of the cohort studied may not have undergone enough chronic stress secondary to this imbalance for it to have any significant effects. This relationship, however, would be expected to increase with age.

Our current study has some limitations. Higher BMI may have decreased the average and peak amplitude of the acoustic emissions from the knee joint due to increased fat deposit thickness between the knee joint and the surface microphone probe. Future studies will incorporate waveform analyses to characterize the sounds recorded, as has been done in other studies (Yiallourides et al. 2021). Sound character may not be impacted by changes in BMI. Furthermore, knee acoustic emissions paint an incomplete picture, in that the knee joint cannot be visualized with this method. Future work could utilize imaging technology, such as ultrasound or magnetic resonance imaging to better understand the state of the knee joint. Measures of electrical bioimpedance can also provide a quantification of inflammation and fluid retention in the joint. Also, BMI is limited in determining whether an individual is obese or not. Future studies can also examine body fat percentage as a more accurate measure of obesity.

#### **CONCLUSION**

In the cohort studied, HQ ratio is not significantly related to biomarkers of knee health (including knee acoustic emissions) and heel strike angle is not significantly related to acoustic emissions from the knee joint. However, we found that an individual's HQ ratio may provide insight into their overall health. This is highlighted by the negative relationship between HQ ratio and BMI. The mechanism to explain this relationship remains unknown. We also found that increased hamstring rigidity shows a significant relationship with decreased heel strike angle on the left leg, which was more commonly the nondominant leg. We found that increased right quadriceps flexibility correlates with increased right heel strike angle. The right leg was more frequently reported as the dominant leg. Therefore, differential levels of upper leg muscle flexibility may contribute to changes in gait. Future work must be done to determine whether these gait changes increase the stress placed on the knee joint during walking.

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