VASCULAR FUNCTION IN PATIENTS WITH CHRONIC ANKLE INSTABILITY AND HEALTHY ADULTS

by

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ABSTRACT

CASEY BRUCE. Vascular function in patients with chronic ankle instability and healthy adults. (Under the direction of DR. ABBEY THOMAS FENWICK)

Introduction: Patients with CAI display impaired sensorimotor output and deficits in afferent/sensory processing which negatively impact motor control and function. While these neural and sensorimotor deficits are well documented in patients with CAI, changes in the vascular structure and function are not commonly understood in this population. With many blood vessels running parallel and in close proximity to the nerves, it is likely that these structures are also damaged during joint injury such as a lateral ankle sprain. Vascular damage may impair blood flow to the nerves and muscles and may potentially be a source of poor sensorimotor function.

Objective: To observe neurovascular function in the ankles of patients with and without chronic ankle instability to determine if vascular damage is present along with changes in neural excitability.

Methods: A total of 10 participants with CAI and 7 healthy control participants participated in this study. Participants reported for a single testing session which contained spinal reflex excitability testing, blood flow analysis, and exercise. Blood flow analysis occurred before and immediately after exercise using the same procedures. Soleus spinal reflex excitability testing was assessed by eliciting a maximal H-reflex and M-response from the muscle. Blood flow was analyzed using an infrared thermal camera

and captured videos of each participant's lower legs and feet before, during, and after exercise. A region of interest (ROI) was selected posterior to the malleoli for both anterior and posterior views. The ROI was used for data analysis. Participants completed exercises containing three sets of ten of calf raises and bodyweight squats. Exercise were counterbalanced between subjects and separated by five minutes of rest. H_{max}:M_{max}ratios were compared between groups and limbs using 2x2 ANOVAs. Thermal imaging data were analyzed using Bland-Altman plots to determine agreement between the temperature of both limbs within each group. The association between peak temperature within each ROI and spinal reflex excitability were analyzed using Pearson Product Moment correlation coefficients were calculated for participants in the CAI group. Statistical analyses were performed using SPSS (IBM, Armonk, NY) and Matlab.

Results: Spinal reflex excitability was not different between limbs or groups. There was no association between spinal reflex excitability and blood flow in the involved (posterior: r=-0.137, *P*=0.706; anterior: r=-0.338, *P*=0.339) or uninvolved (posterior: r=-0.039, *P*=0.916; anterior: r=-0.078, *P*=0.829) limbs of the CAI group. Analysis displays agreement in the surface temperatures obtained from both the posterior and anterior ROI between limbs in the participants with CAI. There was also agreement in posterior and anterior ROI surface temperatures between the limbs in health participants.

Conclusions: Our findings reveal no statistical relationship between spinal reflex excitability and blood flow between limbs or groups when examining patients with CAI

and healthy adults. Therefore, impaired neurovascular function may not contribute to the cycle of recurrent ankle sprains that is observed in patients with CAI.

DEDICATION

To my wife, Kaeleen, and my mother, Eileen without whom this project would not have been possible. Thank you for your support, motivation, and enthusiasm throughout this process.

ACKNOWLEDGMENTS

I would first like to thank Dr. Abbey Thomas for her mentorship and endless patience over the last four years. I truly appreciate all of the guidance and encouragement you have given me throughout this process. This project would not have been possible without your support. I would also like to thank Dr. Luke Donovan and Dr. Susan Trammell for all of their help and support as my thesis committee. I would like to thank all members of the Biodynamics Laboratory for always supporting me throughout the last two years. Finally, I would like to thank all members of the Biomedical Imaging Laboratory.

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CHAPTER 1: INTRODUCTION

Lateral ankle sprains are the most widely reported injury in athletics, occurring at a rate of an estimated 23,000 per day in the United States.[1,2] [3] Further, lateral ankle sprains are suggested to account for up to 45% of injuries in athletics. [2-6] Treatment costs associated with lateral ankle sprains exceed $4.4 billion dollars a year, making these injuries of great concern within the healthcare system.[2,5,7]

Lateral ankle sprains also carry with them a host of residual physical deficits. Up to 74% of individuals experience recurrent episodes of instability characterized by the ankle "giving way" following an initial sprain. [2,3,5,6] This condition, known as chronic ankle instability (CAI), is associated with ligamentous laxity, perceived instability, and repeated dysfunction. [3,6,8-10] What causes CAI is unknown. However, research suggests [6] that patients with CAI display impaired sensorimotor output. These deficits, characterized by Hertel[10], in afferent/sensory processing (proprioception), spinal reflex excitability, efferent/motor output, and feedforward and feedback function, all of which negatively impact motor control following an ankle injury. Individuals with alterations in proprioception are more likely to experience CAI after an initial ankle sprain due to incorrect foot placement at initial contact of the gait cycle [10]. Specifically, alterations in spinal reflex excitability and neuromuscular function are linked to arthrogenic muscle inhibition, or a reflexive "shut down" of musculature that surrounds a joint following injury as well as impaired sensory input [10]. Reductions in spinal reflex excitability have

been observed in the peroneus longus and soleus muscles in patients with CAI compared to healthy adults, which may result in an individual's inability to optimally contract, or control, musculature in an athletic or daily setting. If an individual cannot express total neuromuscular control, he or she may be at greater risk for injury or reductions in performance. [10,11] Understanding how to break the cycle of altered sensory input producing impaired motor output is necessary to stopping CAI and improving function for affected individuals.

While neural and sensorimotor deficits are well documented in patients with CAI,[6,8,10,12] changes in the vascular structure and function are not commonly understood in this population. With the tibial and peroneal arteries running parallel and in close proximity to the nerves, it is likely that these structures are also damaged during joint injury such as a lateral ankle sprain. Vascular damage may impair blood flow to the nerves and muscles. As skeletal muscle contracts, blood flow is mediated as vascular structures within the working muscle vasodilate in order to properly supply adequate nutrients, such as oxygen, to assist in optimal contraction. This concept is known as functional sympatholysis. [12,13]In mammalian physiology, there is a significant association between skin surface temperature and vascular dynamics [14]

With heat dissipation being a well observed effect of exercise and muscular contraction, changes in superficial skin temperature are related to the degree of underlying vasodilation and vasoconstriction from muscular contraction [15]. These changes in superficial skin temperature as a result of exercise can be observed with

infrared thermal imaging. Formenti et al. [15] observed differences in local musculature temperature in trained vs. untrained women and found that trained women responded much quicker to the exercise and rapidly elevated skin temperature. The rapid elevation of skin temperature in trained individuals is hypothesized to be an increase in efficient heat dissipation. [15]

In theory, if individuals with CAI had lower skin temperatures during exercise compared to healthy counterparts could suggest that repeated injury has consequences beyond altered sensorimotor function. Impaired vascular function could negatively impact motor function and perpetuate the CAI cycle. It is imperative to test under both, active (exercise) and resting conditions as lateral ankle sprains occur during athletic and daily movements. The inability to supply adequate blood flow and its nutrients to the nerves following exercise may potentially be a source of poor sensorimotor function.

To date, no study has examined the integrity of the vascular supply to the foot and ankle to determine if it plays a role in the CAI process. Thermal imaging of the surrounding musculature during both, rest and following ankle joint exercises in individuals with CAI may elucidate vascular changes following ankle joint injury. Therefore, the aim of this study is to observe neurovascular function in the ankles of patients with and without chronic ankle instability to determine if vascular damage is present along changes in neural excitability. Findings from the proposed study may further clarify specific mechanisms of CAI. Understanding the relationship between neural and vascular changes following ankle joint injury may enhance our ability to

create therapeutic and performance modalities to enhance quality of life and function.

Specific Aim 1: To determine if there is a difference in blood flow between limbs in patients with CAI and healthy adults.

Hypothesis 1.1: Blood flow will be reduced in the CAI limb of patients compared to the contralateral side.

Hypothesis 1.2: There will be no difference in blood flow between limbs of healthy adults. The lack of difference in blood flow in healthy adults would confirm that any differences observed between limbs in patients with CAI are attributable to the injury.

Specific Aim 2: To determine if blood flow changes immediately following ankle exercise.

Hypothesis 2.1: Blood flow will increase following exercise but will remain reduced in the CAI limb compared to the contralateral side.

Specific Aim 3: To determine if there is an association between spinal reflex excitability and blood flow in patients with CAI.

Hypothesis 3.1: Greater spinal reflex excitability will be associated with greater blood flow.

The results of this study may further clarify specific mechanisms of CAI. Understanding the relationship between neural and vascular changes following ankle joint injury may enhance our ability to treat patients with CAI and break the cycle of recurrent ankle sprains and reduce the prevalence of CAI.

CHAPTER 2: REVIEW OF RELATED LITERATURE

The purpose of this literature review is detail the: 1) anatomy, 2) mechanisms, and 3) consequences of CAI.

2.1 Ankle Joint Anatomy

2.1.1 Articulations

The ankle is composed of 3 primary joints. These are the talocrural joint, the subtalar joint, and the distal tibiofibular syndesmosis. The articulations of the ankle act in unison in order to provide motion in all three planes of movement (sagittal, frontal, and transverse). The primary joint of interest in patients with CAI is the talocrural joint. The talocrural joint is formed by the articulation of the talus with the tibia and is supported by a multitude of ligaments: the anterior talofubular ligament (ATFL), the posterior talofibular ligament (PTFL), the calcaneofibular ligament (CFL), and the deltoid ligament. [9]. The deltoid ligament provides stability to the medial side of the ankle joint, limiting excessive eversion. It is not injured during lateral ankle sprains. The ATFL runs from the lateral malleolus anteriorly to the medial aspect of the talus. The PTFL orients posteriorly from the lateral malleolus to the posterolateral portion of the talus. The CFL runs posteriorly and inferiorly from the long axis of the fibula to the lateral calcaneus. Collectively, these ligaments serve to limit excessive inversion of the ankle (Figure 1). During a lateral ankle sprain, the ATFL is often damaged initially, followed by the CFL. Initial injury to the ATFL has been shown to increase rearfoot internal rotation and, ultimately, transverse plane instability and damage to remaining ligaments. [9,16] Individuals with CAI may exhibit increased joint laxity following ligament damage from

a lateral ankle sprain and chronic instability. [10,17]

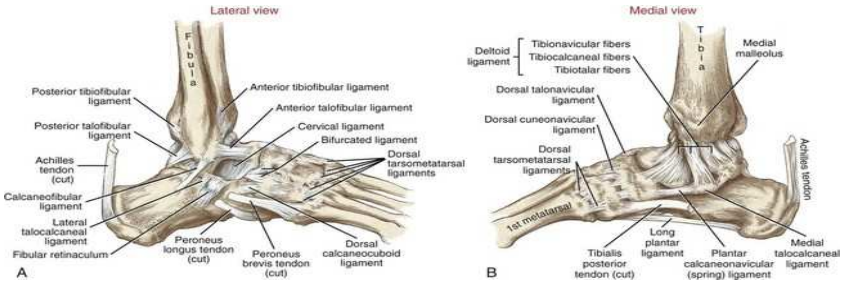

Figure 1. Schematic of ankle ligaments and tendons.
https://musculoskeletalkey.com/structure-and-function-of-the-ankle-and-foot/

2.1.2 Musculature

The ankle complex is comprised of many muscles that provide motion and stability. Anteriorly, the tibialis anterior, extensor hallicus longus, and extensor digitorum longus generate ankle dorsiflexion and toe extension. Medially, the ankle is inverted by the tibialis anterior and posterior, flexor hallicus longus and flexor digitorum longus. Laterally, the peroneus longus and brevis evert the ankle. Posteriorly, the gastrocnemius and soleus plantar flex the ankle. During the gait cycle, the above muscles of the ankle joint complex act synergistically to provide stability and mobility. For instance, the peroneus longus both, everts the ankle and provides sensory input to the brain regarding balance. Researchers have found deficient neuromuscular recruitment patterns in patients with CAI. [8,10,11] Also, deficits in inversion and eversion muscle strength have been reported [10]. Alterations in muscle function may predispose individuals with CAI to further joint injury and decrease daily and athletic function.

2.1.3 Neural Innervation

The peroneus longus and brevis are innervated by the superficial peroneal nerve. The tibialis anterior, extensor hallicus longus, and extensor digitorum longus are

innervated by the deep fibular nerve. The gastrocnemius and soleus are innervated by the tibial nerve [18]. Although a lateral ankle sprain can be multifaceted joint injury, the common factor associated nearly all sprains is an inversion at the ankle (often combined with plantar flexion and/or supination). [9,19-21] During a lateral ankle sprain, nerves innervating the lateral aspect of the ankle may be compressed during the inversion moment. Also, the nerves of the medial aspect of the ankle may be excessively stretched during ankle inversion. Ultimately, these actions may impair neuromuscular control and function.

2.1.4 Vascular Supply

The peroneus longus and brevis are supplied by the peroneal artery. The tibialis anterior, extensor hallicus longus, and extensor digitorum longus are supplied by the anterior tibial artery. The gastrocnemius and soleus are supplied by the sural arteries: popliteal artery, posterior tibial artery, and peroneal artery. The peroneal artery, anterior tibial artery, and sural arteries also supply the ligaments of the talocrural joint [18]. As mentioned above, during a lateral ankle sprain, the common factor is inversion of the ankle joint complex. The vasculature (arteries, arterioles, capillaries, etc.) may experience similar damage as the nerves due to their position next to the nerves (Figure 2). The vasculature supplying the lateral aspect of the ankle may be compressed during the inversion moment. Also, the vasculature of the medial aspect of the ankle may be excessively stretched during ankle inversion. Impaired blood flow to the musculature supporting the ankle joint may hinder the delivery of oxygen and, ultimately, impair the

ability to support and stabilize the ankle during activities of walking and sport.

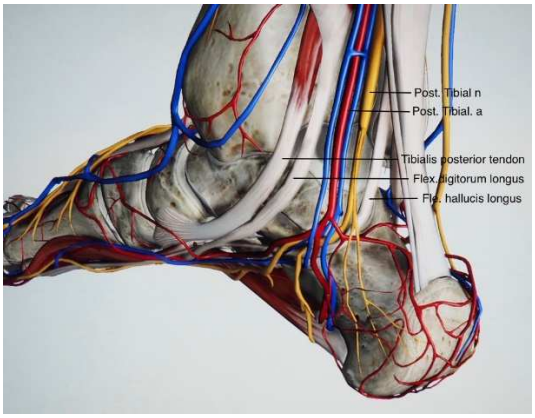

Figure 2. Schematic of the ankle nervous and vascular structure. http://www.bats.ac.nz/detail-foot_and_ankle_surgery-2.

2.2 Mechanisms of Lateral Ankle Sprains and Chronic Ankle Instability

2.2.1 Acute Lateral Ankle Sprain

It is suggested that lateral ankle sprains are caused by excessive ankle inversion with concurrent external rotation of the lower limb during initial contact of the gait cycle.[9,16,20] This position is often described as rolling the ankle inward. [22] This cascade of action during walking, cutting, or landing coupled with the inability of the ligamentous and muscular tissue to withstand the strain result in lateral ankle sprain [9]. The ATFL of the talocrural joint is initially injured during a lateral ankle sprain, with more severe sprains also injuring the CFL and PTFL.

2.2.2 Chronic Ankle Instability

CAI is characterized by repeated bouts of instability described by the ankle "giving way" following an initial sprain and recurrent ankle sprains due to mechanical instability, functional instability, or both.[3,5,8,22] CAI is associated with ligamentous laxity, perceived instability, repeated dysfunction, and sensorimotor deficits. [3,6,8] Using the Hertel CAI model, researchers hypothesize there are two causes of CAI: mechanical

instability and functional instability. Mechanical instability refers to deformation within ankle anatomy that leads to deficiencies in function. In the case of lateral ankle sprain, it is often the result of increased ligamentous laxity of the ATFL and CFL within the talocrural joint. Functional instability refers to negative alterations within neuromuscular system of the ankle. This refers to changes in sensorimotor function such as proprioception, motoneuron excitability, strength, and postural control. [9,10] It is important to note that functional and mechanical instability are not mutually exclusive. The degree at which mechanical, ligamentous damage occurs can vary. For instance, Bowker et al. observed spinal reflex excitability and joint laxity in individuals with CAI, individuals with a history of one ankle sprain with no present deficits, and healthy controls. Researchers found that while spinal reflex excitability was significantly lower in those with CAI, there was no significant difference in laxity among all groups. [17]

2.3 Consequences of Chronic Ankle Instability

2.3.1 Muscle Weakness and Impaired Activation

Impaired muscle activation and neuromuscular control is proposed as a primary sensorimotor deficit in individuals with Chronic Ankle instability (CAI). This is largely characterized as Arthrogenic muscle inhibition (AMI), or, a reflexive response of the muscle surrounding a joint following injury or damage. AMI is measured by estimating the alpha motoneuron excitability (motor output) within a muscle. More so, it is an estimation of the motoneuron pool available for contraction. It is hypothesized that the more alpha motoneruons available, the higher the motor output.[6,8,10] Individuals with CAI have demonstrated decreased motoneuron excitability in the peroneus longus and soleus muscles compared to healthy controls.[3,10] Decreased motoneuron excitability of the soleus may

negatively affect sensory feedback and neuromuscular control.[3] Impaired neuromuscular control may alter and individuals' ability to walk, run, or make postural corrections during perturbations. For instance, Feger et al. observed altered peroneus longus muscle activation timing during walking. Compared to healthy adults, individuals with CAI exhibited an early onset of peroneus longus activation during initial contact of the gait cycle. This may negatively impact the biomechanical role of the peroneus longus and, ultimately, alter motor control and dynamic stability of the ankle. [23] With the neural and vascular structures that supply muscles running parallel and in close proximity. The reductions in neural activation following joint injury may be accompanied by damage and impaired function to the vasculature.

2.3.2 Spinal Reflex Excitability: The Hoffman-Reflex

The primary purpose of eliciting a Hoffman-reflex (H-reflex) is to estimate the alpha motoneuron excitability of Ia sensory fibers. Motoneuron excitability is imperative for measuring how well muscles can be activated and the extent to which AMI has occurred following joint injury. It is important to note that the H-reflex does not directly measure motor output, it only provides an estimate of neural excitability and the potential to which a muscle can be activated.

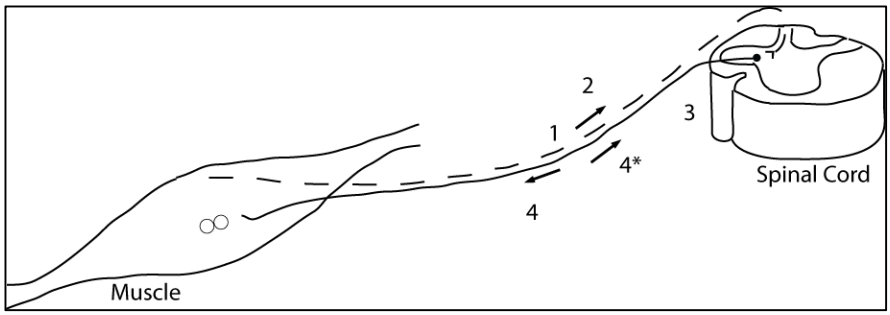

Figure 3. H-reflex and M-wave pathway. The solid line represents the αMN axon and the dashed line represents the Ia afferent axon. If a low-intensity percutaneous stimulus is delivered to a mixed nerve (1), Ia afferents are selectively recruited and action potentials within these fibers travel toward the spinal cord (2). Ia action potentials transmitted to the spinal cord synapse with interneurons and send action potentials toward the muscle belly via the αMN (3). These action potentials are recorded in the muscle belly as an H-reflex via EMG electrodes (open circles). As the stimulus intensity is increased, αMN are recruited at the point of stimulation and action potentials travel to the muscle belly where they are recorded via EMG electrodes as an M-wave(4). Action potentials generated in the αMN at the point of stimulation also travel antidromically toward the spinal cord (4*), colliding with the action potentials generated within the Ia fibers, thereby cancelling out the H-reflex signal. Modified from Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, P., & Dyhre-Poulsen, P. (2002). Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. J Appl Physiol, 92, 2309-2318.[24]

The H-reflex is induced through electrical stimulation (Figure 3). A stimulating electrode is placed over the target nerve and a dispersive electrode is placed on the opposite side of the limb being tested. Electromyography (EMG) electrodes are placed over the target muscle to record the muscle's response to stimulation. When stimulating a mixed nerve, one that has both sensory and motor functions, a low intensity stimulus will selectively recruit Ia afferents and action potentials will travel toward the spinal cord. These action potentials synapse with interneurons in the spinal cord, resulting in the transmission of action potentials toward the target muscle via alpha motoneurons. These efferent action potentials are recorded in the muscle belly as an H-reflex via EMG electrodes. As the stimulus intensity is increased, alpha motoneurons are recruited at the point of stimulation and action potentials travel to the muscle belly where they are recorded

via EMG electrodes as an M-wave. Action potentials generated in the alpha motorneuron at the point of stimulation also travel antidromically toward the spinal cord, colliding with action potentials generated within the Ia fibers, thus cancelling out the H-reflex signal. Because the M-wave signal only travels from the point of stimulation directly to the muscle, whereas the H-reflex signal travels from the point of stimulation, to the spinal cord, and back to the muscle, the M-wave signal is observed to occur prior to the H-reflex signal (Figure 4).

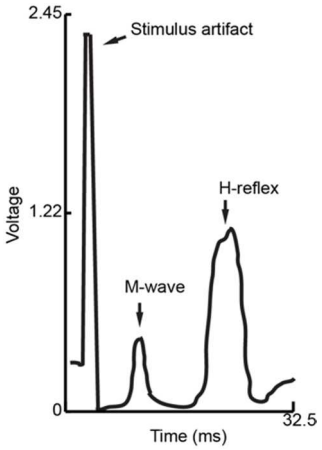

Figure 4. Example of a collected Muscle Response (M-wave) and H-reflex recorded on and electromyograph (EMG).

The H-reflex is often presented as a ratio of H_{max}:M_{max}, or excitability within the target Ia afferents relative to the capability of the entire motoneuron pool. The larger the ratio, the more excitable the muscle.

H-reflex is a significant tool to assess performance and function after injury or during strength training. If H-reflex values decrease from pre- to post-injury, then clinicians and professionals can deduce there is impaired muscle activation and/or reduced motoneuron excitability and proper rehabilitation measures can be taken. [11]

One major assumption regarding H-reflex is while the subject is seated in a reclined position or lying prone, there is no neural descending drive contributing to the measurement of H-reflex values and it is strictly the Ia afferent fibers and the alpha motoneurons of the spinal cord. Thus, generating a presumed measurement of motonueron excitability. However, inhibitory interneurons (Ib inhibitory interneurons and Renshaw Cells) may play a role in limiting H-reflex. [25] The H-reflex occurs at a latency, allowing inhibitory pathways to activate, along with Ia afferents, and potentially suppressing H-reflex values. [25] This inhibition has been found as a result of muscular contraction and may allude to changes or depression in H-reflex values during contraction protocols.

2.3.3 Thermal Imaging

Thermal imaging provides a measurement of the surface temperature of a tissue. Specifically, thermal imaging can be explained using Stefan-Boltzmann law which dictates that the amount of infrared radiation produced from an object's surface is proportional to the fourth power of the object's temperature. Simply put, for a higher object temperature, more thermal radiation emitted. [26] Thermal imaging is often used to observe and interpret underlying physiology and blood flow by imaging with a camera that is sensitive to light in the 8 – 10 micron region of the electromagnetic spectrum. [14] During exercise, sympathetic nervous system stimulation causes a cascade of hemodynamic regulatory processes. One of which is to divert blood flow to the working muscle in order to provide necessary resources for contraction. This is enhanced by arterial vasodilation within working musculature and arterial vasoconstriction of non-working musculature. These processes, ultimately, result in increased heat dissipation

from the enzymatic reactions necessary to produce energy. This increased heat dissipation and body temperature from exercise and muscle contraction within deep regions of the body can manifest to superficial skin layers and, therefore, be accurately measured using infrared thermography.[15,27] Formenti et al. observed changes in skin temperature in trained and non-trained women and found that trained women had a quicker thermal response to exercise. Meaning, local skin temperature of working musculature adapted more rapidly to accommodate exercise blood flow needs (See figure 5).[15] This suggested increase in efficiency supports the notion that trained, healthy

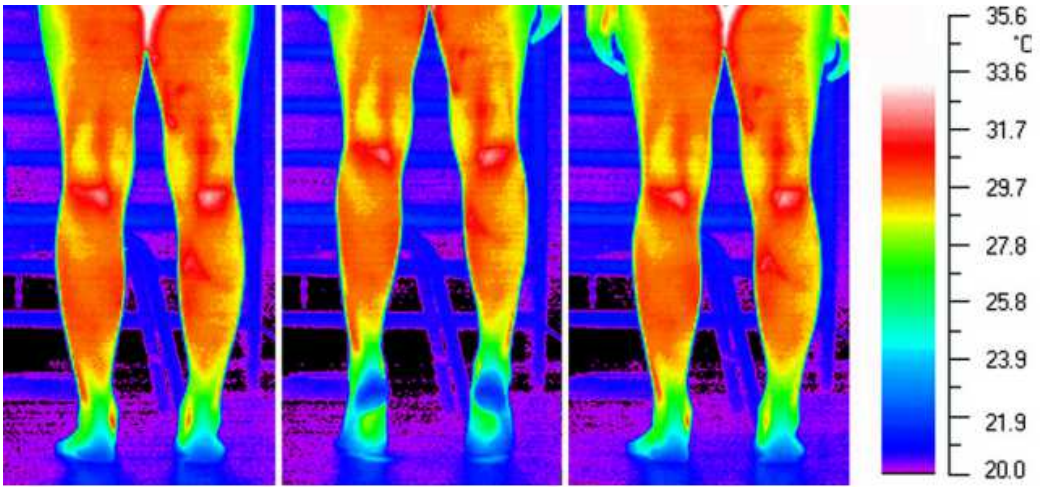

Figure 5. Thermal image changes during a single repetition of a standing calf raise. Formenti, D., et al., Thermal imaging of exercise-associated skin temperature changes in trained and untrained female subjects. Ann Biomed Eng, 2013. 41(4): p. 863-71.

women with no history of ligamentous damage have adequate blood flow and heat dissipation with no hindrance to function and performance.

There are many factors that may influence skin temperature of research participants. The room in which data are collected should maintain a constant temperature between subjects and between sessions. If the subject becomes too cold then

reflexive vasoconstriction may occur and blood flow may divert from the extremities to the body's core.[28]

Skin temperature and heat production in humans is largely variable from subject to subject. However, there are many controllable factors that may help normalize subject data. For instance, it is strongly suggested that subjects avoid using topical and cosmetic ointments on the day of testing. Application of ointments may cause a local change in superficial skin temperature. Also, physical exertion and exercise should be avoided in order to minimize body temperature changes due to repeated bout of muscle contraction and increased body temperature.[28]

2.4 Conclusion

While the neuromuscular deficits associated with CAI are commonly understood, vascular changes resulting from joint injury are not known. Given the proximity of vascular structures to nerves, it is logical vascular damage occurs following injury. Given the high prevalence of ankle injury and large financial burden, it is vital to observe the mechanisms of CAI to develop potential treatment modalities to delay and/or prevent further injury and enhance quality of life.

CHAPTER 3: METHODS

3.1 Participants

A total of 17 participants (n=10 with CAI, n=7 control) completed this study. Participants were recruited from the University of North Carolina at Charlotte community. All participants were required to be between the ages of 18-40 years. Inclusion criteria for CAI included: i) Foot and Ankle Ability Measure (FAAM) score of <90%, ii) Foot and Ankle Instability Measure Sport (FAAM-sport) score of <80%, iii) a history of 2 or more unilateral ankle sprains, with first sprain occurring >1 year prior to the start of the study, iv) Ankle instability Instrument score >4, and v) Identification of Functional Ankle Instability score >10. Exclusion criteria for the CAI group included: i) lateral ankle sprain in the previous 3 months to either ankle, ii) instability in the uninjured ankle, iii) bilateral chronic ankle instability. Exclusion criteria for all participants included: i) body mass index >40 kg/m^2; ii) history of lower limb surgery or fracture; iii) history of neurovascular disorder, fibromyalgia, peripheral neuropathy, or rheumatoid arthritis; iv) impaired balance, inability to consistently comprehend and repeat back directions regarding details of the study; and/or v) current smokers. Individuals in the CAI group were screened in accordance with the recommendations of the International Ankle Consortium.[29] All participants signed a waiver of informed consent and the study

was approved by the Institutional Review Board at University of North Carolina at Charlotte.

3.2 Experimental Design

Pre-tets/post-test observational study. The independent variable was the CAI group. The dependent variable was the blood flow analysis and spinal reflex excitability testing.

3.3 Procedures

Participants reported for testing on one occasion and underwent spinal reflex excitability testing, blood flow analysis, and exercise. Blood flow analysis occurred before and immediately after exercise using the same procedures at all time points (Figure 6).

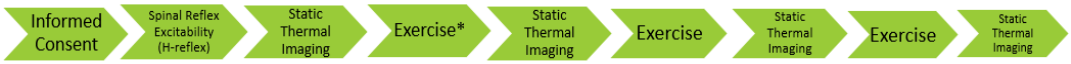

Figure 6. Testing timeline.

3.3.1 Spinal Reflex Testing

Soleus muscle activation was assessed by eliciting a maximal H-reflex and M-response from the muscle. Electromyography (EMG) was recorded using a Biopac MP150 unit and associated AcqKnowledge software (Biopac, Inc., Goleta, CA). EMG electrodes were placed over the belly of the soleus and a ground electrode over the

ipsilateral medial malleolus after the areas were shaved, debrided, and cleaned. A stimulating electrode was placed on the posterior knee and a dispersive electrode over the anterior thigh. Participants were instructed to lie prone and remain as still and relaxed as possible during testing. A single, 1ms long square-wave stimulus (200V max) was delivered (Digitimer DS7AH, Digitimer, Ltd, Hertfordshire, UK). The amplitude of the stimulus was increased until an H-reflex was recordable on EMG. Next, the stimulus intensity was gradually be increased until H-reflex reached its peak. Once a peak H-reflex had been determined, five recordings were taken. Once more, the stimulus intensity was increased until a maximal M-response was recorded. Five trials were performed at this intensity. The data were averaged across trials and utilized to determine the H_{max}:M_{max} ratio. Data were recorded bilaterally with the limb to be tested first determined via coin flip at the start of the testing session.[11]

3.3.2 Blood Flow Analysis

A FLIR SC655 mid-IR camera (array size 640 x 480 pixels; Figure 7) was used to capture videos of each participant's lower legs/feet before, during and after exercise. This camera is sensitive from 7.5 to 14μm, which is ideal for sensing thermal emission from the human body [14]. Participants stood on a carpeted floor with their toes angled out at approximately 45° and their feet shoulder width apart. The images were taken from above the popliteal fossa to the floor to capture blood vessels as they run down the legs and into the feet. The camera was positioned 1.1m from each participant resulting in a spatial

resolution of approximately 0.15cm/pixel and a field of view of 100 cm (horizontal direction) x 75 cm (in the vertical direction). Videos were recorded at a rate of 15 frames per second. Both anterior-posterior and posterior-anterior images were taken with order counterbalanced between individuals. Both the right and left limbs were imaged at the same time. The above procedures were utilized before and after each exercise with video recorded for approximately 15s. To ensure the participant stood in the same position throughout the rest periods between exercises, cutouts of the feet were taped to the floor and the participant was asked to stand on those

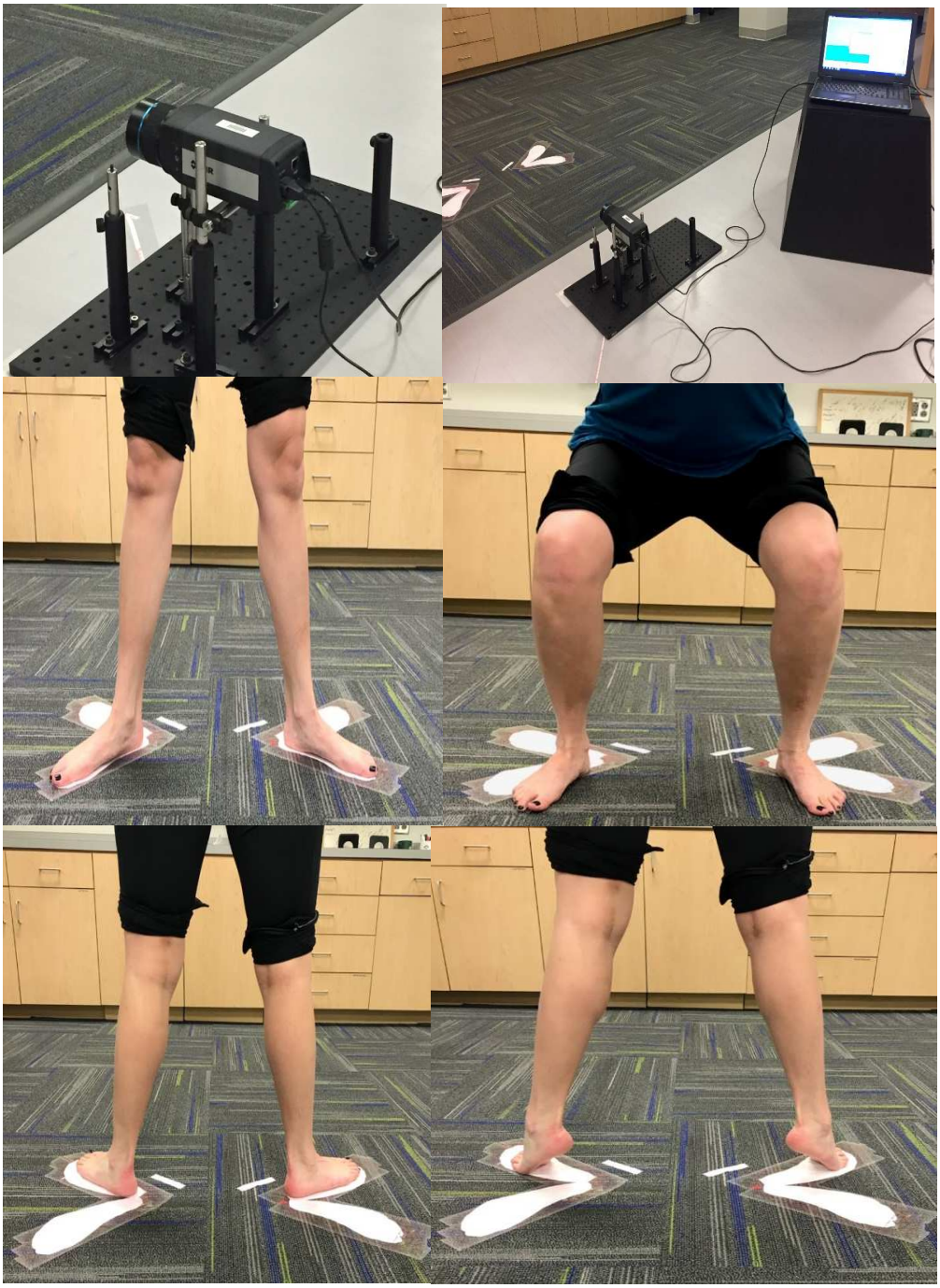

Figure 7. A FLIR SC655 mid-IR camera and participant position during imaging.

during blood flow analysis. To ensure consistency during post-testing data analysis, retroreflective motion capture markers were secured over the most prominent portion of the lateral and medial malleoli. This allowed these landmarks to be easily and accurately identified on the thermal images after they are collected without obscuring the vascular data. Procedures were developed and maximized from previous pilot testing.

Data containing 20 frames per rest period were extracted from the videos, averaged, and stacked using MATLAB software (Mathworks, Inc., Natick, MA). A region of interest (ROI) was self-selected using an ellipsis feature in MATLAB. To ensure consistency, the same researcher selected ROIs for all subjects. The ROIs were located posterior to the lateral malleolus (following the path of the peroneus longus tendon) for the posterior view and posterior to the medial malleolus (over the posterior tibial artery/vein) for the anterior view. This is due to presence of superficial vasculature in those areas. These images were then compared relative to the pre-exercise baseline images and difference images were generated.

3.3.3 Exercise

Exercise consisted of two tasks in a random order. One exercise was calf raises, where the participant rises up onto his toes and lowers his heel back to the ground. The other exercise was squats to a depth such that the proximal femur (upper thigh) is parallel to the floor. Three sets of 10 repetitions of each exercise were performed with while acquiring a video with the thermal camera.[15] The heel raises were performed in conjunction with the posterior-anterior view thermal images and the squats were

performed while the participant was imaged in an anterior-posterior direction. The calf raises were chosen due to common clinical use and provide a clear view of the selected ROI. Squats were chosen due to the ability to occlude blood flow at the bottom of the motion.

3.3.4 Statistical Analysis

Participant demographics were compared across groups using independent samples t-tests. H_{max}:M_{max} ratios were compared between groups and limbs using 2x2 ANOVAs. Thermal imaging data were analyzed using Bland-Altman plots to determine agreement between the temperature of both limbs within each group. Bland-Altman plots graph the data as the average of the peak temperatures in each limb (x-axis) against the difference between the peak temperatures in each limb (y-axis). Finally, to determine the association between peak temperature within each ROI and spinal reflex excitability, Pearson Product Moment correlation coefficients were calculated for participants in the CAI group. Statistical analyses were performed using SPSS (IBM, Armonk, NY) and Matlab.

CHAPTER 4: RESULTS

Participant demographics can be found in Table 1. There were no differences in age, height, or body mass between groups. Patient-reported data indicated worse function in the CAI compared to the control group. Spinal reflex excitability was not different between limbs or group (Figure 8). Further, there was no association between spinal reflex excitability and blood flow in the involved (posterior: r=-0.137, *P*=0.706; anterior: r=-0.338, *P*=0.339) or uninvolved (posterior: r=-0.039, *P*=0.916; anterior: r=-0.078, *P*=0.829) limbs of the CAI group.

Table 1. Participant demographics. Data are presented as mean ± standard deviation.

	CAI (n=10)	Control (n=7)	*P*-value
Age (years)	24.3±5.81	24.14±5.55	0.96
Height (m)	1.74±0.11	1.74±0.13	0.98
Body mass (kg)	74.07±17.44	76.07±14.19	0.81
FAAM	91.78±5.8*	99.83±0.45	0.003
FAAM-Sport	84.06±9.12*	100.00±0.00	<0.001
AII	5.2±1.62*	0.14±0.38	<0.001

CAI: chronic ankle instability
FAAM: Foot and Ankle Ability Measure
AII: Ankle Instability Instrument
*indicates data are significantly different from control group

Bland-Altman analysis reveals agreement in the surface temperatures obtained from both the posterior and anterior ROI between limbs in the participants with CAI (Figures 9 and 10, respectively). Similarly, there was agreement in posterior and anterior ROI surface temperatures between the limbs in control participants (Figures 11 and 12, respectively).

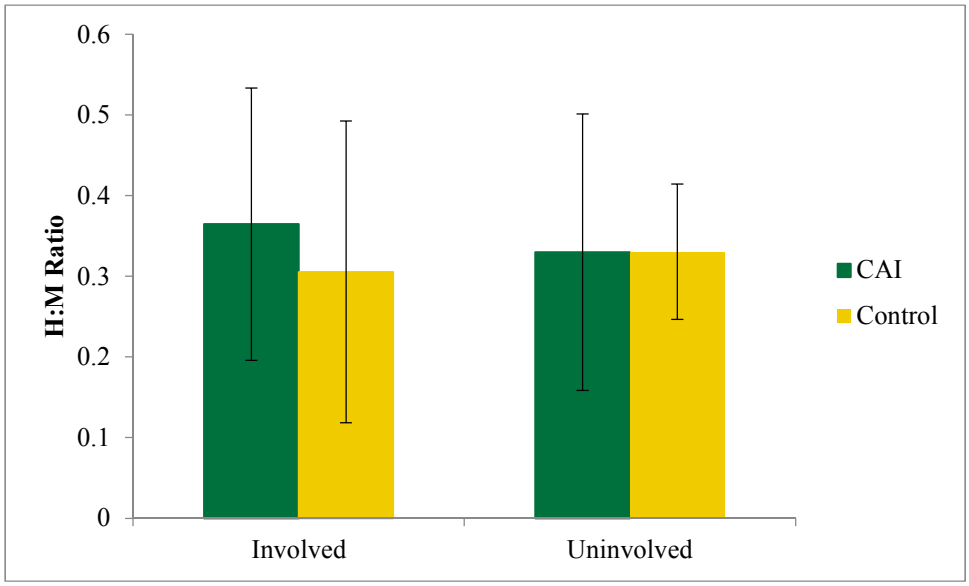

Figure 8. H_{max}:M_{max} ratio for the involved and uninvolved limbs in both groups. Data are presented as mean ± standard deviation.

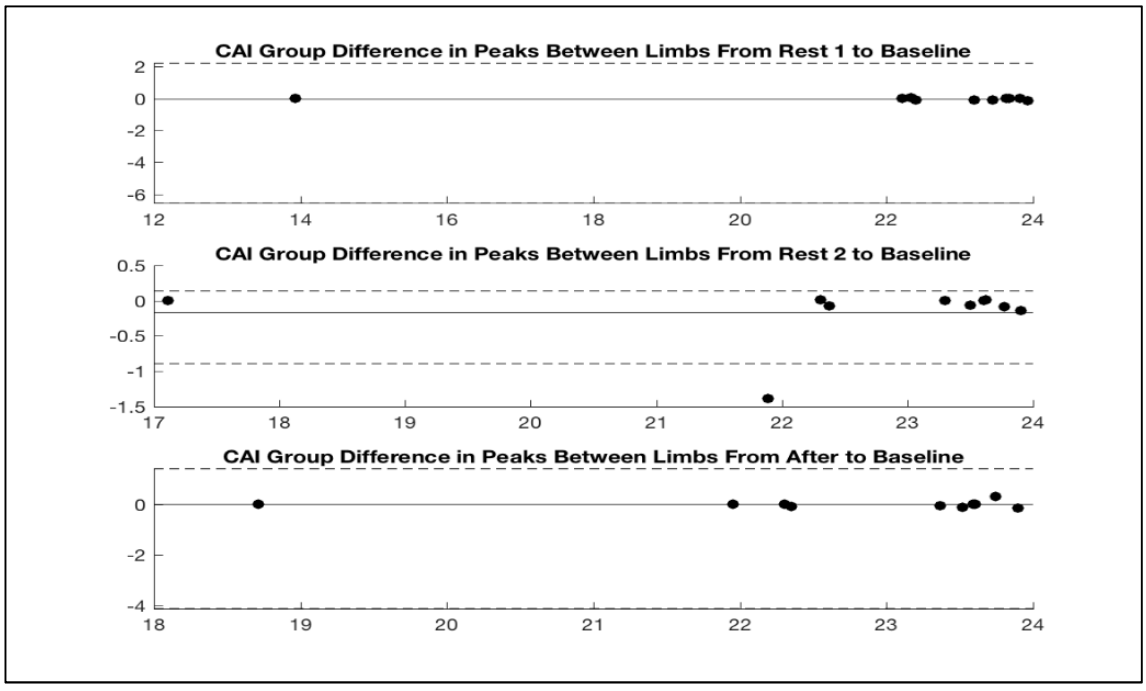

Figure 9. Bland-Altman plot of agreement in posterior region of interest blood flow between limbs for individuals in the CAI group.

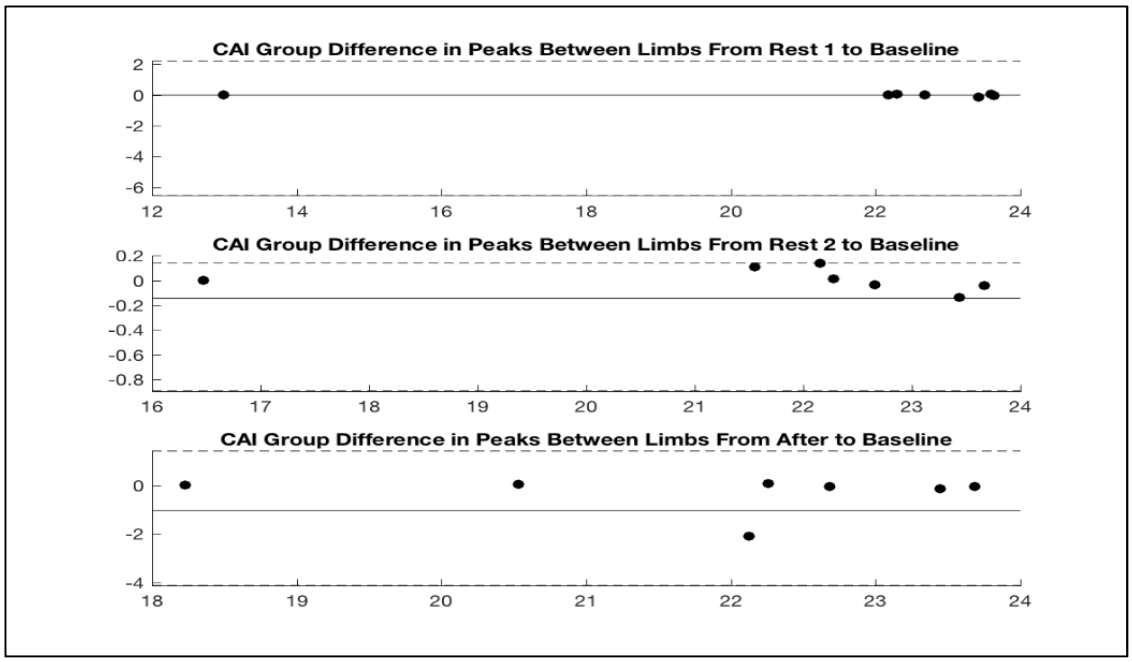

Figure 10. Bland-Altman plot of agreement in anterior region of interest blood flow between limbs for individuals in the CAI group.

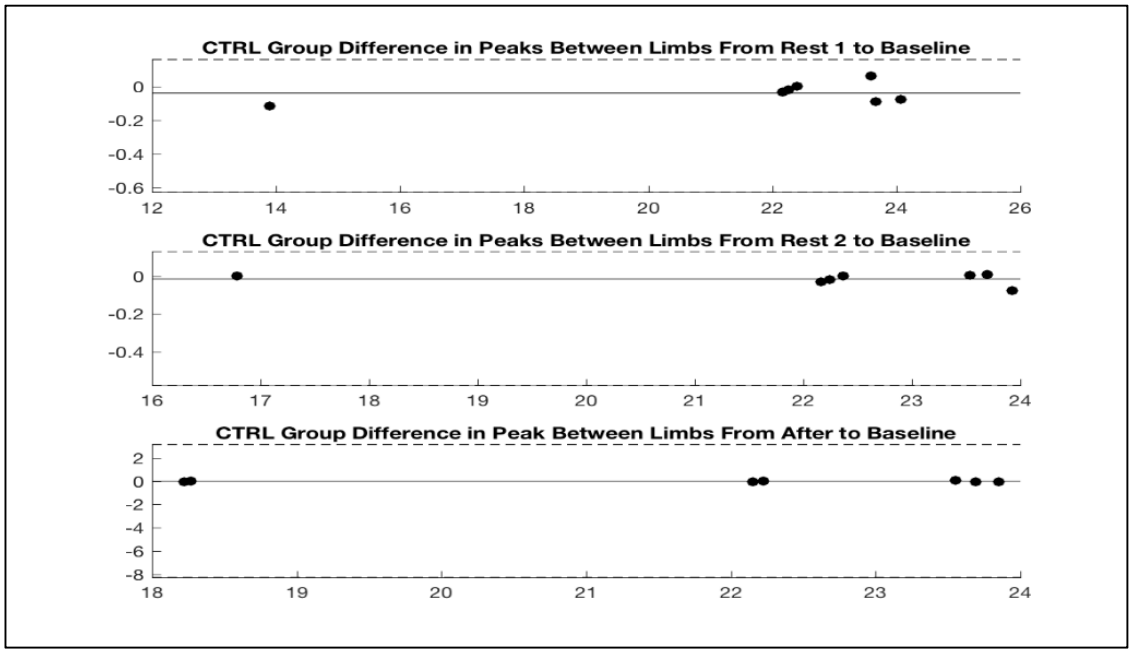

Figure 11. Bland-Altman plot of agreement in posterior region of interest blood flow between limbs for individuals in the control group.

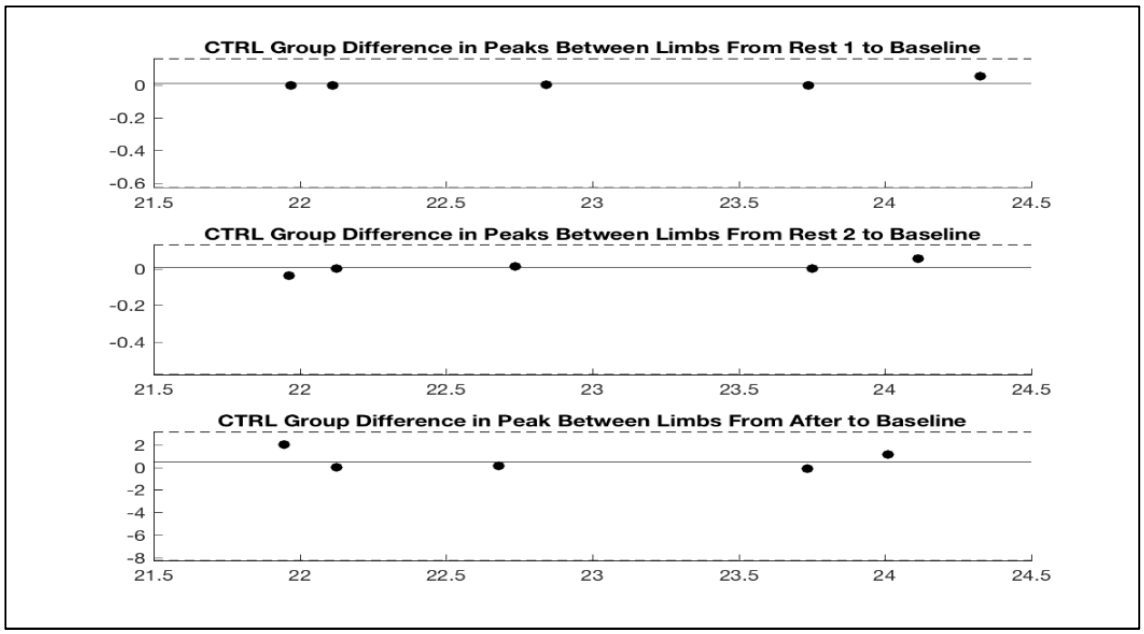

Figure 12. Bland-Altman plot of agreement in anterior region of interest blood flow between limbs for individuals in the control group.

CHAPTER 5: DISCUSSION

This study aimed to elucidate the relationship between blood flow and spinal reflex excitability in patients with CAI compared to healthy adults. Our results suggest that neither blood flow nor spinal reflex excitability differed between limbs or groups.

The lack of differences in soleus spinal reflex excitability is in contrast to our hypothesis and current literature.[8,10,17] Comparing the data collected for the present study to data recently collected by our research team using identical procedures for obtaining the soleus H-reflex suggests that the H:M ratios obtained presently are similar to those previously reported in patients with CAI [17]. However, the healthy control data in the present study are lower in magnitude than in the previously published study. Control participant demographics and self-reported function are similar between the two populations[17]; therefore, it is unclear why the healthy controls in the present study had such low magnitudes of spinal reflex excitability.

Blood flow was also not different between limbs or groups. We are aware of only one other study utilizing thermal imaging in adults following calf raise exercise. That study examined healthy females and suggested that trained individuals demonstrate greater and more rapid heat dissipation (greater change increase in surface temperature) compared to sedentary individuals.[15] Despite having CAI, our participants within that

group were not sedentary and may not have been different enough from our controls in terms of physical activity to detect differences in blood flow between groups. Additionally, while individuals with CAI may experience functional and performance limitations [9,22], other factors such as cardiovascular fitness, blood pressure, muscle fiber composition, etc. may play a vital role in regulation of blood flow and cutaneous skin temperature. For instance, Kalsi et al. [30] observed mechanisms of local tissue blood flow during thermal interventions and found that tissue heating, similar to muscular contraction, was associated with increased adenosine triphosphate (ATP) signaling from erythrocytes. Meaning, ATP release, during contraction or otherwise, from erythrocytes plays a vital role in thermoregulation and blood flow. Thus, adequate ATP availability in both limbs in individuals with CAI may explain the high agreement of peak skin temperatures between limbs.

There are a number of additional possible reasons why blood flow was similar between limbs and groups. First, it is possible that the ROI selected were not optimal for examining blood flow. The thermal imaging technique utilized provides a measure of surface temperature, which serves as a surrogate measure of blood flow in the underlying vasculature. Thus, we selected the ROI that we did due to the superficial nature of the vascular supply in those areas. However, it is possible that other ROI's could have yielded different outcomes. To explore this possibility, we examined areas over the sinus tarsi and dorsal pedal regions of the foot, quadriceps, and popliteal fossa using identical methods as for the primary ROI. These four exploratory ROI also revealed agreement

between limbs and groups suggesting that blood flow is similar throughout the lower extremities of patients with CAI and compared to healthy controls. Second, we chose to examine the peak temperature in each region of interest. It is possible that extracting the minimum value in each region may provide a more meaningful understanding of differences in blood flow and show a larger change in temperature during each rest period. Further analysis of our data would be necessary to confirm this speculation. Third, the nature of the exercises may not have been strenuous enough to warrant changes in blood flow to either increase blood flow to the musculature or shunt blood to the skin for cooling purposes. We selected the calf raises because these are commonly performed during rehabilitation by patients with CAI and the squats due to their ability to occlude blood flow to the lower leg, causing an occlusion/reperfusion cycle. Three sets of 10 exercises is a common clinical prescription, therefore it was selected due to its clinical relevance. However, it is possible that using an exercise like treadmill running may have necessitated changes in blood flow to allow for cooling of deeper tissues and that through this process we could have observed differences in vascular function between limbs and/or groups. Finally, it is possible that the way in which data were processed confounded our results. Data were collected for 15s videos per rest period. An average of 20 consecutive frames across those 15s were extracted and the temperatures from those frames averaged together. Videos were analyzed frame by frame. These methods may have washed out any temporal differences in blood flow that exist between limbs and groups, possibly confounding our results.

Our findings indicate there is no association between spinal reflex excitability and blood flow for either the anterior or posterior ROI in the involved and uninvolved limbs of the CAI group. These findings differ from our original hypothesis stating that the close anatomical proximity in which the nerves and vasculature run will reflect equal damage on both structures and any changes in neural excitability would be associated with impaired blood flow. This hypothesis was driven by evidence from blood flow restriction training that suggests an increase in cortical excitability following an acute bout of blood flow restriction training.[31] Blood flow restriction exercise is characterized by slight occlusion to working musculature during movement followed by occlusion release and a rush of blood flow to the area. The reported increase in cortical excitability suggests blood flow may be modulated by global neurovascular control mechanisms as opposed local structure control. It is possible that had we utilized a different measure of neural excitability, such as examining cortical excitability, our results could have been different.

This study is not without limitations. Thermal imaging measures cutaneous skin temperature as it relates to underlying blood flow. We are not directly able to measure vascular function or damage. Perhaps future studies should utilize other technologies such as ultrasound or laser Doppler imaging to observe vascular structure and perfusion. More so, spinal reflex excitability is only and estimation of motoneuron pool capability and does not directly reflect muscle activation potential. Future studies may observe cortical excitability to reflect neural changes from higher brain centers. Lastly, the exact number and severity of lateral ankle sprains in the CAI group was not observed in the

present study due to the possibility of recall bias on behalf of the participants. Differences in injury severity and time since previous injury may affect vasculature control and blood flow redistribution mechanisms. Future studies could benefit from more closely controlling for severity and number of ankle sprains in patients with CAI.

5.1 Conclusion

Our results suggest that neither spinal reflex excitability nor blood flow differed between limbs or groups when examining patients with CAI and healthy adults. Therefore, impaired neurovascular function may not contribute to the cycle of recurrent ankle sprains that is observed in patients with CAI.

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APPENDIX A: PATIENT REPORTED OUTCOMES

ID:_____ Session:_____ Date:_____

Foot and Ankle Ability Measure (FAAM)

Please answer **every question** with one response that most closely describes to your condition within the past week.
If the activity in question is limited by something other than your foot or ankle mark not applicable (N/A).

	No difficulty	Slight difficulty	Moderate difficulty	Extreme difficulty	Unable to do	N/A
Standing	☐	☐	☐	☐	☐	☐
Walking on even ground	☐	☐	☐	☐	☐	☐
Walking on even ground without shoes	☐	☐	☐	☐	☐	☐
Walking up hills	☐	☐	☐	☐	☐	☐
Walking down hills	☐	☐	☐	☐	☐	☐
Going up stairs	☐	☐	☐	☐	☐	☐
Going down stairs	☐	☐	☐	☐	☐	☐
Walking on uneven ground	☐	☐	☐	☐	☐	☐
Stepping up and down curbs	☐	☐	☐	☐	☐	☐
Squatting	☐	☐	☐	☐	☐	☐
Coming up on your toes	☐	☐	☐	☐	☐	☐
Walking initially	☐	☐	☐	☐	☐	☐
Walking 5 minutes or less						
Walking approximately 10 minutes	☐	☐	☐	☐	☐	☐
Walking 15 minutes or greater	☐	☐	☐	☐	☐	☐

Because of your **foot and ankle** how much difficulty do you have with:

	No difficulty at all	Slight difficulty	Moderate difficulty	Extreme difficulty	Unable to do	N/A
Home Responsibilities	☐	☐	☐	☐	☐	☐
Activities of daily living	☐	☐	☐	☐	☐	☐
Personal care	☐	☐	☐	☐	☐	☐
Light to moderate work (standing, walking)	☐	☐	☐	☐	☐	☐
Heavy work (push/pulling, climbing, carrying)	☐	☐	☐	☐	☐	☐
Recreational activities	☐	☐	☐	☐	☐	☐

How would you rate your current level of function during your usual activities of daily living from 0 to 100 with 100 being your level of function prior to your foot or ankle problem and 0 being the inability to perform any of your usual daily activities?

☐☐☐.0 %

ID:_____ Session:_____ Date:_____

FAAM Sports Scale

Because of your **foot and ankle** how much difficulty do you have with:

	No difficulty at all	Slight difficulty	Moderate difficulty	Extreme difficulty	Unable to do	N/A
Running	☐	☐	☐	☐	☐	☐
Jumping	☐	☐	☐	☐	☐	☐
Landing	☐	☐	☐	☐	☐	☐
Starting and stopping quickly	☐	☐	☐	☐	☐	☐
Cutting/lateral movements	☐	☐	☐	☐	☐	☐
Low impact activities	☐	☐	☐	☐	☐	☐
Ability to perform activity with your normal technique	☐	☐	☐	☐	☐	☐
Ability to participate in your desired sport as long as you would like	☐	☐	☐	☐	☐	☐

How would you rate your current level of function during your sports related activities from 0 to 100 with 100 being your level of function prior to your foot or ankle problem and 0 being the inability to perform any of your usual daily activities?

☐☐☐.0 %

Overall, how would you rate your current level of function?

☐ Normal ☐ Nearly normal ☐ Abnormal ☐ Severely abnormal

ID: _____ Session:_____ Date:_____

Ankle Instability Instrument

Instructions

This form will be used to categorize your ankle instability. A separate form should be used for the right and left ankles. Please fill out the form completely. If you have any questions, please ask the administrator of the survey. Thank you for your participation.

1. Have you ever sprained an ankle? ☐ Yes ☐ No
2. Have your ever seen a doctor for an ankle sprain? ☐ Yes ☐ No

If yes,

2a. How did the doctor categorize your most serious ankle sprain?
☐ Mild (grade I) ☐ Moderate (grade 2) ☐ Severe (grade 3)

3. Did you ever use a device (such as crutches) because you could not bear weight due to an ankle sprain? ☐ Yes ☐ No

If yes,

3a. In the most serious case, how long did you need to use the device?
☐ 1–3 days ☐ 4–7 days ☐ 1–2 weeks ☐ 2–3 weeks ☐ >3 weeks

4. Have you ever experienced a sensation of your ankle "giving way"? ☐ Yes ☐ No

If yes,

4a. When was the last time your ankle "gave way"?
☐ <1 month ☐ 1–6 months ago ☐ 6–12 months ago ☐ 1–2 years ago ☐ >2 years

5. Does your ankle ever feel unstable while walking on a flat surface? ☐ Yes ☐ No
6. Does your ankle ever feel unstable while walking on uneven ground? ☐ Yes ☐ No
7. Does your ankle ever feel unstable during recreational or sport activity? ☐ Yes ☐ No ☐ N/A
8. Does your ankle ever feel unstable while going *up* stairs? ☐ Yes ☐ No
9. Does your ankle ever feel unstable while going *down* stairs? ☐ Yes ☐ No