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Ultrasound characteristics of foot and ankle structures in healthy, coper, and chronically unstable ankles

Short title: Foot and ankle structures in health and sprained ankles

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The study protocol was approved by Ethical Committee of the University of Salford in Salford, UK.

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Abstract

Objective: Ankle sprains constitute approximately 85% of all ankle injuries and up to 70% of people experience residual symptoms. Whilst the injury to ligaments is well understood the potential role of other foot and ankle structures has not been explored. The objective was to characterise and compare selected ankle structures in participants with and without a history of lateral ankle sprain.

Methods: 71 participants were divided into 31 healthy, 20 coper, and 20 chronic ankle instability groups. Ultrasound images of the anterior talofibular and calcaneofibular ligaments, fibularis tendons and muscles, tibialis posterior and Achilles tendon were obtained. Thickness, length, and cross sectional areas were measured and compared between groups.

Results: When under tension the anterior talofibular ligament was longer in copers and chronic ankle instability groups compared to healthy participants ($p <0.001$ and $p = 0.001$ respectively). The chronic ankle instability group had the thickest ATFL and CFL among the three groups ($p < 0.001$). No significant differences ($p >0.05$) in tendons and muscles were observed between the three groups.

Conclusions: The ultrasound protocol proved reliable and was used to evaluate the length, thickness, and CSA of selected ankle structures. The length of the ATFL and the thickness of the ATFL and CFL were longer and thicker in injured groups compared to healthy.

Key Words: ankle ligaments, ankle sprain, chronic ankle instability
Introduction

Ankle injuries rank among the most frequent musculoskeletal problems affecting athletes and the general population\(^1\) and account for 15% – 20% of all sports injuries.\(^2\) Ankle sprains constitute approximately 85% of all ankle injuries\(^2,3\) and up to 90% occur in the lateral ligament complex.\(^1,3,5\) A lateral ankle sprain is caused by inversion of the talus relative to the fibula\(^6\) as a result of an external inversion moment at the ankle. This external moment is dependent on the position and trajectory of the heel and leg relative to the supporting surface\(^7\) and will be resisted by a range of internal structures that create eversion moments, including the lateral ankle ligaments. Of the three lateral ankle ligaments the anterior talofibular ligament (ATFL) is almost always affected, being the weakest and having the lowest modulus of elasticity.\(^8\) The calcaneofibular ligament (CFL) is also affected in 50 - 75% of cases.\(^9\) Damage to the lateral ankle ligaments is associated with residual ankle instability and recurrence rates of up to 70%.\(^10\)

The lateral ligaments are, however, not the only structures involved in resisting ankle inversion moments\(^11\) and tenderness of the fibularis tendons is common post sprain.\(^12\) Indeed, clinical signs of isolated fibularis tendon injuries may be misdiagnosed as ankle sprains,\(^13,14\) and weakness of the fibularis muscles is thought to be a risk factor for lateral ankle sprain.\(^11\) Repeated sprains might lead to a generalised increase in fibularis activity as a strategy to reduce risk of further lateral ankle injuries. This could be especially useful since proprioception is often diminished post sprains\(^15\) and there might be insufficient feedback on ankle position to produce effective fibularis muscle recruitment strategies at the instant of a future inversion incident. Greater use of the fibularis muscles, or weakness in the muscles,
might lead to change in their size over time and be related to differences in sprain incidence.

Other muscles also affect ankle inversion/eversion moments and ankle instability, and might protect against repeated sprains. For example, co-contraction of invertor, plantar-flexor and dorsi-flexor ankle muscles would increase ankle joint compression load and thereby joint stability. Indeed Lam and Lui\textsuperscript{16} reported one case study of undiagnosed rupture of the Achilles tendon associated with severe lateral ankle sprain and Lhoste-Trouilloud\textsuperscript{17} suggested an association with tibialis posterior damage.

Understanding any changes in tendon and muscle function associated with lateral ankle sprains might help identify those at risk of lateral ankle sprain and strategies for rehabilitation post injury. Whilst the ligamentous basis of lateral ankle sprains is well-documented\textsuperscript{5,18} any relationship with other relevant ankle structures is not. Furthermore, individuals with previous sprains demonstrate different risk of recurrent sprains, and this could relate to ligaments and these other structures. So, called “copers” have histories of single ankle sprains\textsuperscript{19,20} and seemingly adopt strategies that do not lead to future sprains, which could include greater use of the fibularises for example. In contrast cases of “chronic ankle instability” (CAI) experience repeated sprains.\textsuperscript{21} The ATFL is the only structure that has been compared between coper and cases of CAI, and it could be that CFL or fibularis muscles play a role in the recurrence of ankle sprains. Mansfield and Neumann\textsuperscript{11} proposed that weakness of the fibularis muscles predisposes the foot to the inversion position that is essential to lateral ankle sprains. As discussed, other structures could be implicated too.
The aim of this study, therefore, was to compare selected ankle structures between healthy, coper and CAI cases for the purpose of understanding whether and how other ankle structures might be implicated in the injury mechanism.

**Materials and Methods**

**Participants**

Seventy-one participants (33 females, 38 male) (mean ± SD age = 27.77 ± 7.13 years, BMI = 24.20 ± 2.47 kg/m²) were recruited from a University community and formed healthy, coper, and CAI groups. All participants provided written informed consent, the rights of participants were protected, and the study was approved by the host institutional ethics committee. Demographics of the three groups are detailed in Table 1.

The inclusion criteria for the healthy group were; physically active based on the general practice physical activity questionnaire from the National Health Service (NHS), self-reported good health and a Cumberland Ankle Instability Tool (CAIT) score ≥ 25.

The coper group was physically active based on the general practice physical activity questionnaire from the NHS, self-reported good health, classified according to Wikstrom and Brown and had a history of a single self-reported lateral ankle sprain diagnosed by a healthcare professional and no weight bearing for at least 3 days at the time of injury. They must have returned to moderate physical activity for at least 1 year without further episodes of giving way or sprain injury, and had a CAIT score of ≥ 24.

The CAI group as physically active based on the general practice physical activity questionnaire from the NHS, self-reported good health, classified according
to Gribble et al.\textsuperscript{24} and had a history of 2 or more self-reported lateral ankle sprains diagnosed by a healthcare professional, with the most recent sprain occurring at least 3 months ago. They also reported several episodes of the ankle “giving way”, had a CAIT score of $\leq 24$. The exclusion criteria from the study included a history of previous surgeries or fractures on the lower limb, or acute lower limb injury in the last 6 weeks (including lateral ankle sprain).

The CAIT questionnaire contains nine questions covering 30 points and identifies the severity of functional instability of the ankle joint.\textsuperscript{25} Eight of the nine questions are designed to evaluate ankle instability during daily and sports activities, while one question is focused on when participants feel pain.\textsuperscript{8} The questionnaire score ranges from 0 to 30, with lower scores representing greater ankle instability.\textsuperscript{20} It has been shown to be reliable and a valid measure of ankle instability.\textsuperscript{26,27}

**Data Collection**

Real time ultrasound scanning was performed with a portable Venue 40 US system (GE Healthcare, UK) and a 5-13 MHz linear array transducer with a 12.7 x 47.1 mm footprint area,\textsuperscript{28} and image depth of 3 cm.

Participants lay in the supine position with the foot held in a neutral position ($0^\circ$ dorsi-/ plantar-flexion) using an ankle foot orthosis (AFO). A strap was placed around the forefoot and the leg placed against a sand bag for stability (Figure 1). After scanning structures in this position, the AFO was removed and the foot manipulated into various positions to place each structure under tension. The ankle was passively and manually moved to the end of the ankle plantar-flexion and inversion range when scanning ATFL, fibularis longus tendon (FLT), fibularis brevis tendon (FBT), fibularis longus muscle (FLM), and fibularis brevis muscle (FBM). The
ankle was moved to the end of its eversion range when scanning for tibialis posterior tendon (TPT), and moved to 10° of dorsiflexion when scanning CFL and Achilles tendon (AT) under tension (Figure 2).

The ATFL was scanned in a longitudinal plane and the proximal edge of the transducer placed over the anterior boarder of the lateral malleolus (LM) and the distal edge over the talus. The full length of ATFL was measured from the origin (LM) to the insertion point (talus) while the thickness was measured halfway between LM and talus (as per protocol by Dimmick and colleagues\textsuperscript{29} (Figure 2. A-B).

For the CFL, the transducer head was placed anterior to the tip of lateral malleolus in an oblique coronal plane such that the distal probe was toward the heel (as described by De Maeseneer et al.\textsuperscript{30}) (Figure 2.C). The measurements of CFL were taken in the longitudinal plane. The full length of the CFL is rarely visible because the origin is underlying the LM. However, the thickness was measured 1 cm from the insertion point (calcaneus) (Figure 2.C).

For the transverse image of fibularis tendons, the transducer was placed slightly inferior to the distal part of the LM and at the posterolateral ankle. The measurement of CSA was taken below LM (De Maeseneer et al.\textsuperscript{30}) (Figure 2.D). Having confirmed the FLT and FBT location (peroneus brevis is located near to the LM while the longus is more superficial (De Maeseneer et al.\textsuperscript{30}), the transducer was rotated 90° to obtain the longitudinal image of tendons to measure the thickness. The longitudinal scan it is the only technique that allows measurement of the distance from the bony attachment to the point where the thickness is measured. Thus, all the measurements will be done at the same point for all participants. The transducer was moved slightly up (toward the dorsum of the foot) to scan the FBT.
and slightly down (toward the plantar of the foot) to scan FLT. Thickness was measured 1 cm below LM (Figure 2.E-F).

For the transverse image of fibularis muscles and to measure its CSA, the transducer was perpendicular to fibula, halfway (50%) between the fibular head and the inferior border of the LM (as in Angin et al.\textsuperscript{28}) (Figure 2.G). The transducer was then rotated 90° to scan the muscles in a longitudinal plane and measure thickness (Figure 2.H).

To scan TPT, the transducer was placed slightly superior to proximal part of the medial malleolus (MM) in an oblique transverse plane to allow CSA measurement (Figure 2.I). The TPT tendon is close to MM and twice the size of flexor digitorum tendon and medial to it.\textsuperscript{17,31} The transducer was then rotated 90° to obtain a longitudinal image of TPT and measured tendon thickness 2 cm above the medial malleolus (Figure 2.J).

To scan the Achilles, the participants moved forward to hang their foot to the end of the bed. The transducer was placed at the posterior aspect of the tendon in a longitudinal plane to measure the thickness at the level where the Achilles tendon separates from calcanei (Figure 2.K), then rotated the transducer 90° to obtain the transverse image of AT and measure its CSA (Figure 2.L).\textsuperscript{32}

To evaluate the intra-examiner reliability of this protocol, ten healthy participants (5 males and 5 females; mean ± SD age, 32 ± 3.59 years; height, 1.64 ± 0.09 m; mass, 62.20 ± 11.83 kg; BMI = 22.92 ± 2.40 kg/m\textsuperscript{2}) and ten participants with lateral ankle sprain (8 males and 2 females; age of 30 ± 8.70 years; height 1.66 ± 0.10 m; weight 69.60 ± 8.92 kg; BMI = 23.21 ± 3.22 kg/m\textsuperscript{2}) were tested on two occasions one week apart by the same sonographer.
**Image Analysis**

Length (mm), thickness (mm), and CSA (mm$^2$) were measured using ImageJ software (National Institute for Health, Bethesda, MD, USA) with sonographer (R.A) blinded to the groups. The thickness of the structure was the linear distance between aponeuroses, while CSA was measured by tracing the inside margin of the connective tissue of the tendon and muscle with an electronic marker in the software.

**Statistical analysis**

Data analysis was performed using the SPSS software version 23.0. The reliability of the protocol was analysed by a two-way fixed model with absolute agreement calculated using the intra-class correlation coefficients (ICC) and Limits of Agreements (LoA). ICC was classified as moderate when > 0.80, and excellent when > 0.90. LoA was calculated (mean difference ± 1.96 x standard deviation) as defined by Bland and Altman. A series of one-way analysis of variance (ANOVA) tests were performed to investigate significant differences in demographics, length, thickness, and CSA of ankle structures between groups (healthy vs coper vs CAI). Post-hoc Bonferroni tests were performed to provide pairwise comparisons with an $a$ priori alpha level set at $p \leq 0.05$. Cohen’s $d$ effect sizes were calculated with $d = 0.20 - 0.49$ to be considered a 'small' effect size, $0.50 - 0.79$ represents a 'medium' and $> 0.80$ a 'large' effect size.

**Results**

Intra-examiner reliability in healthy participants was excellent (ICC range 0.94 – 1.00), and limits of agreement were between 5.0% and 30% of the average
measurement (Table 2). In injured participants, the intra-examiner reliability was moderate to excellent (ICC range 0.85 – 0.98), and the limits of agreement were between 8.0% and 26% of the average measurement.

There was no statistically significant difference in the length of ATFL between the three groups when the ankle was in a neutral position (p = 0.57). However, a statistically significant difference was found with the ankle under tension (p< 0.001 for healthy versus coper and healthy versus CAI, but not coper versus CAI) (Figure 4). The change in neutral length to tension length greater in the coper (4.79 mm) or chronically unstable groups (4.72 mm) was greater than that of the healthy group (3.07 mm).

The ATFL was significantly longer when under tension and thicker in copers (23.61 ± 1.79 mm and 2.44 ± 0.38 mm) and CAI (23.48 ± 0.82 mm and 2.93 ± 0.31 mm) compared to healthy participants (22.22 ± 1.27 mm and 1.90 ± 0.16 mm) (Table 4). The CFL was significantly thicker in CAI (1.82 ± 0.12 mm) compared to healthy participants (1.68 ± 0.15 mm) (p =0.003, d =1.03) (Figure 3). Whilst not statistically significant the thickness of CFL had a large effect size when comparing copers to healthy (p =0.87, d =1.03) and copers to CAI (p = 0.08, d = 0.90). There were no meaningful or significant differences in thickness and CSA of the tendons and muscles between healthy, coper, and CAI participants (p >0.05 and d <0.2) (Table 3 and 4).

**Discussion**

The results of the reliability study are in line with previous studies that have also reported ICC and LoA values for some of these structures.19,36,37 The values for the ligament, tendon and muscle structures are also in agreement with the available
literature. For example, Liu et al.\(^{19}\) reported ATFL thickness of 1.95 ± 0.29 mm for healthy participants, very close to our 1.90 ± 0.16 mm. Hodgson et al.\(^{38}\) reported an Achilles thickness 5.00 ± 0.70 mm in healthy participants, close to our 4.01 ± 0.61 mm found here.

The ATFL was significantly longer in coper and CAI participants compared to the healthy participants when the ankle was in an inversion and plantar flexion position. This is consistent with Croy et al.\(^{39}\) who used stress ultrasonography during inversion and anterior drawer tests. They reported that in CAI cases ATFL length increased by 18% when under tension in anterior and plantarflexion/inversion stress tests, but only 15% in healthy participants. Increases were circa 16% in our healthy group, 25.5% in the coper group, and 25.2% in the CAI group. Increases greater than 20% are thought to cause ligament failure in healthy ankles.\(^{40}\) Hypothetically, lengthening the ATFL leads to reduced constraint on the talus relative to the fibula and tibia, allowing it to translate anteriorly or rotate medially relative the fibula.\(^{41}\) Several differences in normal and remodelled ligament matrix might explain a difference between healthy and injured ankles, including changes in the types of collagen, decreased collagen crosslinks, increased vascularity, abnormal innervation, and presence of inflammatory cell pockets.\(^{42}\)

In line with prior works,\(^{19,39}\) the results suggest there are no differences in ATFL length between ankles with one (coper) and those with multiple sprains (CAI). Given one of our criteria for CAI group was a feeling of “giving away”, this infers that changes in the ATFL are not an obvious explanation for these experiences. Whilst not statistically significant, the ATFL was almost 20% thicker in the CAI group compared to copers. This contrasts with Liu et al.\(^{19}\) who found no such difference, although they only reported a 15% greater thickness in CAI compared to healthy
participants, and the equivalent figure in this work is 57%. The critical difference between Liu et al.\textsuperscript{19} and this work is that in Liu’s CAI participants were selected based on CAIT score regardless the number of previous ankle sprains and the sensation of “giving away”. This is contrary to recent definitions of CAI.\textsuperscript{24} In contrast we did not differentiate participants exclusively by CAIT scores, but used the number of prior sprains and the sensation of “giving away” to differentiate CAI and copers.

The CFL is affected in 50 - 75 % of cases of ankle sprain.\textsuperscript{9} The result of our study showed increased CFL thickness in CAI participants. This is in line with Hua et al.\textsuperscript{43} who used MRI and CT to report thickness increased in acute ankle sprain. Whilst not statistically significant the thickness of CFL had a large effect size when comparing copers to healthy ($p = 0.87$, $d = 1.03$) and copers to CAI ($p = 0.08$, $d = 0.90$).

The tendons and muscles in our injured (coper and CAI) participants were not statistically significantly different than in the healthy participants. Our findings contrast with a report of decreased FLM CSA in laterally sprained ankles compared to healthy ankles.\textsuperscript{37} The age of control and injured participants in Lobo’s study were statistically significantly different ($p < 0.05$) and Kim et al.\textsuperscript{44} and Fujiwara et al.\textsuperscript{45} found that the thickness and CSA of lower extremity muscles changes with age. We used CSA and the thickness as surrogates of the force passing through the structures and their functional role. Increased thickness and CSA could reflect increased mechanical loading on tendons due to increases in muscle strength or use.\textsuperscript{46} In the face of evidence for no differences in muscle or tendon structures, the efficiency of motor control strategies during inversion incidents is perhaps a more likely explanation for differences between copers and CAI.

Limitations
We acknowledge some limitations to this study. We did not differentiate the CAI participants into functional and mechanical instability. It could be that those with functional ankle instability have different characteristics compared to those with mechanical instability. Thus, understanding functional and mechanical ankle instability separately with additional exploration of the sensorimotor and mechanical characteristics related to CAI is needed to improve our understanding of ankle sprain injury. Moreover, the numbers of ankle sprains in injured participants were recorded based on the participant’s recall, we attempted to minimise the impact of this limitation by limiting the participant’s recall to the 24 months prior to the test. This study is cross sectional design and prospective studies would increase our understanding of changes in ligament, muscle and tendon structures as a result of single or multiple lateral ankle sprains.
Conclusions

The ultrasound protocol proved reliable and was used to evaluate the length, thickness, and CSA of selected ankle structures. Of the ATFL and CFL, fibularis tendons and muscles, Achilles and tibialis posterior tendon, only the ATFL and CFL were different in laterally sprained ankles compared to healthy ankles. ATFL was longer and thicker in both coper and CAI participants and thicker but not longer in CAI compared to copers. No differences were found in the selected muscle and tendon structures we measured.
The authors would like to thank King Abdulaziz University and University of Salford for the grant funding for the research to be completed.
References


9. Apoorva D, Lalitha C, Patil G. Morphometric Study of Calcaneofibular...


41. Croy T, Saliba S, Saliba E, W. A, Hertel J. Talofibular Interval Changes After


Table 1. Demographic data of healthy, coper, and CAI participants. Years, weight, height, BMI and CAIT.

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<th>Healthy</th>
<th>Coper</th>
<th>CAI</th>
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<tr>
<td>Number of participants</td>
<td>31</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sex male/ female</td>
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<td>11/9</td>
<td>12/8</td>
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<td>Years (y)</td>
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<td>28.65 ± 0.56</td>
<td>27.70 ± 0.79</td>
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<tr>
<td>Weight (kg)</td>
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<td>69.90 ± 1.006</td>
<td>69.94 ± 1.538</td>
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<td>Height (m)</td>
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<td>0.167 ± 0.10</td>
<td>0.168 ± 0.10</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>23.76 ± 0.12</td>
<td>24.57 ± 0.23</td>
<td>24.54 ± 0.38</td>
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<td>CAIT score</td>
<td>28.75 ± 0.16</td>
<td>27.90 ± 0.186</td>
<td>18.24 ± 0.42 a</td>
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<tr>
<td>Time since last injury</td>
<td>0.0 ± 0.0</td>
<td>18.60 ± 0.473 a,b</td>
<td>07.10 ± 2.57 a</td>
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</table>

Abbreviations: CAIT, Cumberland Ankle Instability Tool; BMI, Body Mass Index.

*Values are mean ± SD

a indicates statistical differences between CAI and coper, and between CAI and healthy

b indicates statistical differences between coper and healthy
Table 2. Limit of agreement for healthy participants in neutral and tension position.

<table>
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<tr>
<th>Structures in neutral position</th>
<th>ICC (95% CI)</th>
<th>LoA %</th>
<th>Structures in tension position</th>
<th>ICC (95% CI)</th>
<th>LoA %</th>
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<td>ATFL thickness</td>
<td>0.96 (0.85-0.99)</td>
<td>11.0</td>
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<td>12.5</td>
<td>CFL thickness</td>
<td>0.94 (0.77-0.99)</td>
<td>12.5</td>
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<tr>
<td>FLM thickness</td>
<td>0.98 (0.88-0.99)</td>
<td>26.5</td>
<td>FLT thickness</td>
<td>0.95 (0.80-0.99)</td>
<td>23.0</td>
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<tr>
<td>FBT thickness</td>
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<td>12.5</td>
<td>FBT thickness</td>
<td>0.94 (0.75-0.98)</td>
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<tr>
<td>TPT thickness</td>
<td>0.99 (0.98-1.00)</td>
<td>05.0</td>
<td>TPT thickness</td>
<td>0.99 (0.95-1.00)</td>
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<tr>
<td>AT thickness</td>
<td>0.98 (0.93-0.99)</td>
<td>08.0</td>
<td>AT thickness</td>
<td>0.99 (0.94-1.00)</td>
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<td>FLT CSA</td>
<td>0.94 (0.76-0.98)</td>
<td>17.0</td>
<td>FLM thickness</td>
<td>0.96 (0.86-0.99)</td>
<td>15.0</td>
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<td>FLM CSA</td>
<td>0.98 (0.92-0.99)</td>
<td>12.0</td>
<td>FBM thickness</td>
<td>0.95 (0.82-0.99)</td>
<td>12.0</td>
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<tr>
<td>FBT CSA</td>
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<td>22.5</td>
<td>FLT CSA</td>
<td>0.98 (0.93-0.99)</td>
<td>23.0</td>
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<td>TPT CSA</td>
<td>0.95 (0.82-0.98)</td>
<td>30.0</td>
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<td>0.94 (0.78-0.98)</td>
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<td>AT CSA</td>
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<td>FLM CSA</td>
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<td>FLM CSA</td>
<td>0.95 (0.81-0.99)</td>
<td>07.5</td>
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</table>

Abbreviations: ICC, Intraclass correlation coefficient; CI, Confidence intervals; LoA, Limit of agreement; ATFL, Anterior talofibular ligament; CFL, Calcenofibular ligament; PLT, Fibularis longus tendon; PBT, Fibularis brevis tendon; TPT, Tibialis posterior tendon; AT, Achilles tendon; PLM, fibularis longus muscle; PBM, Fibularis brevis muscle; CSA, Cross sectional area.
Table 3. Length of ATFL and thickness of selected ligaments, tendons and muscles structures for healthy, coper, and CAI participants.

<table>
<thead>
<tr>
<th>Structures</th>
<th>Healthy</th>
<th>Coper</th>
<th>CAI</th>
</tr>
</thead>
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<tr>
<td>ATFL L</td>
<td>22.22 ± 1.27</td>
<td>23.61 ± 1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.48 ± 0.82&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>ATFL T</td>
<td>1.90 ± 0.16</td>
<td>2.45 ± 0.38&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>2.93 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>CFL</td>
<td>1.68 ± 0.15</td>
<td>1.72 ± 0.10</td>
<td>1.82 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FLT</td>
<td>2.51 ± 0.21</td>
<td>2.50 ± 0.16</td>
<td>2.55 ± 0.20</td>
</tr>
<tr>
<td>FBT</td>
<td>1.71 ± 0.15</td>
<td>1.72 ± 0.09</td>
<td>1.73 ± 0.13</td>
</tr>
<tr>
<td>TPT</td>
<td>2.50 ± 0.19</td>
<td>2.52 ± 0.18</td>
<td>2.54 ± 0.17</td>
</tr>
<tr>
<td>AT</td>
<td>4.01 ± 0.61</td>
<td>4.03 ± 0.53</td>
<td>4.01 ± 0.62</td>
</tr>
<tr>
<td>FLM</td>
<td>5.78 ± 0.45</td>
<td>5.85 ± 0.34</td>
<td>5.85 ± 0.68</td>
</tr>
<tr>
<td>FBM</td>
<td>9.52 ± 1.12</td>
<td>9.67 ± 1.04</td>
<td>9.73 ± 0.54</td>
</tr>
</tbody>
</table>

Abbreviations: ATFL L, Anterior talofibular ligament ligament; ATFL T, Anterior talofibular thickness; CFL, Calcenofibular ligament; FLT, Fibularis longus tendon; FBT, Fibularis brevis tendon; TPT, Tibialis posterior tendon; AT, Achilles tendon; FLM, fibularis longus muscle; FBM, Fibularis brevis muscle.

*Values are mean ± SD in mm

<sup>a</sup>Statistically significant differences (P<0.05, d>0.2) between coper and healthy

<sup>b</sup>Statistically significant differences between CAI and healthy

<sup>c</sup>Statistically significant differences between coper and CAI
Table 4. CSA of selected tendon and muscles structures for healthy, coper, and CAI participants. There were no statistically significant differences between the groups.

<table>
<thead>
<tr>
<th>Structures</th>
<th>Healthy</th>
<th>Coper</th>
<th>CAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT</td>
<td>2.10 ± 0.21</td>
<td>2.11 ± 0.21</td>
<td>2.14 ± 0.23</td>
</tr>
<tr>
<td>FBT</td>
<td>1.57 ± 0.10</td>
<td>1.58 ± 0.08</td>
<td>1.59 ± 0.15</td>
</tr>
<tr>
<td>TPT</td>
<td>1.74 ± 0.16</td>
<td>1.77 ± 0.16</td>
<td>1.77 ± 0.18</td>
</tr>
<tr>
<td>AT</td>
<td>5.40 ± 0.45</td>
<td>5.36 ± 0.34</td>
<td>5.54 ± 0.40</td>
</tr>
<tr>
<td>FLM</td>
<td>7.39 ± 0.42</td>
<td>7.44 ± 0.38</td>
<td>7.47 ± 0.52</td>
</tr>
<tr>
<td>FBM</td>
<td>24.00 ± 3.59</td>
<td>24.40 ± 2.90</td>
<td>25.00 ± 2.65</td>
</tr>
</tbody>
</table>

Abbreviations: FLT, Fibularis longus tendon; FBT, Fibularis brevis tendon; TPT, Tibialis posterior tendon; AT, Achilles tendon; FLM, fibularis longus muscle; FBM, Fibularis brevis muscle.

*Values are mean ± SD in mm.
Figure 1. Right leg held in AFO in neutral position
Figure 2. Transducer position, orientation and sample images for all structures, A-B, anterior talofibular ligament in tension position; C, calcaneofibular ligament in tension position; D, fibularis tendon in tension position; E, fibularis brevis tendon in tension position; F, fibularis longus tendon in tension position; G-H, fibularis muscles in neutral position; I-J, tibialis posterior tendon in neutral position; K-L, Achilles tendon in tension position. LM, lateral malleolus; MM, medial malleolus.
Figure 3. Mean (SD) thickness (mm) of anterior talofibular ligament (ATFL); and calcaneofibular ligament (CFL).

*Statistically difference between CAI and healthy.

**Statistically difference between coper and healthy.

***Statistically difference between coper and CAI.
Figure 4. Length of the anterior talofibular ligament ATFL (mm) in two positions among the three groups. N, neutral; T, tension.

*Statistically difference between CAI and healthy.

**Statistically difference between coper and healthy.