EXAMINATION OF ANTERIOR TALOFIBULAR LIGAMENT THICKNESS
AND THE NUMBER OF PREVIOUS ANKLE SPRAINS IN COLLEGIATE
ATHLETES

by

Lindsay Yates

A thesis submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Master of Science in Exercise Science

Summer 2018

© 2018 Lindsay Yates
All Rights Reserved
EXAMINATION OF ANTERIOR TALOFIBULAR LIGAMENT THICKNESS
AND THE NUMBER OF PREVIOUS ANKLE SPRAINS IN COLLEGIATE
ATHLETES

by

Lindsay Yates

Approved:

Charles “Buz” Swanik, Ph.D., ATC
Professor in charge of thesis on behalf of the Advisory Committee

Approved:

John Jeka, Ph.D., MA
Chair of the Department of Kinesiology and Applied Physiology

Approved:

Kathleen S. Matt, Ph.D.
Dean of the College of Health Sciences

Approved:

Douglas J. Doren, Ph.D.
Interim Vice Provost for the Office of Graduate and Professional
Education
ACKNOWLEDGMENTS

This project would not have been possible without the help and guidance from many individuals in my life. I am so appreciative of all the time and effort each and every person put into helping me achieve such an accomplishment.

I would first like to thank my thesis advisory committee and thesis advisor, Dr. Charles “Buz” Swanik, Dr. Thomas Kaminski, and Dr. Joseph Glutting for all of their valued input. I would especially like to thank my doctoral student mentor, Bethany Wisthoff, who stood by me every step of the way and pushed me to succeed. This was by no means an easy journey for me, and Bethany dedicated so much of her time and energy into making sure I crossed the finish line. Without her continued support, this would not have been possible.

Finally, I would like to thank my family, who has cheered me on my whole life. Their unwavering encouragement, love, and support impelled me to succeed, even when it did not seem possible. I would not be where I am today without them.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>Chapter</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2 METHODS</td>
<td>5</td>
</tr>
<tr>
<td>2.1 Subjects</td>
<td>5</td>
</tr>
<tr>
<td>2.2 Instrumentation</td>
<td>6</td>
</tr>
<tr>
<td>2.3 Procedures</td>
<td>6</td>
</tr>
<tr>
<td>2.4 Statistical Analysis</td>
<td>9</td>
</tr>
<tr>
<td>3 RESULTS</td>
<td>10</td>
</tr>
<tr>
<td>4 DISCUSSION</td>
<td>14</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>18</td>
</tr>
<tr>
<td>Appendix</td>
<td>30</td>
</tr>
<tr>
<td>A INTRODUCTION</td>
<td>30</td>
</tr>
<tr>
<td>B EPIDEMIOLOGY</td>
<td>32</td>
</tr>
<tr>
<td>C ANATOMY</td>
<td>33</td>
</tr>
<tr>
<td>D CHRONIC ANKLE INSTABILITY</td>
<td>36</td>
</tr>
<tr>
<td>E MUSCULOSKELETAL ULTRASOUND</td>
<td>38</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>43</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1: Participants .............................................................................................................. 5
Table 2: Reliability Measures ................................................................................................. 8
Table 3: Average ATFL Thickness and CAIT Scores by Group ............................................. 11
LIST OF FIGURES

Figure 1: Average ATFL Thickness by Group ...........................................12
Figure 2: Average ATFL Thickness and Gender................................. 12
Figure 3: Correlation Between Number of Sprains and CAIT Score ..............13
ABSTRACT

**Context:** In an athletic population, acute ankle sprains are the most common and most prevalent musculoskeletal injury, accounting for up to 40% of all injuries. Research has shown that acute and untreated ankle sprains can lead to chronic ankle instability, which refers to repetitive episodes of instability resulting in recurrent injuries and loss of function. Many ankle sprains occur due to inversion of the ankle, which over-stresses the anterior talofibular ligament (ATFL). Repetitive damage to the ligament, and subsequent inflammation responses results in the formation of scar tissue. Although it has been shown that sprains lead to scar tissue formation, it is unknown whether the frequency of recurrent sprains corresponds with increased tissue thickness as well a loss on function. **Objective:** The purpose of this study was to determine if the number of previous ankle sprains influences the thickness of the ATFL and function. **Design:** Retrospective cohort. **Participants:** 121 NCAA DI student-athletes (178 ankles) (18.6 ± 1.1 yrs, 82.0 ± 20.7 kg, 180.0 ± 10.6 cm) from the University of Delaware was collected for this study. The participants were divided into groups: 0 ankle sprains, 1 ankle sprain, and 2+ ankle sprains. **Methods:** Each participant completed the Cumberland Ankle Instability Tool (CAIT) and an injury history questionnaire to report the number of sprains they have experienced throughout their lifetime. Thickness of the ATFL was measured by musculoskeletal ultrasound (MSUS) using a GE
LOGIQ e. **Statistical Analysis:** A one-way ANOVA was performed to determine the difference between those with or without a history of previous ankle sprains and ATFL thickness, along with a post-hoc test with a Bonferroni adjustment to determine whether statistically significant differences exist. A one-way ANOVA was also performed to determine if there were statistical differences between gender and ATFL thickness. A Spearman’s rho correlation coefficient was used to determine a relationship between the number of sprains and CAIT scores, CAIT scores and ATFL thickness, number of sprains and ATFL thickness, and gender and ATFL thickness. The independent variable was group (0, 1, 2+). The dependent variable was thickness of the ATFL in millimeters (mm). **Results:** There were no statistically significant differences between groups (0, 1, 2+) and ATFL thickness (1.59 ± 0.15 mm vs. 1.63 ± 0.15 mm vs. 1.61 ± 0.14 mm, respectively). There were no statistical differences between gender 0.62 (0.49) and ATFL thickness: 1.6 (0.15) (p= 0.97, F= 2.791 (0 {68} 1.59 [0.16], 1 {110} 1.63 [0.14])). There was a statistically significant correlation between the number of sprains: 1.18 (1.14) and CAIT scores: 27 (3.7) (p< 0.001, -0.614) and there was a statistically significant correlation between gender: 0.62 (0.49) and thickness: 1.6 (0.15) (p= 0.047, 0.149). There was no statistically significant correlation between the ATFL thickness: 1.6 (0.15) and CAIT scores: 27 (3.7) (p< 0.263, -0.084) or between number of sprains: 1.18 (1.14) and thickness: 1.6 (0.15) (p= 0.026, 0.026). **Conclusions:** We did not find a statistically significant difference in
ligament thickness between groups; however, we found that ligament thickness increases after one lateral ankle sprain. We also found a statistically significant correlation between number of sprains and CAIT scores, suggesting that the numbers of sprains affect joint stability. These findings indicate that after just one lateral ankle sprain, morphological changes occur. These changes could result in deficits to the stabilizing properties of the ligament, leaving it more susceptible to re-injury and compromising joint stability.

**Key Words:** Anterior Talofibular Ligament, lateral ankle sprain, chronic ankle instability, diagnostic ultrasound
Chapter 1
INTRODUCTION

Ankle sprains are among the most common injuries in sports.\textsuperscript{1,2} A lateral ankle sprain (LAS) is defined by Delahunt et al.,\textsuperscript{3} and accepted by the International Ankle Consortium\textsuperscript{4} as “an acute traumatic injury to the lateral ligament complex of the ankle joint as a result of excessive inversion of the rear foot or a combined plantar flexion and adduction of the foot.” In an athletic population, acute ankle sprains are the most common type of sport-related trauma, and the most prevalent musculoskeletal injury, which account for up to 40% of all athletic injuries.\textsuperscript{4-6} In National Collegiate Athletic Association (NCAA) student-athletes, ankle sprains account for 15% of all injuries each year.\textsuperscript{7,8} As concluded in a study by Roos et al.,\textsuperscript{9} sprains to the lateral ligamentous complex of the ankle were the most reported type of injury, accounting for 7.3% of all injuries among 25 NCAA sports.

Research has shown that acute and untreated ankle sprains can lead to chronic ankle instability.\textsuperscript{5,10} Chronic ankle instability (CAI) refers to repetitive episodes of instability resulting in recurrent ankle sprains.\textsuperscript{11} The recurrence rates of LASs are high, up to 75% of initial ankle sprains lead to recurrent sprains and chronic symptoms.\textsuperscript{9,12} However, McKay et al.\textsuperscript{13} found that 56.8% of basketball players who sprained their ankles did not seek professional treatment. An estimated 30-40% of
untreated ankle sprains result in CAI, which can result in impaired balance, proprioception, reaction time, strength, pain, loss of play time, medical expenditure, bone fractures, functional disability, limited mobility, and permanent retirement from sports participation. CAI also contributes to ongoing disability and sensorimotor deficits, which may lead to decreased physical activity and quality of life.

The majority of ankle sprains (85%) occur due to inversion of the ankle, which stresses the anterior talofibular ligament (ATFL). The ATFL is a flat, quadrilateral ligament that originates on the fibula and inserts on the talus. It is the weakest of the lateral ankle ligaments, which consist of the calcaneofibular ligament, posterior talofibular ligament, and anterior talofibular ligament. The ATFL originates from the inferior oblique segment of the anterior border of the lateral malleolus, courses anteromedially, and inserts on the talar body just anterior to the lateral malleolar articular surface.

There are various ways of assessing ankle ligaments through imaging, such as magnetic resonance imaging, radiographs, and computed tomography scans; however, musculoskeletal ultrasound (MSUS), when compared to other methods for obtaining images, proves to have acceptable sensitivity, specificity, and accuracy values. There is a greater than 90% accuracy for musculoskeletal ultrasound (MSUS) in identifying injuries to the ATFL. In a study by Khawaji et al., the ATFL was observed to have one band in 22.9% of subjects, two bands in 56.3% of subjects, and three bands in 20.8% of subjects. In regards to this anatomical variation, the overall width of the
ATFL does not significantly vary, which leads to the assumption that this does not change the function of the ligament.\textsuperscript{23} Typically, the ATFL is the first ligament to suffer damage, followed by the CFL, and then the PTFL.\textsuperscript{10,14} Sprains result in damage to the ligament, which initiates a healing response that is distinguished by scar formation.\textsuperscript{24} However, scar tissue is weaker by nature when compared to a normal, healthy ligament,\textsuperscript{24} potentially making the tissue more prone to future injury. Although it is shown that sprains result in scar tissue formation, it is unknown whether the thickness of the tissue increases as the frequency of recurrent sprains increases.

Previous research has shown that ankle sprains can lead to increased thickness in the injured ligament.\textsuperscript{24,25,26} Ligaments are primarily made up of water, collagen, proteoglycans, fibronectin, and cells.\textsuperscript{24} The collagen fibers are organized longitudinally, and are recruited upon ligament loading for length changes in relation to the position of the joint.\textsuperscript{24} However, if the force placed on the ligament exceeds the ultimate strength of the fibers recruited, failure occurs and the ligament is damaged, thus, creating joint instability and initiating the healing response.\textsuperscript{24,27} The first step of the healing process is the inflammatory phase, followed by the proliferative phase and lastly, the remodeling phase. The result of this process is scar tissue, which is weaker than the normal ligament and contains an increase of minor collagens and a decrease in collagen crosslinks, as well as an increase in glycosaminoglycans.\textsuperscript{24} In ligaments, the scar tissue contains smaller collagen fibrils, flaws between fibers, lower matrix-cell ratio, higher cell density, immature collagen cross links, large proteoglycans, and more cell division.\textsuperscript{27} These differences diminish the ultimate strength of scar tissue,
compared to a healthy ligament and alters joint biomechanics.\textsuperscript{27} A study by Liu et al.\textsuperscript{26} found a 0.25-0.33 mm increased thickness of injured ankles when compared with healthy ankles. This finding represents a 16\% increase in ATFL ligament thickness among injured ankles compared to healthy controls, suggesting that morphologic changes in the ligament occur secondary to ankle injuries.\textsuperscript{26} Thus, an irregular ligament could result in biomechanical deficits to the stabilizing properties needed during physical activity.\textsuperscript{26} However, it is unknown if the actual thickness is influenced by a higher frequency of subsequent sprains or if it related to a loss of function.

Therefore, the purpose of this study was two-fold; first was to determine how the number of sustained lateral ankle sprains affects the thickness of the ATFL, and second was to determine if a relationship exists between Cumberland Ankle Instability Tool (CAIT) scores and number of ankle sprains, CAIT scores and thickness, and gender and thickness. We hypothesized that an increased occurrence of ankle sprains would result in the increased thickness of the ATFL and that a correlation would exist between CAIT scores and number of ankle sprains, CAIT scores and thickness, and gender and thickness.
Chapter 2

METHODS

2.1 Subjects

Data from a total of 121 NCAA Division I collegiate athletes, with a total of 178 ankles, were examined. (Table 2.1.1) Each participant completed the Cumberland Ankle Instability Tool (CAIT) and an injury history questionnaire to report the number of sprains they have experienced throughout their lifetime. Participants with a history of bilateral ankle sprains were treated as individual ankles. Each participant read and signed the informed consent approved by the University Institutional Review Board.

The participants were divided into three groups: 0 (zero previous ankle sprains and a CAIT score of 30, bilaterally), 1 (one unilateral ankle sprain), and 2+ (two or more unilateral ankle sprains).

Table 1: Participants

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Participants (n)</th>
<th>Age (Years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>47</td>
<td>18.2 + 0.6</td>
<td>172.1 + 8.3</td>
<td>67.6 + 12.7</td>
</tr>
<tr>
<td>Male</td>
<td>74</td>
<td>18.8 + 1.1</td>
<td>184.8 + 8.6</td>
<td>91.1 + 19.3</td>
</tr>
</tbody>
</table>
2.2 Instrumentation

The Cumberland Ankle Instability Tool (CAIT) is a valid and reliable 9-item questionnaire, with multiple responses designed to evaluate the severity of functional ankle instability. The participants describe their instability of their ankle during sport and daily activities in eight of these questions. The remaining question asks when the participants experience pain. Possible scores range from 0, which is the best, to 30, which is the worst.

Thickness of the ATFL was measured by musculoskeletal ultrasound (MSUS) using a GE LOGIQ e (General Electric Company, Waukesha, WI, USA) using a 12 MHz frequency and 2.5 cm depth. The position of the transducer head will be positioned in the sinus tarsi, obliquely from the distal fibula from the origin to insertion of the ATFL. Ultrasonic gel (Aquasonic 100, Parker Laboratories, Inc. Fairfield, NJ) will be used as a conductive medium for the sound waves to travel from the probe through the skin. Reliability of this measure has been previously reported by Liu et al. (ICC= 0.91).

2.3 Procedures

The same examiner, who has three years of experience working with MSUS, performed all imaging, and a second, novice, examiner performed all the thickness measurements; reliability was determined between the two examiners. The average difference in measurements between the two examiners was 0.077 mm, or 70% agreement. Images of the ATFL were captured by MSUS. Patients were seated on a
treatment table in the long-seated position while three images of the ATFL with the ankle in neutral were taken. To obtain an image using MSUS, the region of the ATFL was scanned in the direction of the ligament fibers, which run anterioinferiorly from the lateral malleolus to the talus. Gentle pressure was applied directly over the ATFL with the ultrasound probe, and a real-time image was captured using a transducer to send high frequency sound waves that reflect back and provide images of the internal structure. The thickness measure was taken from the midpoint of the ligament between the attachments on the lateral malleolus and talus using the built-in measuring tool on the MSUS, using previously identified methodology by Liu et al. An average of the three images were used for analysis.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Examiner 1</th>
<th>Examiner 2</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.567</td>
<td>1.733</td>
<td>0.167</td>
</tr>
<tr>
<td>2</td>
<td>2.100</td>
<td>2.133</td>
<td>0.033</td>
</tr>
<tr>
<td>3</td>
<td>2.000</td>
<td>1.967</td>
<td>0.033</td>
</tr>
<tr>
<td>4</td>
<td>1.900</td>
<td>1.900</td>
<td>0.000</td>
</tr>
<tr>
<td>5</td>
<td>2.000</td>
<td>2.000</td>
<td>0.000</td>
</tr>
<tr>
<td>6</td>
<td>1.933</td>
<td>2.033</td>
<td>0.100</td>
</tr>
<tr>
<td>7</td>
<td>2.167</td>
<td>2.233</td>
<td>0.067</td>
</tr>
<tr>
<td>8</td>
<td>2.367</td>
<td>2.267</td>
<td>0.067</td>
</tr>
<tr>
<td>9</td>
<td>2.167</td>
<td>2.333</td>
<td>0.167</td>
</tr>
<tr>
<td>10</td>
<td>1.767</td>
<td>1.900</td>
<td>0.133</td>
</tr>
<tr>
<td><strong>Average (mm)</strong></td>
<td><strong>1.997</strong></td>
<td><strong>2.050</strong></td>
<td><strong>0.077</strong></td>
</tr>
<tr>
<td><strong>Min</strong></td>
<td></td>
<td></td>
<td><strong>0.000</strong></td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td></td>
<td></td>
<td><strong>0.167</strong></td>
</tr>
<tr>
<td><strong>% Agreement</strong></td>
<td>≤0.1 mm</td>
<td></td>
<td>70%</td>
</tr>
</tbody>
</table>
2.4 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed to determine the difference between those with or without a history of previous ankle sprains and ATFL thickness, along with a post-hoc test with a Bonferroni adjustment to determine whether statistically significant differences exist. A one-way ANOVA was also performed to determine if there were statistical differences between gender and ATFL thickness. A Spearman’s rho correlation coefficient was used to determine a relationship between the number of sprains and CAIT scores, CAIT scores and ATFL thickness, number of sprains and ATFL thickness, and gender and ATFL thickness. The independent variable was group (0,1, 2+). The dependent variable was thickness of the ATFL in millimeters (mm). A-priori level was set to 0.05 in order to determine significance. Descriptive statistics were calculated to provide measures of central tendency and to determine normality of the data.
Chapter 3

RESULTS

The aim of our study was to determine if the number of ankle sprains would affect ATFL thickness, and to determine if a relationship existed between CAIT score and ATFL thickness, number of sprains and ATFL thickness, gender and ATFL thickness, and CAIT scores and number of sprains. 121 NCAA division I student athletes, with a total number of 178 ankles were included in this study. Two ankles were excluded due to the thickness being at least three standard deviations outside the mean. There was no significant difference between the three groups (0, 1, 2+) and ATFL thickness. (Table 3) The 0 group had a mean of $1.59 \pm 0.15$, range of 1.33 to 1.97 mm; the 1 group had a mean of $1.63 \pm 0.15$, range of 1.4 to 2.1 mm; and the 2+ group had a mean of $1.61 \pm 0.14$, range of 1.37 to 1.97 mm. (Figure 3.1) Although we found no statistically significant differences in ligament thickness between groups, we found that ligament thickness increases after one lateral ankle sprain. There were no statistical differences between gender 0.62 (0.49) and ATFL thickness: 1.6 (0.15) (p= 0.97, F= 2.791 0.68 1.59 [0.16], 1 1.63 [0.14]). There was a statistically significant correlation between the number of sprains: 1.18 (1.14) and CAIT scores: 27 (3.7) (p< 0.001, -0.614) (Figure 3.2) and there was a statistically significant correlation between gender: 0.62 (0.49) and thickness: 1.6 (0.15) (p= 0.047, 0.149). There was no statistically significant correlation between the ATFL thickness: 1.6
(0.15) and CAIT scores: 27 (3.7) (p< 0.263, -0.084) or between number of sprains:
1.18 (1.14) and thickness: 1.6 (0.15) (p= 0728, 0.026).

Table 3: Average ATFL Thickness and CAIT Scores by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Ankles (n)</th>
<th>ATFL Thickness (mm)</th>
<th>P-value</th>
<th>CAIT Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52</td>
<td>1.59 ± 0.15</td>
<td>0.272</td>
<td>30.0 ± 0.0</td>
</tr>
<tr>
<td>1</td>
<td>77</td>
<td>1.63 ± 0.15</td>
<td>1.00</td>
<td>26.7 ± 3.4</td>
</tr>
<tr>
<td>2+</td>
<td>49</td>
<td>1.61 ± 0.14</td>
<td>1.00</td>
<td>24.7 ± 4.2</td>
</tr>
</tbody>
</table>

*CAIT score out of 30
Figure 1: Average ATFL Thickness by Group

![Bar chart showing average ATFL thickness by group.]

Figure 2: Average ATFL Thickness and Gender

![Bar chart showing average ATFL thickness and gender.]

Average ATFL Thickness and Gender
Figure 3: Correlation Between Number of Sprains and CAIT Score

![Figure 3: Correlation Between Number of Sprains and CAIT Score](image-url)
The aim of our study was to determine if the number of ankle sprains would affect ATFL thickness, and to determine if a relationship existed between CAIT score and ATFL thickness, number of sprains and ATFL thickness, gender and ATFL thickness, and CAIT scores and number of sprains. We hypothesized that an increased occurrence of ankle sprains would result in the increased thickness of the ATFL and that a correlation would exist between CAIT scores and number of ankle sprains, CAIT scores and thickness, and gender and thickness. We found no statistically significant differences in ligament thickness between groups, however, we found that ligament thickness increases after one lateral ankle sprain. We also found a significant correlation between number of sprains and CAIT scores, meaning that a higher number of sprains correlates to a lower CAIT score. These findings indicate that after sustaining an ankle sprain, morphological changes occur. Our findings also show that thickness on a macroscopic scale does not reflect the loss of function experienced by patients who have a history of ankle sprains.

As ligaments heal after an ankle sprain, normal tissue is replaced with stiffer scar tissue, which may lead to re-injury.\textsuperscript{31,32} Congruent with our findings, previous studies have found that ATFLs in ankles with no previous history of injury are thinner and
experience thickening of the ligament after one sprain. However, the finding that ankles with two or more sprains seem to have decreased ligament thickness has not been researched, warranting a need for further investigation. Chimich et al. conducted a study that examined collagen fibril diameters in healing adult rabbit medial collateral ligaments. The MCL is comparable to the ATFL due to the fact that both are extracapsular ligaments. It was found that despite an increase in scar strength and stiffness, there was no apparent increase in mean collagen fibril diameters. In this study, Chimich et al. compared healing adult rabbit MCLs to healing rabbit Achilles tendons in a study conducted by Postacchini et al. This study speculated that loading plays an important role in the recovery of normal collagen fibril morphology during healing, based on a partial thickness injury model in rabbit Achilles tendons. In the case of the Achilles tendons, it was also speculated that the absence of loading in complete ruptures may inhibit maturation and distribution of collagen. These findings suggest that individuals that place excess stress on the healing tissue, or fail to apply any load during the healing process, may be altering the morphology of the ligament, thus creating a weaker structure. In this case, those that return to play too soon on a healing ligament or fail to properly rehabilitate it, may be inhibiting the maturation of normal collagen diameter distributions. This may ultimately lead to a weaker or abnormal ligament that is more susceptible to re-injury, which could in turn lead to chronic ankle instability.

One theory that may help explain the altered mechanical properties ATFLs after injuries is related to circulation. Ligaments have a limited blood supply which can
change over time due to chronic damage and could ultimately inhibit the healing process. Bray et al. found that scar tissue in the MCL had increased densely disorganized blood vessels. Early on, the scar tissue was hypervasculaular, but as the healing process progressed, the tissue that initially contained thick and disorganized vessels became less vascular. Ultimately, this sequence of events may disrupt cellular physiology and the collagen proliferation needed to restore ATFL strength and ankle biomechanics.

Anterior talofibular ligament thickness was measured using diagnostic ultrasound, similar to the study by Liu et al. Using ultrasound, we measured ligament thickness, which can aid the clinician in determining who is at risk for potential injury or for developing CAI. Diagnostic ultrasound has shown to have comparable sensitivity and specificity when compared to MRI in diagnosing ATFL injuries. It also allows for dynamic and real-time imaging, as well as cost-effectiveness. Additionally, ultrasound is portable, allowing for easy and ready use in a clinic setting. The utilization of diagnostic ultrasound would be useful in the clinical setting to determine both ligament thickness, as well as injury to the ligament and various other structures. The visualization of the ligament would also allow clinicians to determine who is at risk of injury. With this information, athletic trainers would be able to anticipate and work to prevent injury, in addition to tailoring the rehabilitation process to fit the specific patient’s needs. Based on our thickness measurements in the 2+ group, as well as the speculations of Chimich et al. and Postacchini et al., individuals who have sprained their ATFLs numerous times may have disrupted normal collagen maturation
in the tissue, may have an abnormal and injury prone ligament. Ultrasound produced a visualization of these thinner ligaments, which could identify who may be at risk for re-injury and chronic ankle instability. This knowledge allows clinicians to be preventative with their treatment and rehabilitation, as well as possible taping or bracing techniques.

We acknowledge the limitations present in this study. The determined number of ankle sprains for each participant was self-reported in the CAIT, making it subjective and based on their own memory. Additionally, for this study we did not measure mechanical instability to determine the effect of laxity on ATFL thickness, which has been previously examined. Lastly, the time from injury to measurement was difficult to identify due to the self-reporting nature of the CAIT.

Our results showed no significant differences in thickness measurements between the three groups. However, individuals with a previous history of lateral ankle sprains had thicker ATFLs when compared to those with no previous history, as well as lower CAIT scores. The observations and speculations by Postacchini et al.34 regarding excess loading and absent loading may be the reasoning behind decreased ligament thickness in the 2+ group, and warrant the need for more research on the subject relating to the ATFL. With further research, and the use of diagnostic ultrasound, clinicians will be more informed and can be more preventative and knowledgeable in regards to treating ankle sprains, as well as chronic ankle instability.
REFERENCES

1. Garrick JG. The frequency of injury, mechanism of injury, and epidemiology of ankle sprains*.


7. Hootman JM, Dick R, Agel J. Epidemiology of collegiate injuries for 15 sports:


33. Chimich D, Frank C, Shrive N, Dougall H, Bray R. The effects of initial end contact on medial collateral ligament healing: A morphological and


45. Attarian DE, McCrackin HJ, DeVito DP, McElhaney JH, Garrett WE.


58. LiMarzi G, Scherer K, Porrino J. Visualization of the Ankle Lateral Collateral


65. van Rijn RM, van Os AG, Bernsen RMD, Luijsterburg PA, Koes BW, Bierma-


68. Hintermann B, Boss A, Schä D. Arthroscopic Findings in Patients with Chronic Ankle Instability.


71. Oae K, Takao M, Uchio Y, Ochi M. Evaluation of anterior talofibular ligament


Appendix A

LITERATURE REVIEW

A. Introduction

Ankle sprains are among the most common injuries in sports.\textsuperscript{1,2} Approximately 712,000 individuals worldwide sprain their ankles each day.\textsuperscript{3} Of these, 850,000 new ankle sprains occur each year in the United States, with an overwhelming 30,000 that occur each day in the general and athletic population.\textsuperscript{4,5} In an athletic population, acute ankle sprains are the most common type of sport-related traumas and the most prevalent musculoskeletal injury, which account for up to 40\% of all athletic injuries.\textsuperscript{3,6,7} In National Collegiate Athletic Association (NCAA) student-athletes, ankle sprains account for 15\% of all injuries each year.\textsuperscript{8,9} As concluded in a study by Roos et al.,\textsuperscript{10} sprains to the lateral ligamentous complex of the ankle were the most reported type of injury, accounting for 7.3\% of all injuries in 25 NCAA sports.

An acute lateral ankle sprain (LAS) is defined by Delahunt et al.\textsuperscript{11} and accepted by the International Ankle Consortium as “an acute traumatic injury to the lateral ligament complex of the ankle joint as a result of excessive inversion of the rear foot or a combined plantar flexion and adduction of the foot.”\textsuperscript{3} The majority, or 85\%, of ankle sprains occur due to inversion of the ankle, which stresses the anterior talofibular ligament (ATFL). The most commonly injured ligament is the ATFL, since it is the weakest in the lateral ligament complex of the ankle.\textsuperscript{2,12} Ankle sprains result in
damage to the ligament, which then trigger the healing response.\textsuperscript{13} The first step of the healing process is the inflammatory phase; this phase begins almost immediately and continues over the next 48-72 hours.\textsuperscript{13} During this phase, blood collect where the injury occurred and clot formation is initiated.\textsuperscript{13} Overlapping with the inflammatory phase, the proliferative phase begins days 3-5 following the injury.\textsuperscript{13} The proliferative phase of the healing response is characterized by the production of scar tissue, which is made up of dense, cellular, collagen that forms a bridge to repair the damage.\textsuperscript{14} However, excessive healing results in a superfluous build-up of connective tissue which then leads to altered structure and loss of function.\textsuperscript{15} The proliferative phase then merges with the remodeling phase, which can continue for months to years.\textsuperscript{13} During this phase, collagen and ligament matric continue to be overturned by tissue synthesis and degradation.\textsuperscript{13}

Research has shown that acute and untreated ankle sprains can lead to chronic ankle instability (CAI), as well as increased thickness in the injured ligament.\textsuperscript{16,12} There are various ways of diagnosing ankle sprains through imaging; however, musculoskeletal ultrasound (MSUS), when compared to other methods for obtaining images, proves to have acceptable sensitivity, specificity, and accuracy values.\textsuperscript{12} Although research has shown that suffering from a previous ankle sprain results in morphological changes in the ATFL, the number of previous ankle sprains and how it relates to ATFL thickness has not been researched.
B. Epidemiology

In a study by Roos et al., it was found that the most common injury mechanisms for lateral ankle sprains were player contact (41.4%), non-contact (27.4%), and surface contact (22.2%). In this study, 2,429 lateral ankle sprains were reported for a rate of 4.95 per 10,000 athlete exposures, and accounted for 7.3% of all reported collegiate sports injuries during the 2009-2010 and 2014-2015 academic years. An estimated 16,022 lateral ligament complex sprains occurred annually among 25 sports.

A study by Hootman et al. summarized 16 years of NCAA injury surveillance data for 15 sports. It was found that injury rates were statistically significantly higher in games (13.8 injuries per 1000 athlete exposures) than in practices (4.0 injuries per 1000 athlete exposures), and preseason practice injury rates (6.6 injuries per 1000 athlete exposures) were significantly higher than both in-season (2.3 injuries per 1000 athlete exposures) and post-season (1.4 injuries per 1000 athlete exposures) practice rates. It was also discovered that more than 50% of all injuries were to the lower extremity, and ankle ligament sprains accounted for 15% of all reported injuries.

A systematic review by Fong et al. reviewed 227 epidemiological studies on sports injury from 1977 to 2005 in which the ankle was included. It was found that the ankle was the most commonly injured body part after the knee, and that ankle sprains were the most common injuries involving the ankle. Sports such as aeroball, wall climbing, indoor volleyball, mountaineering, netball, and field events in track and field had ankle sprains as the most common injuries. Additionally, Australian football,
field hockey, handball, orienteering, scooter, and squash reported that all ankle injuries were sprains.\textsuperscript{7} It was also found that incidence of ankle injuries and sprains was high in court games and team sports such as rugby, soccer, volleyball, handball, and basketball.\textsuperscript{7}

C. Anatomy

The talocrural joint is formed by the articulation of the dome of the talus, medial malleolus, tibial plafond, and the lateral malleolus.\textsuperscript{17} The lateral side of this joint receives ligamentous support from the calcaneo Tibul ar ligment, posterior talofibular ligament, and anterior talofibular ligament.\textsuperscript{17,18} The calcaneofibular ligament (CFL) originates on the inferior margin of the fibula distal to the anterior talofibular ligament (ATFL) and runs underneath the peroneal tendons and then inserts on the lateral tubercle of the calcaneus.\textsuperscript{19} The posterior talofibular ligament (PTFL) is a thickening of the capsule from the posterior fibula to the lateral tubercle of the posterior process of the talus.\textsuperscript{19}

Of the lateral ankle ligaments, the anterior talofibular ligament (ATFL) is the weakest, demonstrating lower maximal load and energy to failure values under tensile stress when compared to the other lateral ligaments.\textsuperscript{17,20} It is a flat, quadrilateral ligament that originates from the inferior oblique segment of the anterior border of the lateral malleolus, courses anteromedially, and inserts on the talar body just anterior to the lateral malleolar articular surface.\textsuperscript{21,22,19} When the foot is in the anatomical position, the ATFL runs approximately horizontally, but when it is plantarflexed, the
ligament is nearly parallel to the long axis of the leg.\textsuperscript{2} This plantarflexed position is when the ATFL is the most vulnerable to injury due to the amount of strain placed upon it.\textsuperscript{2,23-27} Approximately two-thirds of all ankle sprains are isolated injuries to the ATFL.\textsuperscript{17,23,28}

In a study by Khawaji et al.,\textsuperscript{29} the ATFL was observed to have one band in 22.9\% of subjects, two bands in 56.3\% of subjects, and three bands in 20.8\% of subjects. In regards to this anatomical variation, the overall width of the ATFL does not significantly vary, which leads to the assumption that this does not change the function of the ligament.\textsuperscript{29} Taser et al.\textsuperscript{21} reports that the ATFL courses from the anterior margin of the lateral malleolus to a talar attachment, making a mean angle of 25° (ranging from 5°-45°) with horizontal plane, and a mean angle of 47° (ranging from 45-56°) with sagittal plane. Burks et al.\textsuperscript{22} found the average width of the ATFL to be 7.2 mm and average length to be 24.8 mm. This study also had an inconsistent finding of a distinct inferior band of the ATFL that had an average length of 20 mm and average width of 4.6 mm in some specimens.\textsuperscript{22}

Ligaments are primarily composed of water, collagen, proteoglycans, fibronectin, and cells.\textsuperscript{30} Approximately two-thirds of ligaments are water and the remaining one-third solid, along with the water that is responsible for cellular function and viscoelastic behavior.\textsuperscript{14} They are covered by the epiligament, which is the vascular outer layer that merges into the periosteum of the bone around the site where the ligaments attach.\textsuperscript{14,31} At the microscopic level, the collagen fibers are organized longitudinally and are recruited upon ligament loading for length changes in relation
to the position of the joint. The collagen presents crimp, which is thought to relate to ligament loading, where uncrimping allows the elongation without damage. However, if the force placed on the ligament is greater than the number of fibers recruited, failure occurs and the ligament is damaged, thus, initiating the healing response which leads to scar tissue formation.

Injury to the lateral ankle ligaments is traditionally described as inversion trauma. The most common mechanism of injury for lateral ankle sprains is excessive supination of the rearfoot about an externally rotated lower leg soon after initial contact of the rearfoot during gait or landing from a jump. This mechanism, coupled with excessive inversion and internal rotation of the rearfoot, results in strain to the lateral ankle ligaments, and excessive strain leads to ligamentous damage. When excess force is applied to the foot in this position, the ATFL is vulnerable to full or partial tears, which are often graded 1-3 depending on the severity of the injury. Typically when a ligament is injured, it often occurs at one site. However, recurrent twists could induce the injury at multiple sites. A contributing factor for the more frequent occurrence of lateral ankle sprains is the fact that the medial malleolus is short and there is a natural tendency for the ankle to invert rather than evert. Typically, the ATFL is the first ligament to suffer damage, followed by the CFL, and then the PTFL. Sprains result in damage to the ligament, which generates a healing response that is distinguished by scar formation. Scar tissue is weaker by nature when compared to a normal, healthy ligament, making the tissue more prone to future injury. Although it is shown that sprains result in scar tissue
formation, it is unknown whether the thickness of the tissue increases as the frequency of recurrent sprains increase.

D. Chronic Ankle Instability

Chronic ankle instability refers to repetitive episodes of instability resulting in the recurrent ankle sprains.\textsuperscript{17} Research has shown that acute, as well as untreated, ankle sprains can lead to chronic ankle instability (CAI).\textsuperscript{6,38} CAI is typically thought to have two potential causes: mechanical instability and functional instability.\textsuperscript{17} Mechanical instability is a cause of CAI due to pathologic laxity after a sprain occurs.\textsuperscript{39} Functional instability is the occurrence of recurrent ankle instability and sensation of joint instability due to the contributions of proprioceptive and neuromuscular deficits.\textsuperscript{40} Approximately one-third of those who experience an acute ankle sprain will suffer from long term residual symptoms.\textsuperscript{41–44} Furthermore, it has been reported in a study by Anandacoomarasamy\textsuperscript{45} that 74\% of individuals exhibited at least one residual symptom of CAI for 1.5 to 4 years after the initial ankle sprain. The recurrence rates of LASs are high, which lead to a large percentage of individuals who suffer from LAS to develop CAI.\textsuperscript{46} An estimated 30-40\% of untreated ankle sprains result in CAI\textsuperscript{38} which can result in impaired balance, proprioception, reaction time, strength, pain, loss of play time, medical expenditure, bone fractures, functional disability, limited mobility, and permanent retirement from sports participation.\textsuperscript{7,47} CAI also contributes to ongoing disability and sensorimotor deficits, which result in decreased physical activity and quality of life.\textsuperscript{46}
A controlled laboratory trial by Son et al. examined movement strategies among groups of chronic ankle instability, copers, and a control group. A total of 66 active individuals: 22 CAI patients, 22 ankle sprain copers, and 22 healthy controls, were recruited to participate in this study. The ankle sprain coper group consisted of those with a history of ankle sprains, but are able to perform a highly demanding sports maneuver without complaint of chronic residual symptoms. The age range was 18 to 35 years old and all participants were matched for sex, height, weight, and leg dominance. It was shown that those with CAI have less of a plantarflexion angle from the initial to midlanding phase when compared to copers and controls. A restricted dorsiflexion angle from the midlanding to mid side-cutting phase, increased hip flexion angle during most of the landing/cutting phase, unique sagittal-plane joint moment movement strategies, less inversion during most of the landing/cutting phase, and altered frontal-plane hip angle and moment movement strategies were also present. It is clinically important to note these kinematic alterations due to the fact that patients with CAI are more susceptible to ankle sprains.

A study by Hintermann et al. performed arthroscopic examinations between the years of 1993-1999 on 148 individuals with symptomatic chronic ankle instability that had lasted 6 months or more. Of these ankles, a rupture or elongation of the ATFL was noted in 86% of ankles, of the CFL in 64%, and of the deltoid ligament 40% of individuals. Of these injuries, 102 ankles (69%) were asymptomatic and 46 patients (31%) had chronic pain, tenderness, swelling, or induration, causing disabilities in sports and daily activities. Eighty-eight patients or 59%, had daily giving way, 40
(27%) had monthly episodes of instability, and 20 (14%) had weakness.\textsuperscript{49} Sixty-six (44%) had pain at rest, 38 (26%) had to use analgesics for chronic pain, 92 (62%) reported pain and instability during walking and 130 (88%) reported pain and instability during sports.\textsuperscript{49} This study concluded that abnormalities of different structures were involved with chronic ankle instability and that there was no one single cause. The CFL was not visible in 42 cases (28%), the ATFL was not visible in 4 ankles (3%), a combined lesion of the ATFL and CFL was found in 95 ankles (64%), and in 59 ankles (40%) there was a lesion of the deltoid ligament found.\textsuperscript{49}

**E. Musculoskeletal Ultrasound**

Research has shown that ankle sprains can lead to increased thickness in the injured ligament.\textsuperscript{50,16} This thickness may be the result of scar tissue due to the healing of the ligament, or the ligament may have become thick due to the retraction when the ligament tears at either side when injured.\textsuperscript{51} The width of the ATFL is approximately 2 mm, so a thickness of more than 20% is thought to be significant.\textsuperscript{21} A study by Liu et al.\textsuperscript{16} found a 0.25-0.33mm increased thickness of injured coper (an ankle that experienced a single previous sprain at least 12 months ago, returned to a preinjury level of activity, scored \( \geq 26 \) of the Cumberland Ankle Instability Tool, and had no episodes of re-injury) and unstable ankles when compared with healthy ankles. This finding represents an increase of nearly 16% in ligament thickness compared with the ATFL of an ankle that has never been injured.\textsuperscript{16}
In a study by Dimmick et al., the mean thickness of the ATFL was found to be $2.19 \pm 0.6$ mm. In men, the mean thickness of a normal ATFL is $2.44 \pm 0.49$ mm. In women, this measurement is $2.16 \pm 0.47$ mm.

Musculoskeletal ultrasound has been used to identify injuries to the ATFL. Advantages of using musculoskeletal ultrasound (MSUS) include cost-efficiency, shorter examination time, and the ability for real-time and dynamic imaging. It also utilizes no ionizing radiation and is portable, as well as widely available. In a case report by Oae et al., MSUS demonstrated a 91% accuracy in diagnosing an ATFL injury. The use of MSUS has shown good to excellent interrater and intrarater reliability in the linear measurement of the ATFL under stress positions and it can be used to examine both laxity and thickness of the ATFL.

A study by Croy et al. in 2012 used MSUS to measure the change in ATFL length during simulated anterior drawer and ankle inversion stress tests in 60 individuals. These individuals were divided into 3 groups: control subjects without a previous history of ankle injuries, ankle copers, and subjects with CAI. The anterior drawer test resulted in length changes that were greater in the CAI and coper groups compared to the control group and the ankle inversion test resulted in greater ligament length change in the CAI and coper groups, with no difference in length when compared to each other. This study concluded that stress ultrasonography identified greater length changes of the ATFL in both cope and CAI groups compared to the control group.
A prospective cross-sectional study by Lee et al.\textsuperscript{58} in 2017 aimed to evaluate the feasibility of point-of-care ankle ultrasound compared with magnetic resonance imaging for diagnosing major ligament injuries in patients with recurrent ankle sprain and CAI in 85 patients. As a result of this study, point-of-care ultrasound showed acceptable sensitivity (96.4-100\%), specificity (95.0-100\%), and accuracy (96.5-100\%).\textsuperscript{58} Lee et al. concluded that point-of-care ankle ultrasound is as precise as MRI for detecting major ankle ligament injuries and that it clearly demonstrates the anatomy and extent of injury to the ankle ligaments.\textsuperscript{58,59,60}

Placement of the transducer head for Imaging the anterior talofibular ligament.\textsuperscript{16}
Liu et al.\textsuperscript{16} in 2015 sought to examine whether morphologic differences exist in the thickness of the ATFL in healthy, coper, and unstable ankle groups in 80 NCAA D1 athletes. The coper group was defined as an ankle that experienced a single previous sprain at least 12 months ago, returned to a preinjury level of activity, scored $\geq 26$ of the CAIT, and had no episodes of re-injury.\textsuperscript{16} MSUS was used to take an image of the ATFL and the thickness was measured at the midpoint of the ligament between the attachments on the lateral malleolus and talus. It was found that the ATFLs of the injured limbs for the coper group ($2.20 \pm 0.47$ mm) and unstable group ($2.28 \pm 0.53$ mm) were thicker than the “matched side” limb of the healthy group ($1.95 \pm 0.29$ mm). This finding represents an increase of nearly 16\% in ligament thickness compared with the ATFL of an ankle that has never been injured, thus, this study suggests that morphologic changes to the ligament occur secondary to ankle injuries.\textsuperscript{16}
Thickness compared to laxity has been researched, however, it is unknown if that thickness in unstable ankles has a higher frequency of subsequent sprains.

A case report by Battaglia et al. examined a 28-year old man with left ankle pain 5 days after an ankle injury. The patient presented with an inability to fully weight-bear, pain while walking, moderate localized swelling over the left lateral malleolus, and point tenderness over the left lateral malleolus. The injury was diagnosed as a grade II ATFL sprain and the patient underwent conservative treatment for stabilization and proprioception. When the patient’s pain returned 12 weeks later, musculoskeletal ultrasound was used to examine the joint. Through the use of musculoskeletal ultrasound, a full thickness ATFL tear was found, as well as laxity and partial thickness tear of the CFL, ankle joint and talonavicular osseous hypertrophic changes consistent with arthritis, remote deltoid ligamentous complex injury, peroneus and posterior tibialis tenosynovitis, and a large ankle joint effusion with synovial hypertrophy and synovitis. Ultrasonography provided a real-time dynamic examination of the impaired ligamentous structures and enabled visualization of ankle joint and talonavicular osteoarthritis. Thus, this case report exemplified the value and utility of ultrasonography in diagnosing ligamentous, tendinous, articular, and osseous injuries of the ankle.
REFERENCES

1. Garrick JG. The frequency of injury, mechanism of injury, and epidemiology of ankle sprains*. 


27. Marder RA. Current methods for the evaluation of ankle ligament injuries. Instr


41. Thompson JY, Byrne C, Williams MA, Keene DJ, Schlussel MM, Lamb SE. Prognostic factors for recovery following acute lateral ankle ligament sprain: a


3758.38.2000295.