Title

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Isometric Hamstrings:Quadriceps Strength Ratio, Flexibility, and Gait Pattern as Predictors of Knee Health

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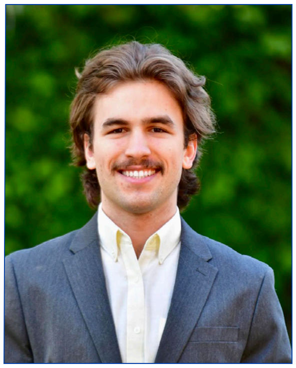
ABSTRACT

The knee joint faces daily stresses that cause its overall health to degrade and pathologies to develop. I hypothesized that increased stress on the knee joint and imbalance in thigh musculature would positively correlate with increased acoustic emissions from the knee joint, a biomarker of inflammation 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 and measured quadricep and hamstring flexibility, hamstring and quadricep maximum voluntary isometric contraction, and heel strike angle during self-selected walking gait. Heel strike angle does not correlate with increased acoustic emissions from the knee, but BMI negatively correlates with the hamstrings:quadriceps strength ratio. Furthermore, left hamstring flexibility positively correlates with left heel strike angle. Finally, we found that right quadricep flexibility had a positive correlation with right heel strike angle. Since the hamstrings:quadriceps strength ratio is an important biomarker for knee health, this finding may indicate evidence of progressing knee pathology in individuals with higher BMI. 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.

KEYWORDS: knee health, BMI, strength, stability

FACULTY MENTOR - Dr. Erica Heinrich, Department of Biomedical Sciences

Dr. Erica Heinrich is an Assistant Professor at the UC Riverside School of Medicine, Division of Biomedical Sciences. She trained as a comparative physiologist and currently studies the control of breathing and inflammatory signaling in human populations at high altitude.



LORENZO BAZZANI

Lorenzo Bazzani completed his Bachelor of Science in Cell, Molecular, and Developmental Biology, Magna Cum Laude, in June 2023. In 2022, he began researching physiological systems in the Heinrich Lab. Using the knowledge and questions sourced from his time as a hospital volunteer and scribe, he designed and conducted his Honors Capstone under Dr. Erica Heinrich's supervision. He is now a medical assistant at a sports medicine clinic while applying to medical schools.

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INTRODUCTION

The knee joint is a complex weight-bearing joint used extensively in everyday life. Its stability is maintained by ligaments, cartilage, and the muscles surrounding the joint. Due to its frequent use, the joint is prone to a myriad of painful conditions. Among the most common of these conditions is osteoarthritis (OA). OA most often affects weight-bearing joints and is characterized by pain, limitations in range of motion, and muscular weakness (Coaccioli et al., 2022). As of now, the only cure for OA is reconstructive surgery. Other therapies focus on managing the symptoms associated with the condition. OA has long been regarded as a relatively simple condition that arises due to chronic overuse. However, recent findings indicate that factors related to metabolic and cardiovascular health contribute to osteoarthritis development. These include chronic joint inflammation, cholesterol imbalances in the blood, and blood vessel dysfunction (Coaccioli et al., 2022). Therefore, understanding the progression of knee health throughout an individual's life may provide enhanced preventative treatment options for knee pathologies. The hope is that if factors that exacerbate knee OA are present in a younger individual, these may be corrected before the disease has progressed significantly.

A critical factor in the knee joint's function is the hyaline cartilage, which coats the articular surfaces of synovial joints (Nahian, 2022). It allows for the femur and tibia to glide across each other smoothly, facilitating movement and absorbing impact. Cartilage is a tissue with little nutrient supply from blood vessels and is composed of chondrocytes and the cartilaginous matrix. Unlike other cells, chondrocytes respond to increased stress by dying, which reduces cushioning and increases stress on the underlying bone. Bone cells, conversely, respond to increased stress by producing more bone (Nahian, 2022). This bone modification, coupled with chondrocyte death, transforms the once-smooth articular surface into a rough and poorly cushioned environment. These rough surfaces grinding together cause significant pain in individuals with OA.

The knee joint may face increased stress during locomotion due to abnormal gait patterns. Studies indicate that increased heel strike angle increases the force exerted on the knee joint during walking (Levinger et al., 2008). Repetitive, increased stress contributes to cartilaginous degradation and subchondral bone modification (Hurley, 1999). Therefore, gait analysis may provide valuable insight into knee OA progression.

Thigh musculature also contributes to knee stability, especially during movement. The thigh musculature includes quadricep muscles in the front of the thigh, which contract to straighten the knee, and hamstring muscles in the back of the thigh, which assist in bending the knee. Evidence suggests that weaker thigh muscles stabilize the knee joint less effectively, therefore increasing stress on cartilage during locomotion and movement (Hurley, 1999). Multiple studies indicate that while quadricep weakness is frequently present in those with knee OA, it is not always secondary to pain. Weakness may therefore be both a predictive and etiologic factor (Øiestad et al., 2015). Hence, it is reasonable to suspect that thigh muscle characteristics contribute to knee joint health.

Knee acoustic emissions (AEs) have been utilized as convenient, cost-effective, and non-invasive biomarkers to objectively quantify the state of the knee joint (Yiallourides et al., 2021). Unlike X-rays or magnetic resonance imaging (MRI), AEs do not require large, costly machines. Furthermore, AEs are quickly and easily measured using small microphones placed on the surface of the skin. Studies indicate that increased peak and average knee AEs during flexion and extension suggest progressed pathology (Yiallourides et al., 2021). Hence, this study will include the use of knee AEs to examine knee health.

The goals of this research project are (1) to determine if hamstrings:quadriceps (HQ) strength ratio correlates with biomarkers of knee health, including knee AEs, and (2) to determine if gait patterns, measured by heel strike angle, correlate with increased knee AEs. I hypothesize that a larger HQ ratio and a larger heel strike angle will both demonstrate a positive correlation with increased AEs from the knee.

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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 provided informed consent, including the benefits and risks of study participation, in their native language (English) before participation.

Study participants:

27 moderately active, healthy university students (16 males, 11 females) with a mean age of 22 years (SD: 3.85 years) 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 lack of English fluency. Prior to their appointment, participants were asked to shave the areas of microphone placement and wear shorts and form-fitting clothing.

Variable	Men (N=16)	Women (N=11)
Age (years)	22.19 (4.02)	21.91 (3.58)
Height (cm)	177.05 (6.93)	161.35 (4.35)
Weight (kg)	78.66 (12.77)	59.52 (6.42)
Body Mass Index (kg/m^2)	25.08 (3.46)	22.96 (3.18)

Table 1. Participant demographics. Data are 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). We then measured physiological parameters including height, weight, and blood pressure. Following these procedures, participants completed a series of maneuvers in the following order: knee audio emission, flexibility measures, strength measures, and gait analysis.

Knee acoustic emission:

Two cardiac microphones were placed on the medial and lateral aspect of the right knee joint (MLT201, ADInstruments, Dunedin, FL, USA) and secured using cloth medical tape and a knee brace, as depicted in Figure 1. Hair removal was performed with a razor at the site of microphone placement if necessary to ensure accurate acoustic emission recording. The microphone detected audio signals and sent these to a data interface (Powerlab 8/35, ADInstruments) which transcribed digital voltage measures to analog data which was collected using LabChart 8 software (ADInstruments). Subjects were instructed to perform five seated knee extensions, resting for at least one second between each repetition. After this, subjects were instructed to perform five sit-to-stand repetitions, again resting for at least one second between each repetition. This procedure was repeated for the left leg. To quantify AEs from the knee joint, peak and mean signal amplitudes were measured during each movement in LabChart 8.

Figure 1. Experimental setup for the knee acoustic emission portion.

Flexibility measures:

Angles were measured using a 12” goniometer (Ever Ready First Aid, Brooklyn, NY). Quadricep flexibility was measured using a modified Thomas test (Harvey, 1998) Briefly, each participant was instructed to lie on their back and bring one knee to their chest, grasping their shin with both hands. Each was instructed to relax the opposite leg while keeping their lumbar spine in contact with the bench during the entire maneuver. Once the participant confirmed their leg was relaxed, the popliteal joint angle was measured. Then, the testing knee was flexed until they reported discomfort, and the joint angle was re-measured.

We measured hamstring flexibility with a passive knee extension test (Gnat et al., 2010). The participant was placed on the bench with their hip flexed at 90 degrees. They were then instructed to extend their knee as far as they voluntarily could, and the popliteal joint angle was measured. We then further straightened the participant's knee until they reported discomfort and re-measured the joint angle.

Strength measures:

Maximal voluntary isometric contraction (MVIC) measures were determined for both the quadriceps and the hamstrings. Surface electromyography (EMG) probes were placed on participants' rectus femoris muscles. The participants were seated upright on an exercise bench with their knees at a 90-degree angle. The exercise bench was anchored in place to prevent movement. The participants were then instructed to maximally contract their quadriceps for three seconds, timed using a stopwatch. Their knees were reset to 90 degrees between trials and the left and right legs were tested separately. The hamstring MVIC test followed quadricep testing. EMG probes were placed on the semitendinosus muscle. The participants were placed supine with one knee on the bench at 90 degrees and the opposite foot resting on the ground for support. They were then instructed to maximally contract their hamstrings for three seconds, timed using a stopwatch, after which they were allowed to relax their hamstrings to minimize unwanted fatigue. During each contraction, peak force production was measured using a scale that measured maximum force output (Klau OCS-L Weighing Scale). MVIC measures were conducted in triplicate and averaged for each leg.

Gait analysis:

The participants removed their shoes, and we placed high-contrast markers on various aspects of the subjects' feet, legs, and hips. Markers were placed on the greater trochanter of the femur, the lateral knee joint line, the lateral malleolus of the ankle, the lateral calcaneus, the distal aspect of the fifth metatarsal, the first distal phalange, the distal aspect of the first metatarsal, and on the tibialis anterior tendon. The participants were 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). The participants were instructed to fixate on a point at eye-level straight in front of them and rest their arms on their shoulders. After they walked through once, they were instructed to repeat the same procedure, but facing the opposite way.

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 R4/L4 and R5/L5.

Statistical analyses:

All statistical analyses were performed in R Studio (R version 4.2.2). To determine the relationship between knee joint AEs and our variables of interest (heel strike angle and HQ ratio), we first checked the normality of each variable's distribution using a Shapiro-Wilks test 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 ±3.8 years) were recruited for this study. Figure 2 shows a negative correlation between HQ strength ratio and BMI on the right leg with a similar trend on the left leg. N=20 participants reported that they were right-leg dominant.

To determine if BMI and HQ ratio were related, Pearson correlation coefficients were calculated for both the left and right HQ ratios. Correlation coefficients in Figure 2 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 AEs from the microphones placed on both

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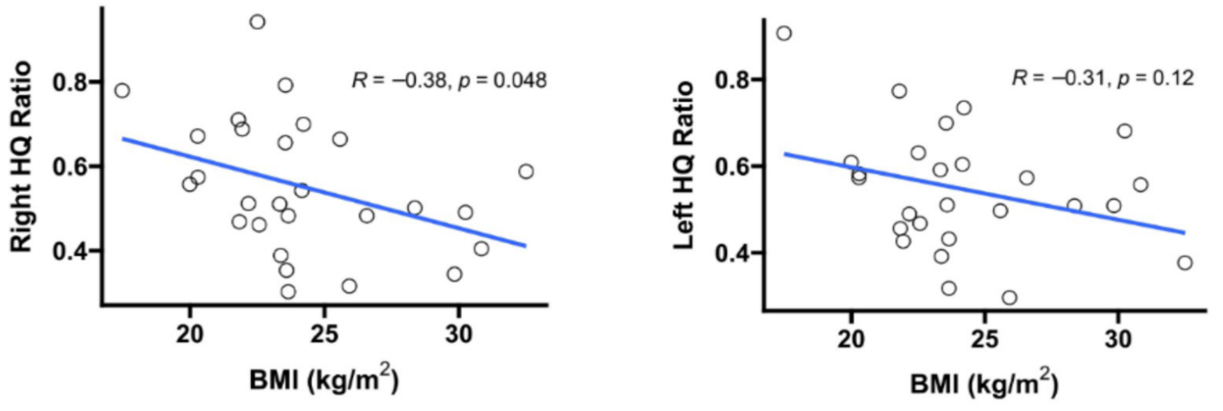

Figure 2. Negative correlation observed between BMI and HQ ratio. R and p values for a Pearson correlation analysis are provided. Each point represents data from a single participant, with one measurement plotted per individual.

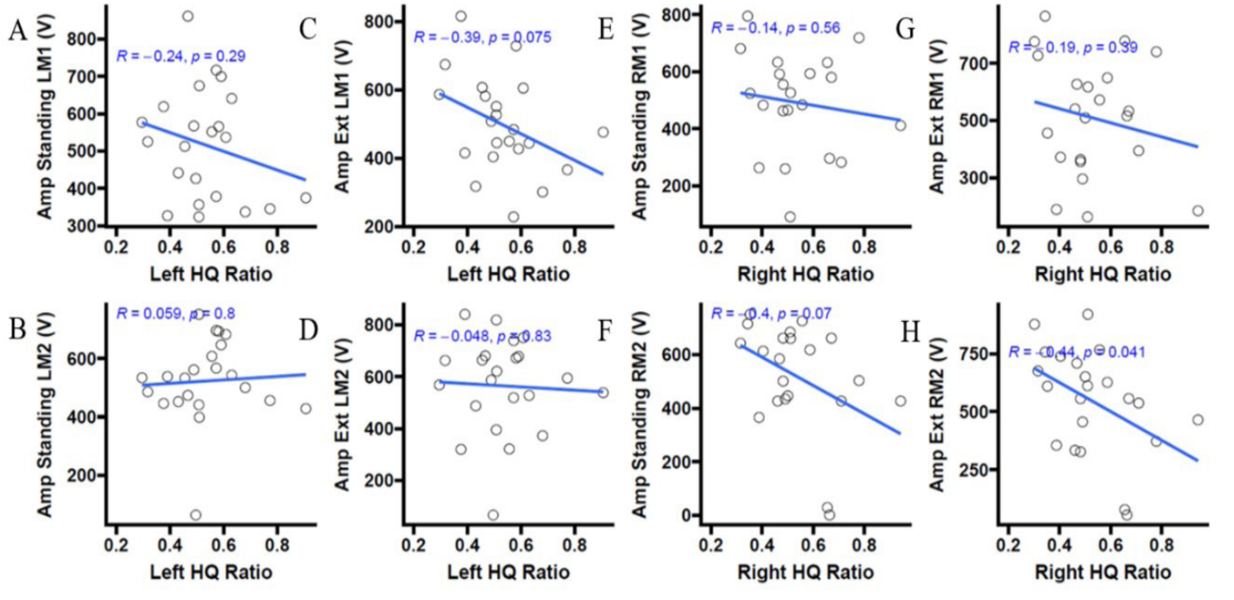

Figure 3. Results for average acoustic emission from the knee joint and HQ strength ratio. Plots represent data collected during seated knee extension (B, D, F, H) or standing from a seated position (A, C, E, G). Data is recorded from a microphone located on the medial joint line (M1) and the lateral 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.

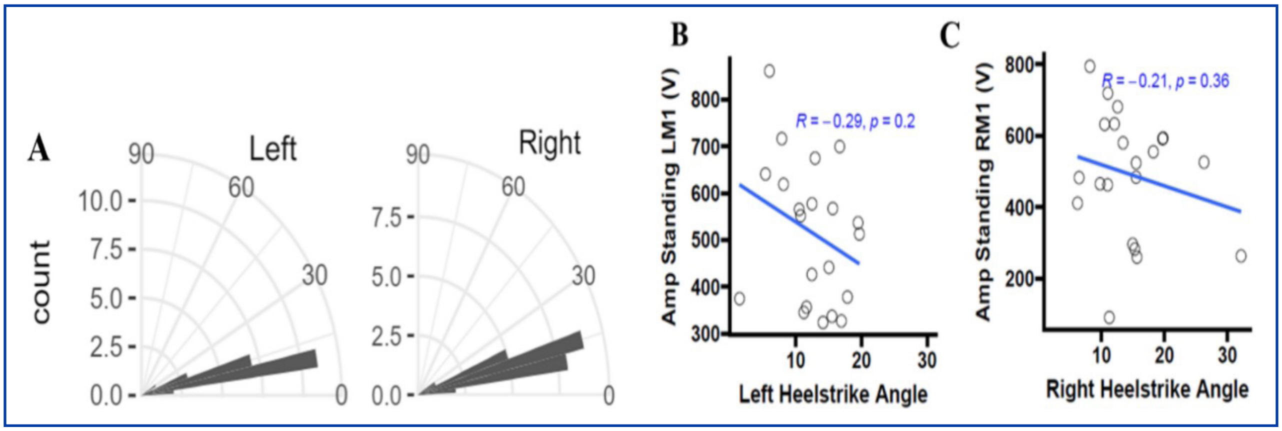

Figure 4. Representative results for average acoustic emission from the knee joint and heel strike angles. Histogram showing the distribution of heel strike angles across participants (A). Representative data from one microphone position compared to heel strike angles from left foot (B) and right foot (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.

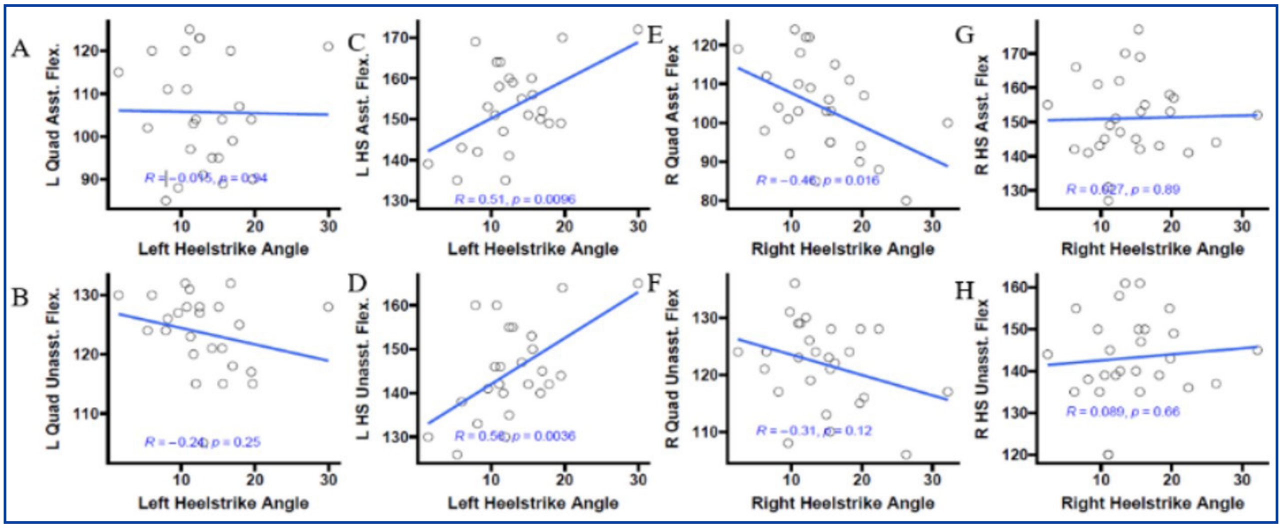

Figure 5. Representative results for quadricep/hamstring flexibility and heel strike angles. Plots represent data collected during either the modified Thomas test (A, B, E, F) or during the passive knee extension test (C, D, G, H). For hamstring flexibility measures, larger angles represent increased flexibility. For HQ 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.

the medial and lateral joint line. There may be a relationship between decreased HQ strength ratio and reduced AEs from the knee (Figure 3H). To determine if heel strike angle and knee AEs were related, we performed a Pearson correlation analysis between the two variables. Heel strike angle may have a weak relationship with mean AE amplitude from the knee (Figure 4).

We performed Pearson correlation analyses to determine if quadricep and hamstring flexibility had any relationship with heel strike angle. We compared both hamstring flexibility and quadricep 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 5C, D). Right assisted quadricep flexibility has a strong positive correlation with right heel strike angle (Figure 5E). Unassisted right quadriceps flexibility showed a weaker correlation with right heel strike angle (Figure 5F).

DISCUSSION

The goal of this study was (1) to determine if the HQ strength ratio relates to biomarkers of knee health, including knee AEs, and (2) to determine if gait patterns, quantified by heel strike angle, are associated with increased knee AEs. Our findings corroborate much of the existing literature regarding the relationship between obesity and an increased risk of eventual OA (Pottie et al., 2006). However, such a correlation between HQ strength ratios and BMI was unexpected in the cohort studied here, considering that the study employed healthy, moderately active young adults. It has long been established that individuals with higher BMIs face increased stress on the knee joint. However, this study suggests that the negative effects of this stress are exacerbated by imbalances in the HQ ratio. Hence, not only do individuals with a higher BMI place increased stress on their knees, their imbalanced musculature also renders the joint less stable. This instability can exacerbate the progression of knee OA if left uncorrected.

The mechanism behind the negative correlation between BMI and HQ ratio remains unclear. The quadricep muscles are more involved than the hamstring muscles 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 hamstrings 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 observed trend. Future work may also incorporate more accurate measures of body fat percentages, such as DXA scans or skinfold calipers, to better elucidate the relationship between body mass and HQ ratio.

While we expected to see a relationship between heel strike angle and knee joint AEs, there was no significant correlation between these two variables. Hence, we 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 an observed relationship 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.

The relationship between upper leg muscle flexibility and gait is complex and multi-joint. Furthermore, there is little data examining how muscle flexibility affects gait in healthy individuals. This makes it difficult to draw conclusions from the available data. Additionally, muscle stiffness itself may not be pathologic, but the collected data indicates an association between flexibility and gait patterns in this cohort. Therefore, chronic thigh muscle stiffness may lead to lasting effects on gait. If these changes in gait cause chronic increased stress on the knee joint, OA may progress more quickly 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.

Our current study has some limitations. A higher BMI may have decreased the average and peak amplitude of the AEs from the knee joint due to increased subcutaneous fat thickness between the knee joint and the surface microphone probe. Future studies will incorporate waveform analyses to characterize the acoustic emissions recorded, as other studies have done (Yiallourides et al., 2021). Waveforms may not be impacted by changes in BMI. Furthermore, knee AEs 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.

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. Increased hamstring stiffness shows a significant relationship with decreased heel strike angle on the left leg, which was more frequently reported as the nondominant leg. We found that increased right quadricep flexibility correlates with increased right heel strike angle. 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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