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In vivo quantification of the shear modulus of the human Achilles tendon during passive loading using shear wave dispersion analysis

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Abstract

The shear wave velocity dispersion was analyzed in the Achilles tendon (AT) during passive dorsiflexion using a phase velocity method in order to obtain the tendon shear modulus (C_{55}). Based on this analysis, the aims of the present study were (i) to assess the reproducibility of the shear modulus for different ankle angles, (ii) to assess the effect of the probe locations, and (iii) to compare results with elasticity values obtained with the supersonic shear imaging (SSI) technique. The AT shear modulus (C_{55}) consistently increased with the ankle dorsiflexion ($N = 10$, $p < 0.05$). Furthermore, the technique showed a very good reproducibility (all standard error of the mean values $<$10.7 kPa and all coefficient of variation (CV) values $\leqslant$0.05%). In addition, independently from the ankle dorsiflexion, the shear modulus was significantly higher in the proximal location compared to the more distal one. The shear modulus provided by SSI was always lower than C_{55} and the difference increased with the ankle dorsiflexion. However, shear modulus values provided by both methods were highly correlated ($R = 0.84$), indicating that the conventional shear wave elastography technique (SSI technique) can be used to compare tendon mechanical properties across populations. Future studies should determine the clinical relevance of the shear wave dispersion analysis, for instance in the case of tendinopathy or tendon tear.

Keywords: *in vivo* tendon, ultrasound elastography, guided shear wave

(Some figures may appear in colour only in the online journal)

Introduction

Tendons transmit muscle force to the skeleton. They also act as a mechanical buffer to prevent the muscle from damage resulting from a sudden-high load (Konow and Roberts 2015) and they contribute to improving the mechanical efficiency of the muscle fibers through storage/restitution of elastic energy (Ishikawa *et al* 2005, Lichtwark *et al* 2007, Cronin and Lichtwark 2013). As such, they have a crucial role in the production of human movement. It is well accepted that the mechanical properties of the tendon play a key role in these processes. Development of techniques to accurately quantify tendon mechanical properties in a noninvasive way is therefore of primary interest for both basic and clinical science.

Tendon stiffness is classically inferred from the forces applied on the tendon and the associated displacements of the tendinous tissue measured *via* ultrasound imaging (for reviews see Magnusson *et al* 2008, Seynnes *et al* 2015). However, these forces cannot be directly measured and are therefore estimated. Various methods to estimate tendon force have been proposed but to date, there is no consensus on the best method to be used (Seynnes *et al* 2015). In addition, this methodology characterizes the global properties (albeit indirectly) of the whole tendon, while tendon pathologies (e.g. tear or tendinopathy) are thought to induce local changes. Therefore, alternative methods that are able to accurately quantify elastic properties of a localized area of the tendon are needed.

Ultrasound shear wave elastography techniques measure the shear wave velocity to estimate the localized elastic properties of various tissues *in vivo* (Nightingale *et al* 2002, Sandrin *et al* 2003, Bercoff *et al* 2004). The supersonic shear imaging (SSI) technique generates shear waves using the acoustic radiation force of focused ultrasound beams. The shear wave propagation is measured by using ultrafast ultrasound imaging, typically thousands of frames per second. Shear wave velocity (V) is then measured on the acquired shear wave propagation movie. By considering the medium as elastic and homogeneous, the shear modulus (μ) given in kPa is deduced using the equation: $\mu = \rho V^2$, where ρ is the density (assuming $\rho = 1000\,\text{kg m}^{-3}$) (Bercoff *et al* 2004). This technique has been used to assess tendon mechanical properties in healthy volunteers (Aubry *et al* 2011, 2013, Hug *et al* 2013, DeWall *et al* 2014) and in people with Achilles (Aubry *et al* 2015) and patellar tendinopathy (Zhang *et al* 2014). However, the SSI technique has two main limitations when applied to the tendon. First, as tendon is stiff, the shear wave speed is high. Hence, measurements saturate at relatively low tension levels, e.g. about 20° in plantar flexion during passive stretching (Hug *et al* 2013). Second, as the shear wavelength ($\lambda \sim 25\,\text{mm}$ in the parallel direction) is larger than the mean tendon thickness ($h \sim 4\,\text{mm}$ for Achilles tendon), the wave propagation is then guided along the tendon due to the successive reflections at the tendon boundaries (Brum *et al* 2014). Consequently, the group velocity of the shear wave estimated under the assumption of an elastic and homogeneous medium is not necessarily linked to the actual shear modulus, in contrast to what is assumed by the SSI technique. In a recent study, Brum *et al* (2014) reported a technique based on the measurement of shear wave dispersion to characterize tendon viscoelastic properties using a model that takes into account that waves propagation is guided. Taking advantage of this new technique, the present study aimed to: (1) assess the reproducibility of this technique; (2) determine the effect of Achilles tendon stretching on tendon shear elasticity; (3) assess the variability of tendon elasticity between two locations (i.e. proximal versus distal) and (4) to compare results obtained with this shear wave dispersion analysis with those obtained with the shear wave elastography mode of the conventional SSI technique.

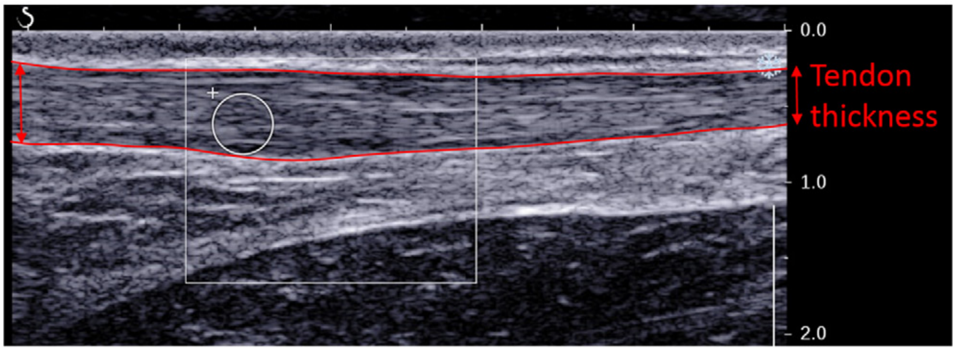

Figure 1. Ultrasound image (Bmode) acquired with SL 15-4 probe on the tendon for subject #9 at an angle of $-25°$. Thickness was measured on the left on right part (red arrows) to obtain a mean value of the tendon thickness.

Materials and methods

Participants

Twelve healthy young males (23.2 ± 3.3 years, 178 ± 7 cm, 73.8 ± 7.5 kg) with no history of Achilles tendon (AT) injury volunteered to participate in this study. The local ethics committee approved the study (CPP-MIP-008), and all the procedures were adhered to the latest revision of the Declaration of Helsinki.

Materials

An isokinetic dynamometer (Con-Trex MJ dynamometer, CMV AG, Dübendorf, Switzerland) was used to passively set the ankle angles. Participants were positioned prone with the right foot attached to the footplate and the lateral malleolus aligned with the dynamometer axis of rotation. The knee angle remained fully extended throughout the experiment.

An Aixplorer ultrasound scanner (V8, Supersonic Imagine, Aix-en-54 Provence, France) and a linear transducer array (SL15-4, Supersonic Imagine, Aix-en-Provence, France) were used in 'shear wave elastography mode' (i.e. standard MSK preset). The ultrasound device also included a specific home-made preset designed for tendons, which was based on ultrasound sequences previously described in Brum *et al* 2014. This preset provides an analysis of the phase velocity of propagating shear waves (i.e. 'phase velocity mode'). Mean tendon thickness was also measured using the B-mode ultrasound images (averaging of one measure on the left side of the image and one on the right side, figure 1). The thickness value was used in the phase velocity method (see Data Analysis section).

Protocol

All data were collected while the participants remained relaxed (i.e. plantar flexors in passive state). First, five slow ($5° \text{ s}^{-1}$) passive loading/unloading cycles between 50° of plantar flexion and 10° of dorsiflexion (0° of ankle dorsiflexion = foot perpendicular to the leg) were performed to account for conditioning effects (Nordez *et al* 2008). In order to assess

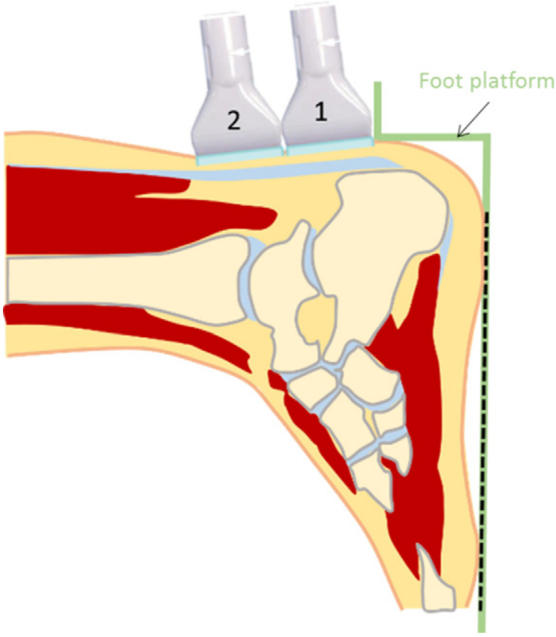

Figure 2. Achilles tendon shear modulus was measured at different ankle positions and US probe locations. Position 1 (distal) corresponds to the probe position used to assess the effect of ankle angle, test the reproducibility and to compare the two techniques (i.e. commercial mode and phase velocity mode). Position 2 (proximal) was used to test the spatial variability of the measurements.

the effect of tendon tension on the shear modulus measured using the dispersion analysis (i.e. phase velocity mode), measurements were performed every 5° from 50° of plantarflexion (i.e. initial position) to 10° of plantar flexion. These measurements were started immediately after the conditioning cycles. At each ankle angle, a 60 s rest period preceded each data collection. This time was used to determine the probe location, and to minimize the viscoelastic stress relaxation (Freitas *et al* 2015). The ultrasound probe was hand placed over the distal insertion of the AT, about 0.5 cm from the calcaneus and against the foot platform of the ergometer (i.e. position 1 in the figure 2). It corresponded to the most distal measurement that can be performed using the ergometer. Between each measurement the ankle was repositioned to the initial test position at 50° of plantar flexion. Data were collected by the same experienced examiner.

The reproducibility of the 'phase velocity mode' was tested on 7 participants at three ankle angles in a randomized order (45°, 35°, and 25° of plantar flexion). For each angle, five measurements were performed over the position 1 (figure 2). Between each measurement the probe was removed and replaced over the same location. Between each tested angle, the ankle was repositioned to the initial test position at 50° of plantar flexion.

The comparison of the two probe locations (i.e. distal versus proximal) was performed using the 'phase velocity mode' at three ankle angles (45°, 35°, and 25° of plantar flexion). The first probe location was the most distal location that can be measured (i.e. position 1 in the figure 2), while the second location corresponded to the probe over the myotendinous junction of the soleus muscle. The probe was placed just in the continuity of the first position corresponding to a probe length (5 cm) from the foot platform of the ergometer, i.e. position 2 in the figure 2). Before each measurement, the ankle was returned to its resting position, and measurements started after a 60s resting period with the ankle angle set in the desired position.

To compare results obtained with the shear wave dispersion analysis with those obtained with the conventional 'shear wave elastography mode' (the 'conventional mode' refers in this paper to the SSI technique), shear wave elastography acquisitions (as defined in Bercoff *et al* 2004) were performed at three ankle angles (45°, 35°, and 25° of plantar flexion). The software of the ultrasound scanner allowing to define a circular region of interest (Q-Box function, Supersonic Imagine, France) was used to measure mean shear wave speed and then deduce the shear modulus (under the assumption of elastic and homogeneous medium) across circular regions. Before each measurement the ankle was released to its resting position, and measurement, started after a 60-s rest period.

Data analysis

For the data measured using the 'phase velocity mode', the shear wave velocity dispersion curves were extracted from the acquired shear wave propagation using a 2D-Fourier analysis to 1200 Hz. At low frequencies, values were removed when frequencies were smaller than c/h (where c is the shear wave speed for each frequency and h the mean thickness of the tendon). Then, as proposed by Brum *et al* (2014) for the AT, the model of guided wave propagation along a transversely isotropic elastic plate with wave propagation direction parallel to the fiber direction was used to estimate the shear elasticity. The compression (C_{13}) and the shear (C_{55}) moduli, defined from the Christoffel's elasticity tensor (Royer *et al* 1999), were quantified by fitting the experimental phase velocity dispersion curve with this guided wave model. The mean thickness of the AT measured from the B-mode images was used in the model as the plate thickness, and the elastic constants C_{11} and C_{33} were held fixed in accordance with Kuo *et al* (2001) ($C_{11} = 2.89\,\text{GPa}$ and $C_{33} = 3.72\,\text{GPa}$).

For the conventional shear wave elastography acquisitions, a representative shear wave modulus (μ) value was obtained by averaging shear wave speed values over circular ROIs (circles with about 4 mm of diameter depending on the tendon size). The apparent shear modulus (μ) was then calculated based on the elastic and homogeneous medium hypothesis ($\mu = \rho V^2$, Bercoff *et al* 2004).

Statistics

The IBM SPSS Statistics 20.0 (IBM Corporation, New York, USA) software was used for the statistics procedures. All data are reported as mean ± SD. Normal data distribution was tested by using the Kolmogorov-Smirnov test.

Reproducibility. The reproducibility of the tendon shear modulus was determined from 'phase velocity mode'. To this end, the interclass coefficient correlation (ICC), standard error of measurement (SEM), and coefficient of variation (CV) were calculated across the three repetitions performed for each ankle angle (45°, 35° and 25° of plantar flexion) (Hopkins 2000).

Effect of passive stretching. In order to assess the effects of passive tension (i.e. ankle angle) on shear (C_{55}) and compression (C_{13}) moduli, two separate Friedman tests (variables: 9 ankle angles (each 50°, from 50° of plantar flexion to 10° in dorsiflexion)) were performed due to the non-normal distribution of data. Separate Wilcoxon signed-rank tests with Bonferroni adjustment were used for post-hoc analysis.

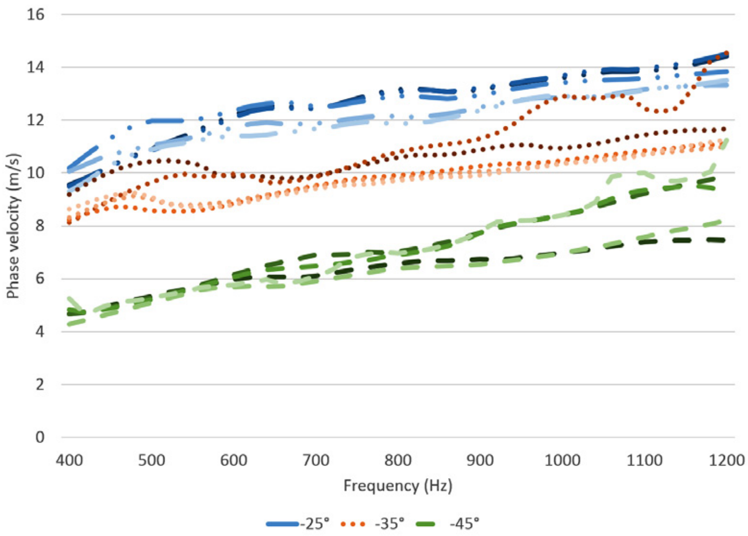

Figure 3. Reproducibility data—phase velocity versus frequency at 3 ankle angles for one participant (#9). At each angle, 5 measures were performed.

Difference between the distal and proximal region. To test the spatial variability of the measurements, two two-way repeated measures ANOVAs (factors: ankle angle (45°, 35°, and 25° of plantar flexion) and location (distal and proximal)) were performed for both the shear (C_{55}) and compression (C_{13}) modulus.

Comparison between conventional shear wave elastography mode and shear wave dispersion analysis. To compare shear modulus values measured by the two techniques, a repeated measures ANOVA (factors: ankle angle (45°, 35°, and 25° of plantar flexion) and techniques (conventional and phase velocity)) was performed. Main effects of variables (the effect of a variable averaged across levels of another variable) were determined.

Post-hoc analyses were performed when appropriated using Bonferroni correction method for multiple comparisons. The statistical significance was set at $P < 0.05$.

In addition, the relationship between the measurements performed using the 2 methods was tested using the Pearson's product correlation.

Results

One participant was not able to reach 50° in plantarflexion without pain. One subject stopped the experiment before reproducibility measurements. Another participant exhibited very high shear wave velocity values and therefore it was not possible to perform measurements at plantarflexed position <20° (See discussion). Therefore, 7 subjects were considered for the reproducibility and 10 for all the other analyses.

Reproducibility

A typical example of the reproducibility of the phase velocity dispersion curve is depicted in figure 3. Although some variabilities of the phase velocity exist, it seems to have little

Table 1. Reproducibility results for $n = 7$. Results of reliability for C_{13} and C_{55} (ICC: intra-class correlation coefficient; SEM: standard error of measurements (SEM); CV: coefficient of variation, Hopkins 2000).

	C_{13} (MPa)			C_{55} (kPa)		
Ankle angles	45°	35°	25°	45°	35°	25°
Mean ± STD	3282.53 ±2.77	3280.49 ±3.25	3281.01 ±2.65	118.01 ±67.88	258.65 ±124.88	509.14 ±148.51
ICC	0.42	0.73	0.85	1.00	1.00	1.00
SEM	2.14	2.04	1.37	10.69	6.45	3.50
CV (%)	0.81	0.60	0.48	0.07	0.05	0.05

Note: Ankle angles analyzed corresponded to plantar flexed positions.

influence on moduli (C_{55} and C_{13}) after data analysis. Overall, the results showed a very good reproducibility of the technique for both C_{13} and C_{55} (table 1). Notably, reproducibility was higher (higher ICC and/or lower SEM/CV) at 25° of plantarflexion than 45° of plantarflexion.

Effect of passive stretching

The phase velocities plotted as a function of the frequency (figure 4) are depicted for one typical participant before fitting the parameters to obtain the compression (C_{13}) and the shear (C_{55}) moduli. The phase velocity increased as both the ankle plantar flexion (i.e. into dorsiflexion) and the frequency increased. This behavior was observed for all the participants and trials.

Although the compression modulus (C_{13}) was not significantly affected by the ankle angle (average value across the angles: 3274 ± 12 MPa), the shear modulus (C_{55}) consistently increased with the ankle dorsiflexion ($P = <0.001$; figure 5). Post-hoc analysis indicated that C_{55} values at 40° and below were significantly different compared to the starting value at 50° of plantar flexion.

Difference between the distal and proximal region

There was no interaction between ankle angle and probe location ($P = 0.932$) on the compression modulus (C_{13}, figure 6). Similarly, there was no significant main effect of ankle angle ($P = 0.471$) or probe locations ($P = 0.586$).

Both significant main effects of ankle plantar flexion ($P < 0.001$) and probe locations ($P < 0.001$) were observed for the compression modulus (C_{55}). The compression modulus was significa ntly higher in proximal than distal region (range: 22.3% to 48.1%; figure 6). No interaction was observed ($P = 0.234$).

Comparison between conventional shear wave elastography mode and shear wave dispersion analysis

There was both a significant main effect of the technique ($P < 0.001$) and a significant interaction between ankle angle and technique ($P <0.001$). Post hoc analysis showed that the tendon shear modulus was systematically estimated higher when it was deduced from the dispersion shear wave analysis rather than using the conventional SSI technique (figure 7). The correlation coefficient of the linear relationship between shear moduli C_{55} and μ was high ($r = 0.844$, $P < 0.001$).

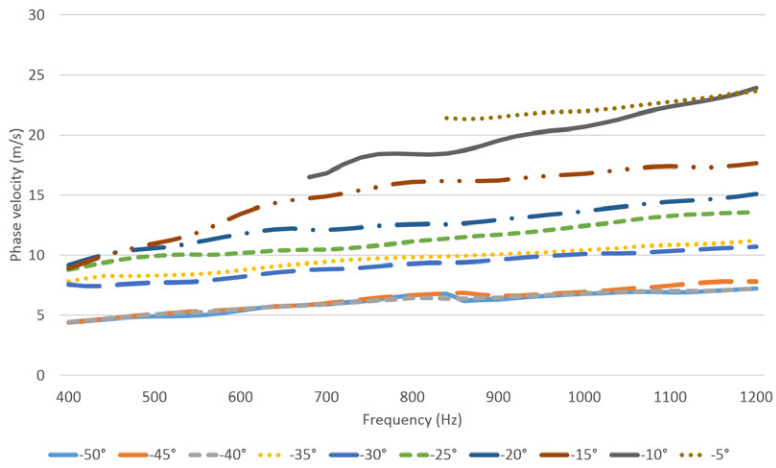

Figure 4. Typical individual example of the relationship between phase velocity and frequency at different ankle angles.

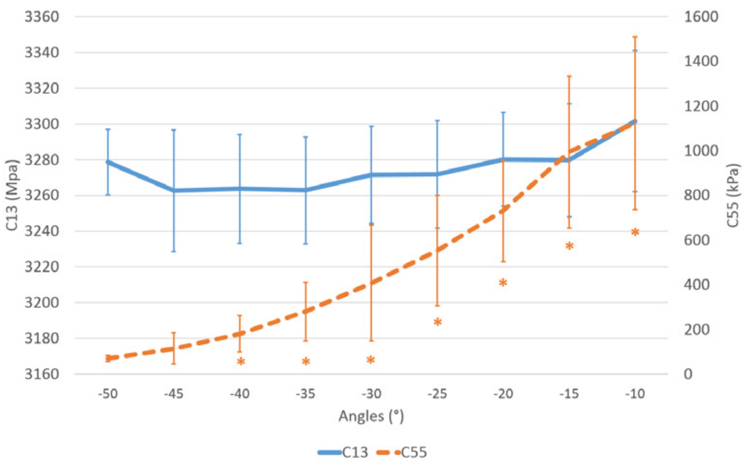

Figure 5. Mean compression (C_{13}) and shear (C_{55}) moduli versus ankle angles for 10 subjects ($N = 10$). *: significantly different ($P < 0.05$) than the more plantar flexed angle (i.e. $-50°$) for C_{55}.

Discussion

Here we used a new technique based on guided wave propagation ('phase velocity mode', Brum *et al* 2014) to quantify both the compression (C_{13}) and longitudinal shear (C_{55}) moduli of the Achilles tendon at different ankle angles (i.e. different level of tension). First, the shear modulus increased with the dorsiflexion and all those measurements were reproducible. Second, the tendon shear modulus was significantly higher at the proximal location (position 2) compared to a more distal location (position 1). Finally, shear modulus values measured using this new 'phase velocity mode' technique were systematically higher than the values measured using the conventional SSI technique. Notably, the values provided by the two techniques were highly correlated.

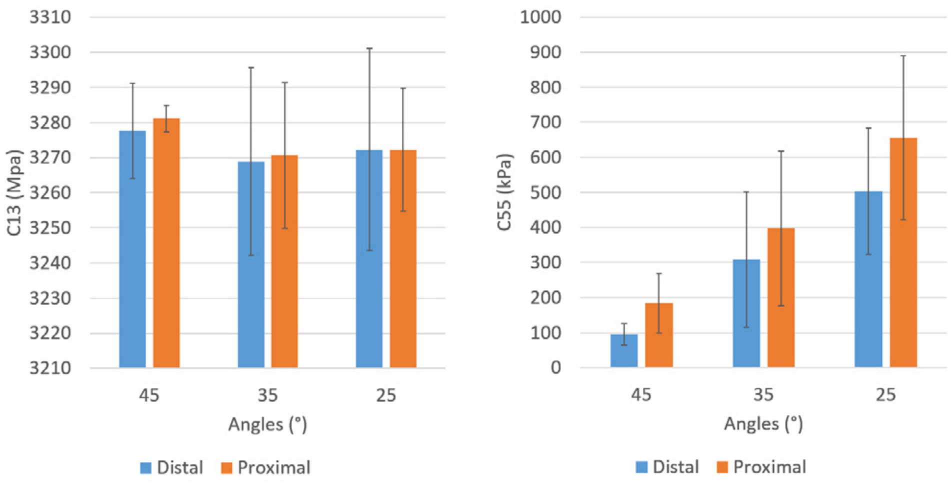

Figure 6. Effects of probe location—compression (C_{13}) and shear (C_{55}) moduli versus 3 ankle angles and at two locations: distal (position 1 of the probe) and proximal (position 2 of the probe).

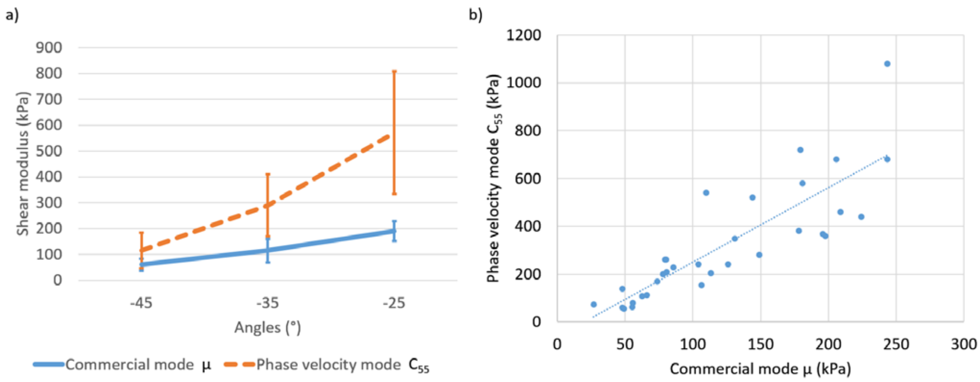

Figure 7. (a) Comparison data—shear moduli obtained with the wave phase velocity mode (C_{55}) and the commercial shear wave elastography mode (μ) versus the ankle angles. (b) Linear relationship between shear moduli C_{55} and μ with a correlation coefficient of $r = 0.844$.

Because the Achilles tendon is a stiff tissue, the shear waves propagate at a high velocity making its measurement challenging. This is the reason why measurements performed with the conventional SSI elastography technique on the Achilles tendon saturates from a plantar flexion of about 30° (Aubry *et al* 2011, 2013, 2015, Hug *et al* 2013, DeWall *et al* 2014). The phase velocity mode used in the present study uses only one pushing location to follow the shear waves, allowing the ultrasound acquisition rate to be increased up to 17 000 frames s^{-1} (i.e. the maximal capability of the current version of the device). It allowed us to measure the shear modulus at up to 10° of plantar flexion during passive stretching. Future development of the device should focus on the ultrasound framerate to provide measurements of tendon modulus closer to maximal range of motion (e.g., Andrade *et al* 2016) and during contractions where active forces will likely give rise to much higher shear wave speeds.

To our knowledge, only one study has measured tendon mechanical properties using the same phase velocity mode (Brum *et al* 2014). Brum *et al* (2014) used a different participant's positioning (sitting position with 90° and 0° of the knee and ankle joint angles, respectively). Despite this difference, C_{13} and C_{55} values obtained were similar and within the same range (3.21 ± 0.20 GPa and 919 ± 330 kPa, respectively in mean over the all angles) with those observed in the present study. Regarding C_{13} coefficient, small changes were observed within the present study. This was already observed and described by the work of Kuo *et al* (2001). This parameter can be assumed a constant parameter for future investigation.

The present study showed a substantial underestimation of the shear modulus measured using the conventional SSI technique when compared to modulus measured using the shear wave dispersion technique (figure 2). As the shear wave propagation is guided in Achilles tendon (Brum *et al* 2014), the tendon thickness is likely to influence the group shear wave speed used by the conventional SSI technique to estimate the shear modulus (Brum *et al* 2014). This should explain the differences observed between both measurements in the present study. Therefore, actual C_{55} values should be obtained using the shear wave dispersion technique. However, there is a high significant correlation between measurements performed with the two techniques ($r = 0.84$), indicating that the direction of change in tendon stiffness may be reliably estimated with any of those techniques. It also indicates that the conventional shear wave elastography technique can provide a good estimate of the inter-individual variability of tendon shear modulus. Thus, this conventional technique remains relevant for tendon characterization, and particularly in clinical studies (e.g. Aubry *et al* 2015). Notably, one advantage of the conventional SSI elastography technique is to provide a 2D mapping of shear modulus, while the shear wave 'phase velocity mode' gives access to only one measurement, representative of the entire length of the probe.

The Achilles tendon shear modulus measured using the 'phase velocity mode' exhibited an increase with ankle angle as previously reported using the SSI technique (Aubry *et al* 2011, 2013, Hug *et al* 2013). This increase in shear modulus at more dorsiflexed angles reflects the well-known strain-stiffness behavior of tendinous tissues (Fung 1993). Using the conventional SSI technique, two previous studies showed that the slack length (considered as the onset of the increase in tension) of the Achilles tendon occurred at a higher ankle plantar flexion (43° of ankle angle; Hug *et al* 2013) than the slack length of both the gastrocnemius (24° in plantarflexion; Hug *et al* 2013) and soleus muscles (2° in dorsiflexion, Hirata *et al* 2015). As these muscles are supposed to be arranged in series with the tendon, this result was not expected. The results of the present study are important to ensure that this previous result was not due to the limitations of the SSI conventional technique. For instance, the group shear wave speed of the tendon might have been influenced by its shape (Brum *et al* 2014). Using the dispersion shear wave analysis, the present study confirmed that the Achilles tendon is stiffened/tensioned very early during the passive dorsiflexion (figure 5). Further studies are thus required to provide a better understanding of structures other than *triceps surae* muscles (e.g. nerves, fascias) that could stretch the Achilles tendon in plantar flexed angles (Hug *et al* 2013).

In the present study, tendon shear modulus was compared between two locations. Our results showed that the proximal part of the Achilles tendon (next to the myotendinous junction of the Soleus muscle) is stiffer than the distal part, regardless the ankle angle. Since it was obtained using the phase dispersion analysis, this change in shear modulus should be independent from change in tendon geometry and boundary conditions. This result differs from those of DeWall *et al* (2014) performed using the SSI technique. In this latter study there was no significant difference between the similar regions than in the present study. In addition, DeWall *et al* (2014) showed that the tendon group shear wave velocity is dramatically decreased when measured more proximally (i.e. closer to the gastrocnemius muscle).

The frequency characteristics of the probe used in the present study did not allow us to investigate more proximal locations where the tendon is very thin. Further experiments are required to map the spatial variability in C_{55} within the Achilles tendon. For instance, as myotendinous junctions are frequently injured, measurements performed close to muscle insertions might provide clinical relevant information.

The present study showed that the group velocity classically measured using shear wave elastography techniques provides a significant lower estimation of shear modulus than the shear wave dispersion analysis combined with a model that account for the guided shear wave propagation. It indicates that the guided propagation of the shear waves precludes the traditional shear wave elastography techniques to provide accurate shear modulus estimation. It casts doubt about the validity of the previous results obtained with traditional shear wave techniques. However, the large correlation between the shear modulus values obtained with the two techniques indicates that changes in tendon shear modulus can be appropriately described using the conventional group velocity method. It is therefore likely that previous conclusions about the tendon slack length (Hug *et al* 2013), the effect of tendinopathy on tendon elasticity (Aubry *et al* 2015) remain valid. It is now recommended that the shear wave dispersion analysis is used to accurately quantify the effects of musculoskeletal conditions on tendon mechanical properties.

Acknowledgments

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Conflict of interest

MT is founder and shareholder of Supersonic Imagine. JLG is scientific consultant for Supersonic Imagine.

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