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Ultrasound shear wave elastography of the anterior talofibular and calcaneofibular ligaments in healthy subjects

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Abstract

Aim of study: Most sprained lateral ankle ligaments heal uneventfully, but in some cases the ligament's elastic function is not restored, leading to chronic ankle instability. Ultrasound shear wave elastography can be used to quantify the elasticity of musculoskeletal soft tissues; it may serve as a test of ankle ligament function during healing to potentially help differentiate normal from ineffective healing. The purpose of this study was to determine baseline shear wave velocity values for the lateral ankle ligaments in healthy male subjects, and to assess inter-observer reliability. **Material and methods:** Forty-six ankles in 23 healthy male subjects aged 20–40 years underwent shear wave elastography of the lateral ankle ligaments performed by two musculoskeletal radiologists. Each ligament was evaluated three times with the ankle relaxed by both examiners, and under stress by a single examiner. Mean shear wave velocity values were compared for each ligament by each examiner. Inter-observer agreement was evaluated. **Results:** The mean shear wave velocity at rest for the anterior talofibular ligament was 2.09 ± 0.3 (range 1.41–3.17); and for the calcaneofibular ligament 1.99 ± 0.36 (range 1.29–2.88). Good inter-observer agreement was found for the anterior talofibular ligament and calcaneofibular ligament shear wave velocity measurements with the ankle in resting position. There was a significant difference in mean shear wave velocities between rest and stressed conditions for both anterior talofibular ligament (2.09 m/s vs 3.21 m/s; $p < 0.001$) and calcaneofibular ligament (1.99 m/s vs 3.42 m/s; $p < 0.0001$). **Conclusion:** Shear wave elastography shows promise as a reproducible method to quantify ankle ligament stiffness. This study reveals that shear waves velocities of the normal lateral ankle ligaments increased with applied stress compared to the resting state.

Introduction

Lateral ankle ligaments, normal anatomy, function and injuries

The lateral ankle ligamentous complex is composed of the anterior talofibular ligament (ATFL), calcaneofibular ligament (CFL), and posterior talofibular ligament (PTFL) (Fig. 1).

The ATFL stabilizes the talus, originating at the anterior lateral malleolus and running anteromedially to insert on the talar body, closely related to the ankle joint capsule[(1)]. The ATFL can be composed of one to three separate bands, however it is most commonly composed of two separate bands[(1,2)]. The number of bands does not seem to impact the overall width of the ATFL[(2)].

The CFL stabilizes the subtalar joint, extending from the lateral malleolar tip to the trochlear eminence of the calcaneus, crossing deep to the peroneal tendons and sheaths[(3)]. The proximal attachment of the CFL is found just below the ATFL, with interconnecting fibers between these ligaments frequently observed[(2)].

The PTFL extends from the lateral tubercle of the posterior talus to the fibular malleolar fossa, which is located at the deep surface of the lateral malleolus[(2,4)]. The PTFL is multifascicular and, therefore, does not insert on one specific area of the posterior talus[(2)].

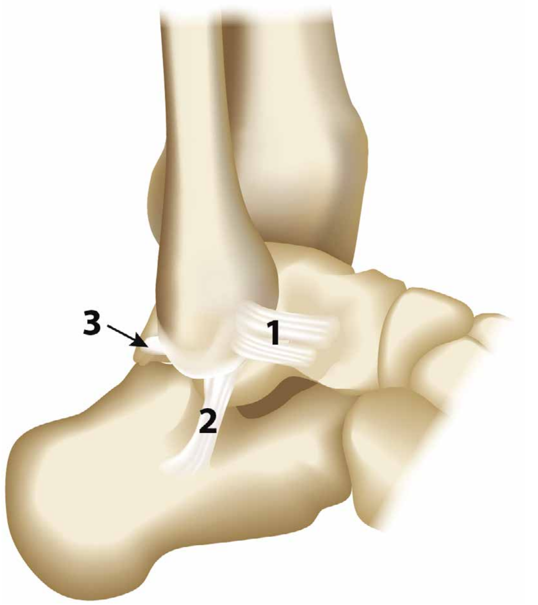

Fig. 1. *Diagram of the lateral ankle ligaments. The lateral ligamentous complex of the ankle is composed of the (1) anterior talofibular ligament (ATFL) which extends from the anterior lateral malleolus to the talar body, (2) calcaneofibular ligament (CFL) which extends from the lateral malleolar tip to the trochlear eminence of the calcaneus, and (3) posterior talofibular ligament (PTFL) which extends from the lateral tubercle of the posterior talus to the fibular malleolar fossa located at the deep surface of the lateral malleolus*

Within the normal population in the United States, the incidence rate of all types of ankle sprains has been reported to range from 2.15 to 7 per 1,000 person-years[(5,6)]. Of these sprains, 77% are of the lateral ankle ligaments[(7)]. Low lateral ankle sprains commonly occur during plantar flexion and inversion with excessive ankle supination[(8)]. The majority of low lateral ankle sprains are related to the ATFL[(7,9)], with the remainder being the combination of the ATFL and CFL. The PTFL is most frequently damaged in frank dislocations.

Low lateral ankle sprains not only have a high prevalence but can lead to complications and increased healthcare burden. They have a high rate of recurrence and can develop into chronic ankle instability in up to 70% of cases, which is associated with decreased physical activity levels and quality of life[(10)]. Post-traumatic ankle osteoarthritis can also occur and lead to profound physical limitation[(10)].

Shear wave elastography

Shear wave elastography (SWE) is an increasingly explored ultrasound (US) technique that measures elastic tissue properties. This technique can add information to the conventional gray-scale and Doppler US techniques, providing a quantitative assessment of tissue elasticities[(11)]. SWE is being applied to both research[(12–15)] and clinical musculoskeletal (MSK) applications[(16–18)].

Shear waves (SW) are generated using focused acoustic radiation force from a linear US array. They propagate at a much slower velocity through the adjacent tissues in the transverse plane, which is perpendicular to the primary wave, while causing shear with temporary tissue displacement. The tissue displacements are used to calculate SW velocity and the shear modulus. This relationship is expressed as a color bar (elastogram) on the US screen, either in velocity (meters/second) or units of pressure (kilopascals). On the color elastograms, red is usually defined for encoding hard consistency, blue encodes soft consistency, and green and yellow encode intermediate stiffness.

SWE could serve as the basis of a novel functional test of lateral ankle ligament healing after ankle sprain. It would differentiate the ligaments that are healing normally from those that will heal without restoration of their elastic properties and thus lead to chronic ankle instability. The first steps in the development of SWE as a functional test of lateral ankle ligament healing are to assess the elastic properties of the normal ligaments in their healthy state, and to demonstrate that SWE of the lateral ankle ligaments is reproducible. While there has been some investigation into the elastic properties of healthy ankle ligaments[(19)], the in vivo material properties of the lateral ankle ligaments have not been well characterized.

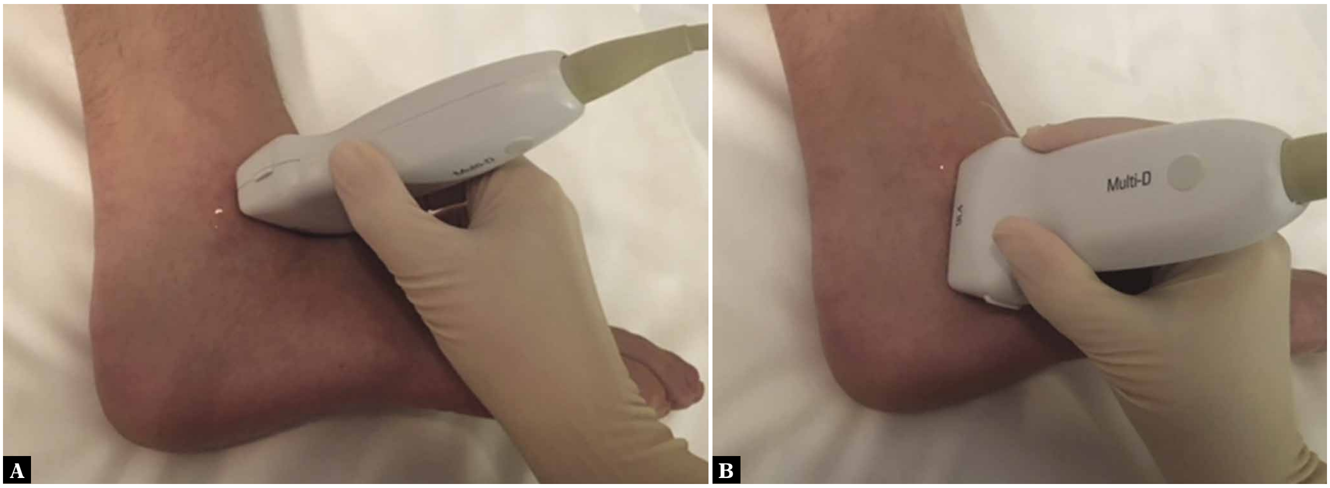

Fig. 2. *Positioning of the foot and ultrasound transducer for the evaluation of the (***A***) ATFL and (***B***) CFL in the long axis*

The primary aim of this study was to determine SW velocity values of the ATFL and CFL in young healthy males at rest and under stress. Our secondary aim was to determine the inter-observer variability in SW velocities to evaluate the reproducibility of these measurements.

Materials and methods

Subjects

Institutional review board approval was obtained for this prospective US SWE study (Protocol Number 1000000966R007). Written informed consent for the participation in the trial was obtained according to the principles issued by the Declaration of Helsinki. Twenty-four right foot dominant male subjects between the ages of 20–40 years old (mean 30 years) without previous history of ankle injury were enrolled in the study. The subjects were recruited through a university announcement in the local community, and their informed consent was obtained. All subjects reported no history of prior ankle sprain including trauma to the lateral ankle ligaments. None of the subjects were athletes, and were only involved in recreational sports activities. Based on the initial US exam, one subject was excluded due to torn bilateral ATFLs and CFLs diagnosed on the conventional grayscale and power Doppler examination, for a total of 23 subjects remaining in the study. Both right and left ankles were examined in each subject, accounting for a total of 46 ATFLs and 46 CFLs.

Ultrasound examination and SWE

For the US examination, each subject was placed supine on the US stretcher, with the lateral aspect of the imaged ankle exposed to the examiner. All US examinations were performed on a Siemens S3000 unit (Siemens Medical Systems) with a high-resolution 9-MHz linear transducer (Fig. 2). Grayscale and power Doppler US examination of each ATFL and CFL was performed prior to SWE. The ligaments were evaluated using 10 mm of US gel between the transducer and skin surface, which has been previously shown to be adequate for the evaluation of superficial structures using probes of this frequency[20].

The ATFLs were imaged in their long axes at the anterolateral aspect of the ankle between their talar and lateral malleolus attachment sites, and the CFLs in their long axes at the lateral aspect of the ankle between their calcaneal and lateral malleolus attachment sites, deep to the peroneal tendons[21]. The US examination of these ligaments was not performed in the short axis, as this is usually not part of the routine US examination of these structures due to their small size. The ligaments were considered normal if they had a fibrillar echogenic appearance on grayscale US images (Fig. 3A, Fig. 4A) without hyperemia on power or color Doppler interrogation. They were considered abnormal if they appeared hypoechoic or thickened, or showed partial or complete discontinuity of the fibers[21].

The US examination of each ATFL and CFL was performed independently by two fellowship-trained MSK radiologists. The first examiner had 24 years MSK ultrasound and 4 years of SWE experience, and the second examiner had 3 years MSK ultrasound and 3 years of SWE experience. For each subject, both examiners repeated SWE imaging of the ATFL and CFL three times, each time with the ankle in the same resting position (Fig. 3B, Fig. 4B). The first examiner performed three additional SWE measurements of the same ligaments with manually applied stress (Fig. 3C, Fig. 4C). For each SW measurement, three regions of interest (ROIs) were placed in similar locations in each ligament on the selected recorded color elastogram US images, and the mean SW velocity was calculated.

Ankle stress maneuvers

All ankle stress maneuvers were performed by a single fellowship-trained orthopedic foot and ankle surgeon

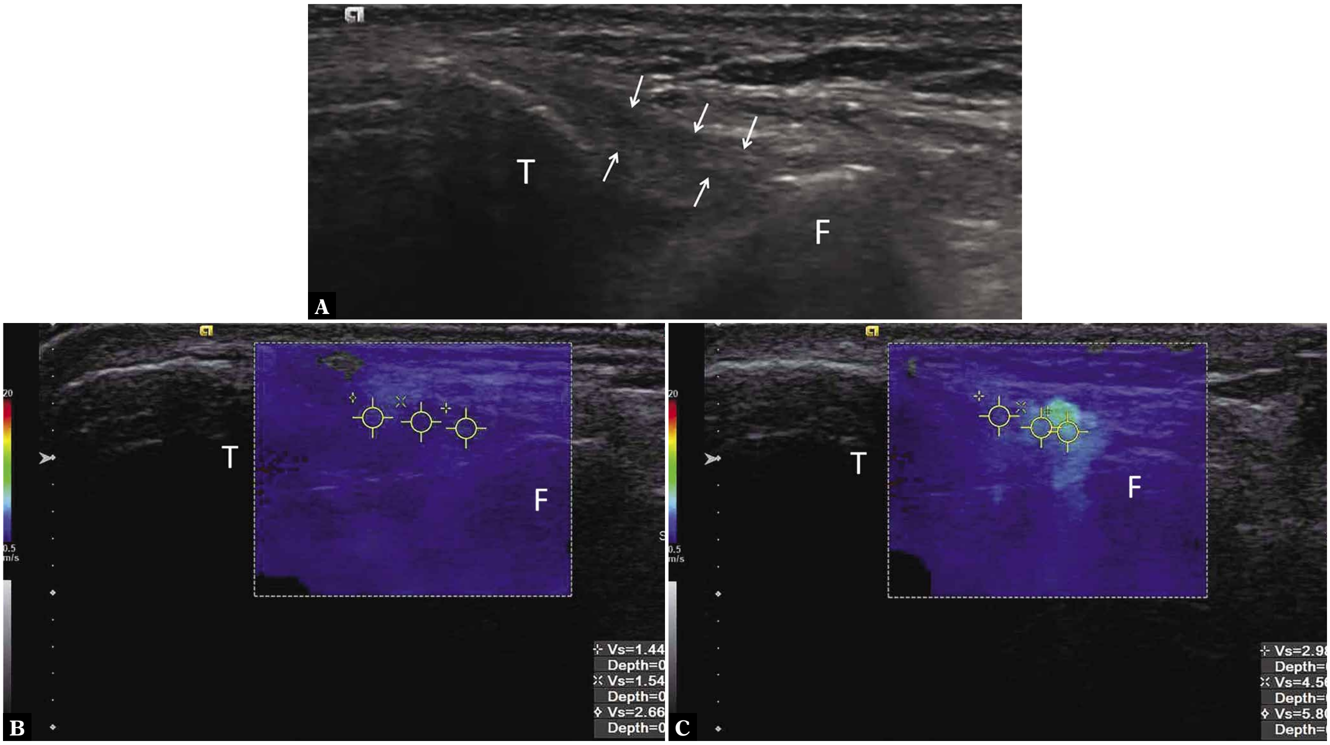

Fig. 3. *Grayscale and SWE US images of the normal ATFL in an asymptomatic volunteer obtained at rest and with stress. (***A***) Long axis grayscale US image of the ATFL (arrows) at rest shows normal echogenic fibrillar appearance. SWE in same region at rest (***B***) and with stress (***C***) show higher SW velocities in (c), consistent with increased stiffness in the contracted ligament with applied manual stress. Three ROIs are placed within the ATFL where the SW velocity measurements were obtained. Note the reference color bar at the side of images (***B***,* ***C***) defining the quantitative color elastogram with velocities ranging from 0.5–20 meters/second. Blue color denotes low, red high, and green and yellow intermediate SW velocities. SWE – shear wave elastography, SW – shear wave, T – talus, F – fibula*

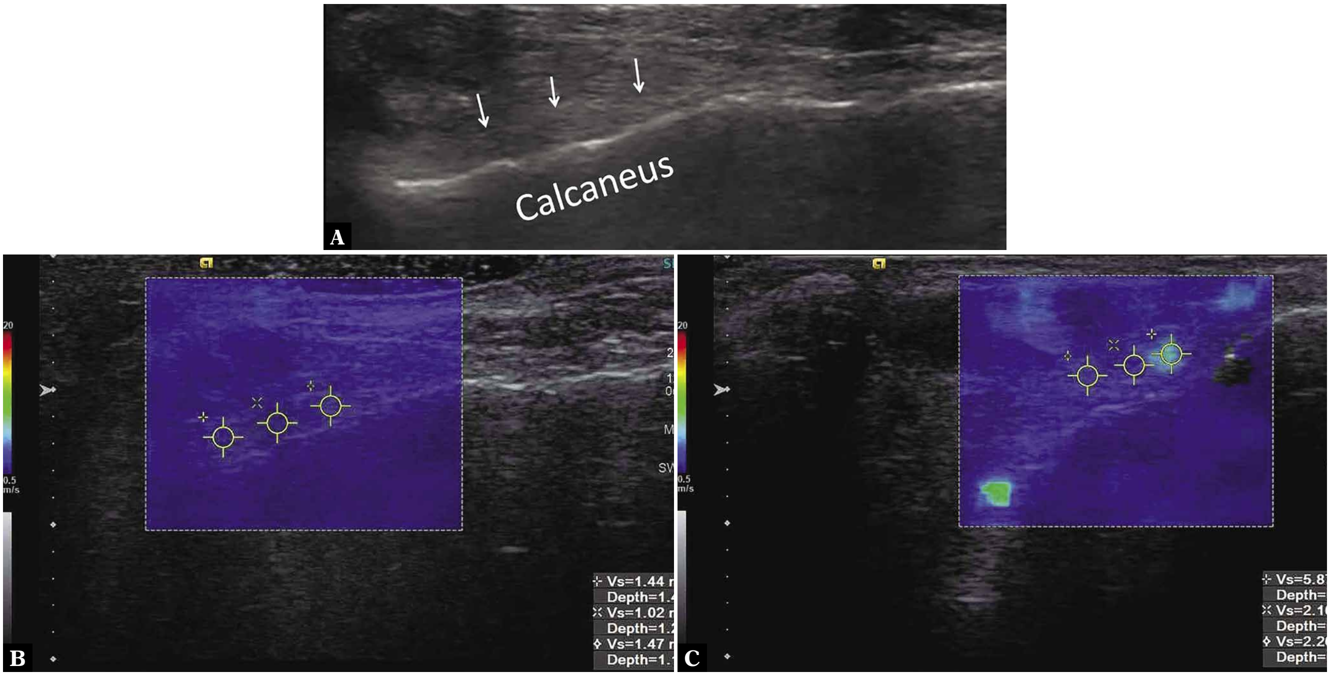

Fig. 4. *Grayscale and SWE US images of the normal CFL in an asymptomatic volunteer obtained at rest and with stress. (***A***) Long axis grayscale US image of the CFL (arrows) at rest shows normal echogenic fibrillar appearance. SWE in same region at rest (***B***) and with stress (***C***) show higher SW velocities in (***C***), consistent with increased stiffness in the contracted ligament with applied manual stress. Three ROIs are placed within the CFL where the SW velocity measurements were obtained. Note the reference color bar at the side of images (***B***,* ***C***) defining the quantitative color elastogram with velocities ranging from 0.5–20 meters/second. Blue color denotes low, red high, and green and yellow intermediate SW velocities. Left side of the image is proximal*

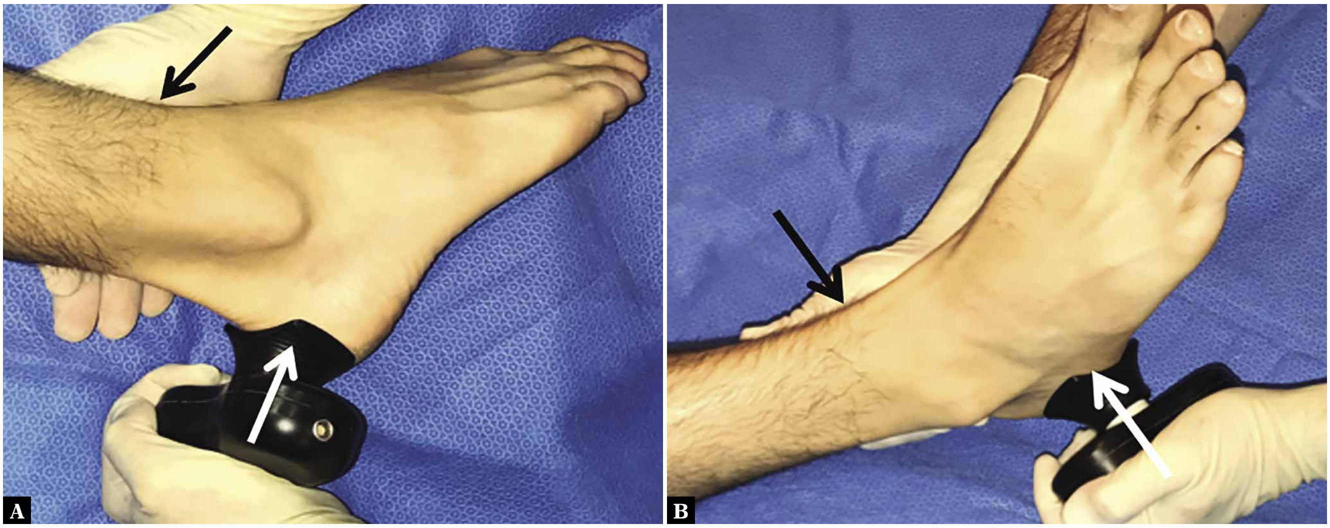

Fig. 5. *Ankle stress maneuvers of the ATFL and CFL. Black and white arrows show the direction of applied force. The (**A**) ATFL was stressed using the anterior drawer test, with the orthopedic surgeon applying an anteriorly directed force to the posterior calcaneus, while the other hand stabilized the distal tibia. The (**B**) CFL was stressed using the talar tilt test, with the orthopedic surgeon applying a medially directed force to invert the heel, while the other hand stabilized the tibia. Note the DataLog Boot Screen myometer used to ensure that 100 N of external force was applied to each stressed ligament*

with 10 years of experience. A handheld digital myometer (Biometrics MyoMeter, Ladysmith VA) was used to ensure that 100 N of external force was applied to the examined ligament at the time of SWE measurement.

The ATFL was stressed using the anterior drawer test (Fig. 5A). With the patient in the supine position and knee in a flexed position to relax the calf muscles, the orthopedic surgeon applied a force to the posterior calcaneus to pull it anteriorly, while the other hand stabilized the anterior distal tibia and fibula.

The CFL was stressed using the talar tilt test (Fig. 5B). With the patient in the supine position with the knee in full extension, the orthopedic surgeon applied a lateral force to invert the heel, while the examiner's other hand stabilized the distal medial tibia.

Statistical analysis

All statistical analyses were performed by a PhD statistician with over 30 years of experience. Inter-observer agreement was assessed on a Bland-Altman plot. A paired t-test was used to assess differences in SW velocities for the ATFLs and CFLs between the two examiners at rest, and between rest and stressed conditions for the examiner 1. Correlation between SW velocity and subject age was assessed using Pearson correlation.

Results

A total of 23 healthy male subjects were included in the study for a total of 46 ATFLs and 46 CFLs. The mean age was 29.8 years ± 5.2 (range 20–40). All subjects were right foot dominant.

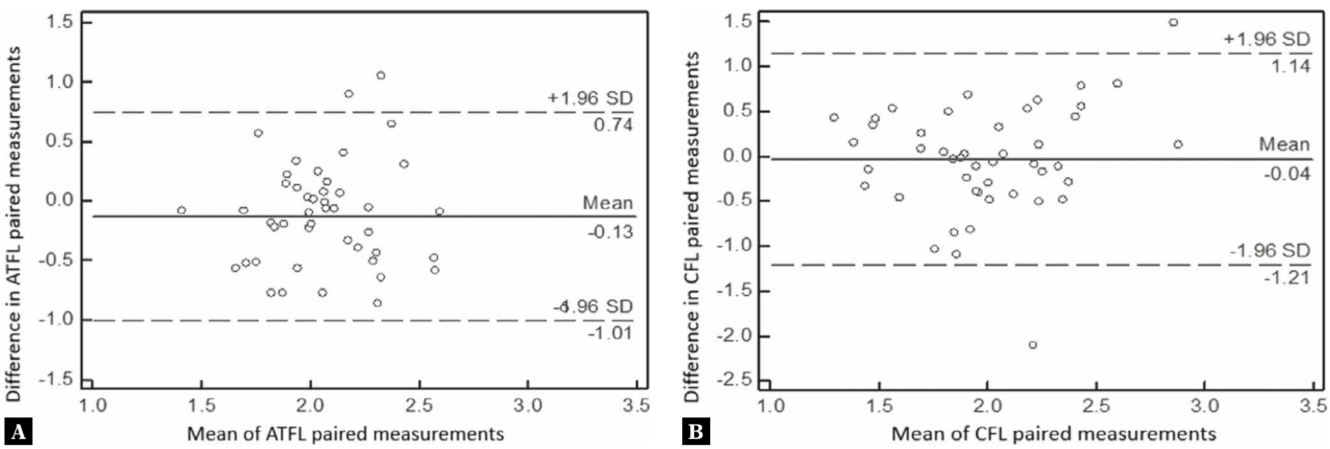

Fig. 6. *Inter-reader agreement for SW velocity measurements. Bland-Altman plot of the (**A**) ATFL and (**B**) CFL demonstrate good inter-reader agreement between the two radiologists for SW velocity measurements, with the majority of measurements falling within 2 standard deviations of the mean*

Tab. 1. *Comparison of SW velocities (m/s) of the ATFL and CFL at rest between radiologist 1 and radiologist 2, using the paired t-test*

		Reader 1	Reader 2	*T-value*	*P-value*
ATFL	Mean	2.02	2.15	*2.06*	*0.05*
	SD	036	0.39		
	Min	1.36	1.45		
	Max	2.85	3.62		
CFL	Mean	1.98	2.02	*0.4118*	*0.68*
	SD	0.51	0.43		
	Min	1.16	1.07		
	Max	3.60	3.26		

ATFL – anterior talofibular ligament; CFL – calcaneofibular ligament

All examined ATFLs and CFLs showed normal hyperechoic fibrillar echotexture on the grayscale US images and no hyperemia on color Doppler interrogation apart from those of the one subject who was excluded from the study.

The mean SW velocity of the ATFLs at rest with both readers combined was found to be 2.09 ± 0.3 (range 1.41–3.17); of the CFLs 1.99 ± 0.36 (range 1.29–2.88). Good interobserver agreement was found for the ATFL and CFL SW velocity measurements with the ankle in resting position, with the majority of measurements falling within 2 standard deviations of the mean (Fig. 6). There was a slight but significant difference ($t = 2.06$, $p = 0.05$) between the ATFL SW velocity measurements at rest between the examiner 1 (mean = 2.02) and examiner 2 (mean = 2.15) (Tab. 1). There was no significant difference ($t = 0.4118$, $p = 0.68$) between the CFL SW velocity measurements at rest between the examiner 1 (mean = 1.98) and examiner 2 (mean = 2.02, Tab. 1).

The mean SWE velocity of the ATFLs for the stressed condition averaged across subjects was found to be 3.21 ± 0.9 (range 1.72–5.97). The mean SWE velocity of the CFLs for the stressed condition was found to be 3.42 ± 1.01 (range 1.72–8.01).

There was a significant difference between the SW velocities at rest and stress when combined measurements from both radiologists were analyzed using a paired t-test (Tab. 2), with the ATFLs at rest and stress ($t = 7.580$, $p < 0.001$); and for the CFL at rest and stress ($t = 8.349$, $p < 0.0001$). An additional paired t-test was performed for the ATFL and CFL SW velocity measurements at rest and stress for the examiner 1 (Tab. 3), and showed significant differences for the ATFL ($t = 7.062$, $p < 0.0001$) at rest (mean = 2.15) versus with stress (mean = 3.21); and for the CFL SW velocity measurements ($t = 8.635$, $p < 0.0001$) at rest (mean = 2.02) versus with stress (mean = 3.42). There were no significant differences between the SW velocity measurements for the right and left foot for the ATFL at rest or with stress or the CFL at rest or with stress using combined data from both radiologists. No significant correlation was seen with the SW velocity measurements and subjects' age using combined data from both radiologists, with correlation coefficients ranging from 0.01 to 0.29 for the ATFL and CFL at rest and with stress.

Discussion

Our study defined the normal US SWE velocity values for the ATFL and CFL in young male subjects. There were significant differences between the SW velocities of the two ligaments at rest and stress, with increased velocities observed with applied stress. In addition, we demonstrated that the SW velocities of the lateral ankle ligaments can be measured repeatably.

A recent study by Hoftiel *et al.*[19] evaluated the ATFL in 60 healthy athletes, 32 females and 28 males, in neutral ankle position. The average SW velocity for the male subjects was 1.85 ± 0.31 m/s, similar to our results of 2.09 ± 0.3 m/s in the resting position. However, this study did not evaluate the ATFL under stress or the CFL. In our study, we did not include female subjects or athletes.

Previous studies have shown a promising clinical role of SWE in evaluating MSK structures. A study by Aubry and collaborators showed significantly softer Achilles tendons with tendinosis compared to normal tendons[16]. Another study demonstrated a positive correlation with SWE pressure and functional outcome in torn Achilles tendons after repair[22]. A study by Carpenter and collaborators (2015) demonstrated that the mean SW velocity of lower extremity musculature was significantly lower in patients with inclusion body myopathy 2 (GNE-related myopathy) when compared to healthy controls[23]. A recent study showed increased stiffness of the tibial nerve in patients

Tab. 2. *Comparison of mean SW velocities (m/s) of the ATFL and CFL at rest and with stress*

		Rest	Stress	*T-value*	*P-value*
ATFL	Mean	2.09	3.21	*7.580*	*<0.001*
	SD	0.30	0.92		
	Min	1.41	1.72		
	Max	3.17	5.97		
CFL	Mean	1.99	3.42	*8.349*	*<0.0001*
	SD	0.36	1.09		
	Min	1.29	1.72		
	Max	2.88	8.01		

ATFL – anterior talofibular ligament; CFL – calcaneofibular ligament

Tab. 3. *Comparison of SW velocities (m/s) of the ATFL and CFL at rest and with stress using data from a single radiologist*

		Rest	Stress	*T-value*	*P-value*
ATFL	Mean	2.15	3.21	*7.062*	*<0.0001*
	SD	0.39	0.92		
	Min	1.45	1.72		
	Max	3.62	5.97		
CFL	Mean	2.02	3.42	*8.635*	*<0.0001*
	SD	0.43	1.09		
	Min	1.07	1.72		
	Max	3.26	8.00		

ATFL – anterior talofibular ligament; CFL – calcaneofibular ligament with diabetic peripheral neuropathy compared to healthy subjects and subjects with diabetes but without peripheral neuropathy[24].

As previously mentioned, the elastic properties of the ankle ligaments have not been well characterized. A recent study by Wu and collaborators showed increased thickness and stiffness of the coracohumeral ligament in shoulders with symptomatic adhesive capsulitis when compared to asymptomatic shoulders[25]. In this study, the coracohumeral ligaments were examined in the relaxed neutral position and in the tightened external rotation position in 30 healthy subjects and 20 patients with a clinical diagnosis of unilateral adhesive capsulitis on a Supersonic Imagine. The researchers measured SW pressures of the coracohumeral ligaments along the long axis, demonstrating greater SW pressures in symptomatic shoulders (median of 234.8 kPa) compared to asymptomatic shoulder (203.3 kPa). However, the study population was not uniform, including both men and women over a wide age range.

Another study by Mhanna and collaborators demonstrated stiffer transverse carpal ligaments in pianists compared to non-pianists[12]. Their study was performed on an Acuson S2000 US scanner on 10 female pianists and 10 female non-pianists. Significantly increased SW velocities were seen in the pianists versus non-pianists (5.52 m/s in pianists versus 5.01 m/s in non-pianists; $p < 0.05$). The subject selection was uniform, selecting all younger female subjects. Similarly to our protocol, this study measured SW velocities.

In our study, we chose to include only young subjects, 20 to 40 years of age, for a more uniform study population. This decision was based on prior studies showing age-related changes in US SWE measurements. A 2015 study showed increasing SW velocities in the Achilles tendon with increasing age in the relaxed and tension states[26]. Another study of the patellar tendons demonstrated decreased SW velocities in the oldest of three examined groups[27]. It has also been found that the shear modulus of the biceps brachii muscle increases with advancing age[28].

Our study included only male subjects to create a more uniform study population. A prior study from 2011 demonstrated the mean elasticity values for several muscles and tendons were higher in men versus women when obtained in the longitudinal plane[13]. Similarly, another study showed the shear modulus in the biceps brachii muscle was higher in women than men[28].

In our study, we obtained SW velocities only along the long axis of the ligament, primarily due to their small cross-sectional size. The two previous studies on US SWE of the upper extremity ligaments were also performed in the long axis[12,25]. Additionally, it has been previously reported that shear waves propagate faster along the long axis of the tendon versus the short axis due to tendon anisotropy[11,29], which may potentially apply to the ATFL and CFL. In order to minimize anisotropy of the ligaments evaluated, and to ensure we were as parallel to the ligaments as possible, the ATFL was scanned with the forefoot in slight inversion, and the CFL was scanned with the foot in dorsiflexion as per guidelines of the European Society of Musculoskeletal Radiology[30]. In addition, the transducer was toggled to minimize anisotropy.

While there are not many studies yet published on US SWE of ligaments, other studies have been published evaluating US SWE of muscles in the relaxed versus contracted state. One study demonstrated an increase in the Young modulus in the muscles of the lower extremity in one individual during muscle contraction[31]. An additional study showed that SW velocities were higher in the contracted vastus intermedius muscles than those that were relaxed[32]. Another study reported increased stiffness of the Achilles tendon with stretching[29].

Similarly to other investigators[29,31,32], we have demonstrated expected increased SW velocities and tissue stiffness in the ATFL and CFL with applied stress in young healthy males. We have also defined the normal US SW velocities of the ATFL and CFL, which are the two major and most commonly injured lateral ankle ligaments. Based on our study results, the SW velocities of the normal ATFL range from 1.41 to 3.17 m/s, with mean of 2.09 m/s at rest; and of the normal CFL range from 1.29 to 2.88 m/s, with mean of 1.99 m/s at rest. Both ligaments become stiffer with stress, with SW velocities for the ATFL ranging from 1.72 to 5.97 m/s, with mean of 3.21 m/s, and for the CFL ranging from 1.72 to 8.01, with mean of 3.42.

While US SWE is an increasingly explored technique for the measurement of elastic tissue properties, there are also magnetic resonance (MR) sequences being used to explore ligament and tendon healing. These novel MR sequences include ultrashort echo time (UTE) MR imaging, quantitative MR imaging biomarkers such as collagen, UTE T2* mapping, kinematic MR, and MR elastography[33–36]. At the time of this manuscript, we could find no relevant literature using these novel MR sequences in evaluating the ATFL and CFL, as most available studies focus on the evaluation of muscles and tendons[34,36].

Our study has several limitations. The sample size is relatively small, with 46 of each ATFLs and CFLs examined. Only young, healthy male ankles were included in the study, without history of prior trauma. We did not examine these ligaments in the short axis. We limited our evaluation to only two ankle ligaments, the ATFL and CFL, with other ligaments not interrogated. Additionally, it is possible that low-grade trauma of the ligaments leading to areas of scar, remodeling, or microstructural abnormalities may influence SWE measurements; however they may not always be visible on US. Corresponding MR imaging to ascertain the presence of these changes in our subjects would have been helpful.

Also, anatomic variations of the ligaments may affect SW measurements, and it is, therefore, crucial to have a thorough understanding of these variations. The interconnecting fibers between the proximal attachments of the CFL and ATFL may influence SW measurements if taken in these regions. Similarly, other interconnections between the ligaments and surrounding structures would affect the SW measurements obtained. For example, the superior peroneal retinaculum can have connections to the ATFL in multiple varying locations[37]. Additionally, the peroneal tendons and sheaths superficially cross the CFL, leaving a concavity over the ligament in this region, which would also influence SW measurements, as the transducer may not be completely parallel to the ligament in this region.

There are also limitations related to the ligaments themselves. The proximal portion of the CFL may be incompletely visualized on US examination, which is a known limitation of the US study. Finally, although every attempt was made to image the ATFL and CFL with the transducer as parallel to the ligaments as possible, it is difficult to completely remove anisotropy, which may also impact SWE measurements.

Reproducibility and comparability of the SWE results in this study may depend on many different factors, such as probe and equipment used, protocols, and differences in acquisition such as pressure applied, acquisition time, and region of interest. The methods used in this study ensured to minimize variability as much as possible. All subjects were evaluated in one day by the same two musculoskeletal radiologists. The same US machine was used to obtain the SW measurements. In addition, a single orthopedic surgeon evaluated and stressed all ankles.

Future directions in research should include additional prospective studies with a larger number of subjects including the SWE of the other ankle ligaments. Further studies are needed to evaluate the ligaments in their relaxed and tension states. Differences in SW velocity measurements of the ankle ligaments based on age and gender should be explored. Ideally, the ligaments should be evaluated in both long and short axes. We would need to correlate the US SWE findings of the ankle ligaments with traumatic injuries, biochemical factors, and histology, as well as correlate the findings with how tissue stiffness may change with tissue healing and different treatments. We should compare US SWE with other novel MR sequences such as MR elastography in the evaluation of the lateral ankle ligaments. Finally, future directions should help to elucidate the role that US SWE can play in the clinical setting.

Conclusions

Our study defined the normal US SWE velocity values for the ATFL and CFL in young male subjects, with significantly increased velocities for both ligaments with applied stress. We demonstrated that the SW velocities of the lateral ankle ligaments can be measured repeatably. US SWE shows promising results as a reproducible method to quantify ankle ligament stiffness.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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