Original Article
Elasticity of healthy Achilles tendon decreases with the increase of age as determined by acoustic radiation force impulse imaging

Zhengmin Ruan[1], Bin Zhao[2], Hengtao Qi[2], Yuan Zhang[3], Feixue Zhang[1], Mei Wu[1], Guangrui Shao[4]

[1]Department of Ultrasound, Second Hospital of Shandong University, Shandong University, Jinan, P.R. China; [2]Shandong Medical Imaging Research Institute, Shandong University, Jinan, P.R. China; [3]Center of Evidence-based Medicine, Second Hospital of Shandong University, Jinan, P.R. China; [4]Department of Radiology, Second Hospital of Shandong University, Jinan, P.R. China

Abstract: Aims: The objective of the study was to investigate the value of acoustic radiation force impulse (ARFI) and its application in Achilles tendon elasticity of healthy volunteers. Methods: Bilateral Achilles tendons of 56 healthy individuals were included in the study. The subjects were divided into four age groups. Shear wave velocities (SWVs) of each tendon in the states of relaxation and tension were measured using ARFI technology. We selected the middle section of the Achilles tendon for ARFI measurement. Each Achilles tendon was examined by a musculoskeletal radiologist. Longitudinal images of each tendon were obtained by ultrasound and ARFI elastography. A quantitative assessment of the tissue stiffness was made with virtual touch tissue quantification. Results: Significant differences in SWVs of Achilles tendon were found between any two age groups in the same status ($P < 0.05$) and between different status in subjects aged 25-35 years ($P < 0.05$) and 36-45 years ($P < 0.05$). SWV of Achilles tendon increased with increasing age in both states of relaxation and tension, which was consistent with the histological feature of ageing tendons. However, there were no significant differences between men and women within any group in the same status ($P > 0.05$) or between different states in subjects aged 46-55 years ($P = 0.308$) and 56-65 years ($P = 0.362$). Conclusions: ARFI imaging provides quantitative information about tendon stiffness and represents an excellent supplementary technique to B-mode ultrasound. Furthermore, the elasticity of healthy tendon decreases with increasing age in subjects without disease and long-term heavy load lifting.

Keywords: Acoustic radiation force impulse, Achilles tendon, shear wave velocity, elasticity

Introduction

Elasticity varies between different tissues in the body or in the same tissue in different pathological states. With conventional ultrasound, it is difficult to distinguish changes in tissue elasticity because the tissue often possesses the same echogenicity as the surrounding healthy tissue [1]. In the last 10 years, several ultrasound-based non-invasive methods for elasticity assessment have been developed, such as transient elastography, real-time elastography, and acoustic radiation force impulse (ARFI) imaging.

ARFI is a new tissue elasticity imaging technology that makes use of sound waves to quantify tissue elastic properties [2]. ARFI technology represents a relatively new imaging method that is able to non-invasively determine the elastic properties of target tissues. This technology differs from previous elastographic techniques in that it allows for the evaluation of tissues without the need for external compression [3]. The acoustic wave is sent into the tissue independent of the examiner pressing the button to start measurement. It is presumed that external compression causes a change in the elasticity of normal tissues [4]. Therefore, it can be claimed that ARFI technology reduces operator dependence. The calculation of the shear wave speed mainly reflects the elasticity and viscosity of the target tissue [5, 6]. Soft materials allow for large displacement amplitudes, whereas hard materials typically create low displacement amplitudes [7]. The wave propaga-

Table 1. SWVs of tendons for healthy men and women in the state of relaxation

Age groups (No for men/women)	SWVs (m/s)		t	P
	Men (58 tendons)	Women (54 tendons)		
1 (18/16)	2.49 ± 0.87 (0.72-3.38)	2.48 ± 0.84 (0.77-3.33)	0.034	0.973
2 (18/18)	3.74 ± 0.49 (2.52-4.62)	3.77 ± 0.53 (2.45-4.55)	0.176	0.861
3 (10/8)	5.07 ± 0.88 (3.08-6.29)	4.91 ± 0.99 (3.01-6.31)	0.358	0.721
4 (12/12)	6.71 ± 1.42 (3.72-8.36)	6.61 ± 1.33 (3.85-8.34)	0.178	0.860
F	54.510	49.741	0	
P	0.000	0.000		

Note: Statistically significant differences were observed between any two of the four groups ($P < 0.05$).

Table 2. SWVs of tendons for healthy men and women in the state of tension

Age groups (No for men/women)	SWVs (m/s)		t	P
	Men (58 tendons)	Women (54 tendons)		
1 (18/16)	2.98 ± 0.69 (1.12-3.65)	3.08 ± 0.95 (1.06-4.30)	0.534	0.726
2 (18/18)	4.26 ± 0.62 (2.72-5.32)	4.34 ± 0.75 (2.74-5.68)	0.349	0.729
3 (10/8)	5.24 ± 1.11 (3.61-6.98)	5.44 ± 0.93 (3.75-6.75)	0.407	0.689
4 (12/12)	7.00 ± 1.45 (4.28-8.67)	7.04 ± 1.34 (4.14-8.72)	0.070	0.945
F	45.160	38.970		
P	0.000	0.000		

Note: Statistically significant differences were observed between any two of the four groups ($P < 0.05$).

Table 3. SWVs of tendons for healthy volunteers in the states of relaxation and tension

Age groups (N)	SWVs (m/s, 112 tendons)		t	P
	Relaxation	Tension		
1 (34)	2.49 ± 0.84 (0.72-3.38)	3.03 ± 0.81 (1.06-4.30)	2.698	0.009
2 (36)	3.75 ± 0.51 (2.45-4.62)	4.30 ± 0.68 (2.72-5.68)	3.882	0.000
3 (18)	5.00 ± 0.90 (3.01-6.31)	5.33 ± 1.01 (3.61-6.98)	1.035	0.308
4 (24)	6.66 ± 1.34 (3.72-8.36)	7.02 ± 1.37 (4.14-8.72)	0.920	0.362
F	397.90	317.40		
P	0.000	0.000		

Note: Statistically significant differences were observed between any two of the four groups ($P < 0.05$).

tion speed is an intrinsic and reproducible property of a tissue, and thus, the quantitative implementation of ARFI imaging generates objective and reproducible data [8]. ARFI imaging technology includes virtual touch tissue imaging and virtual touch tissue quantification (VTQ) [9]. VTQ involves the mechanical excitation of tissue by short-duration acoustic pulses in a region of interest (ROI) chosen by the examiner, which produces shear waves that propagate through the tissue in the ROI, generating localized, microscale tissue displacements (1-10 µm), and uses a "strain" algorithm to determine tissue stiffness [10]. The displacements result in lateral shear-wave propagation away from the region of excitation and are tracked using ultrasonic correlation-based methods [11]. By measuring the time to peak displacement at each lateral location, the shear wave velocity (SWV) within the tissue can be calculated (measured in meters per second), which is proportional to the square root of tissue elasticity [9, 11]. Therefore, VTQ can provide an objective numerical evaluation of tissue stiffness.

Recently, ARFI technology has become of practical importance in elasticity imaging, and its feasibility and utility are being gradually recognized [12]. Numerous studies have shown promising ARFI results in the evaluation of the thyroid, breast, liver, kidney, and pancreas [9, 13-16]. However, most studies about elastography on musculoskeletal structures are based on visual and qualitative assessments through color coding. There are few prior reports on SWV of Achilles tendon for obtaining quantitative ultrasound elastographic measurements [17]. In this study, we investigate the potential value of ARFI imaging by evaluating Achilles tendon elasticity in healthy volunteers at different ages with states of relaxation and tension of the tendon.
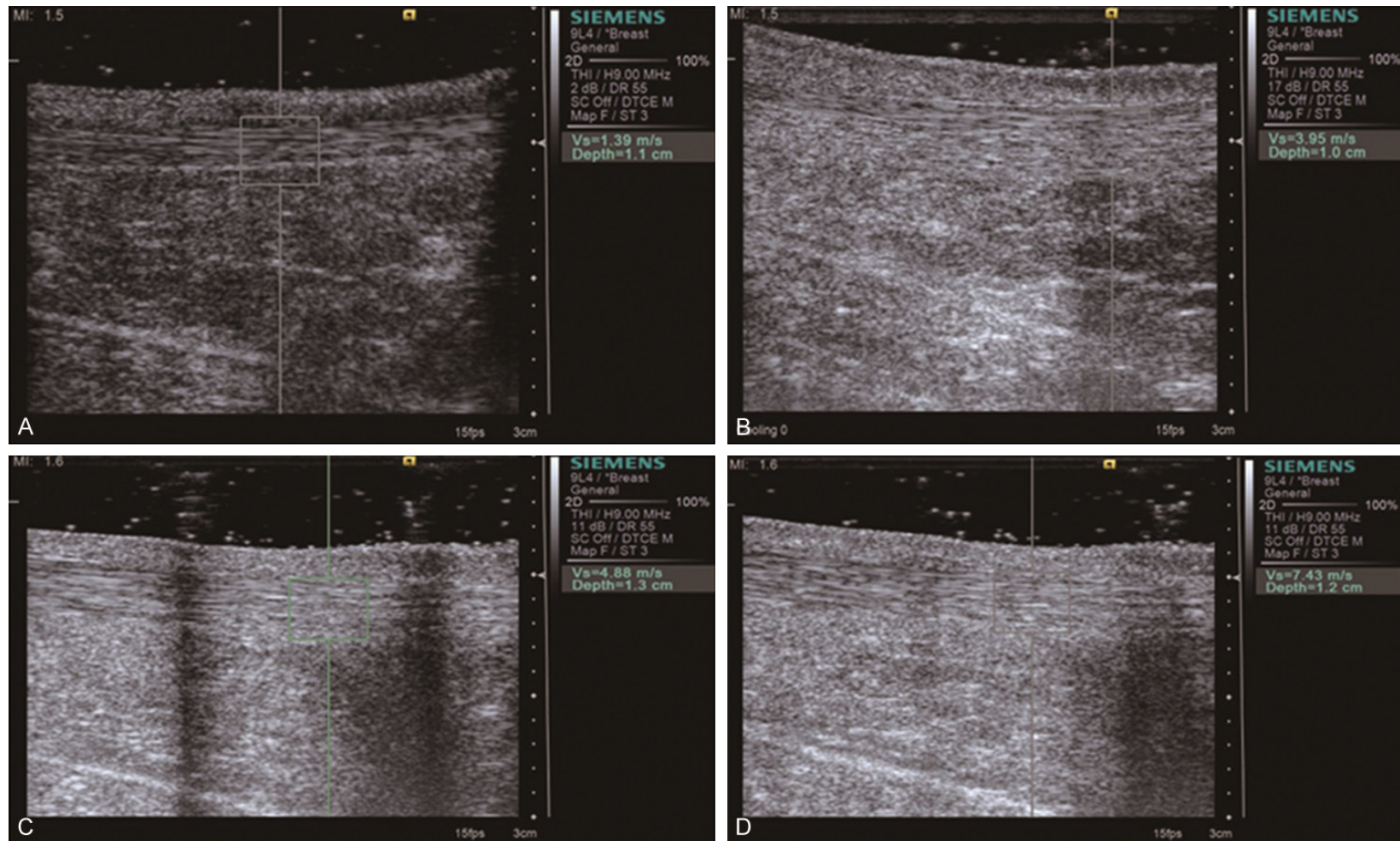
Figure 1. ARFI imaging of the Achilles tendons in a state of relaxation in healthy men at ages of (A) 30 years old (SWV = 1.39 m/s), (B) 42 years old (SWV = 3.95 m/s), (C) 51 years old (SWV = 4.88 m/s), and (D) 60 years old (SWV = 7.43 m/s). ARFI measurement was performed with a Siemens Acuson S2000™ ultrasound system (Siemens Medical Solutions, Mountain View, CA, USA). A linear array transducer (9L4) was equipped for B-mode ultrasound and ARFI elastography examinations. The ROI was located approximately 3 cm above the insertion on the calcaneus.

Materials and methods

Subjects

The study was approved by the Institutional Ethics Committee, and all volunteers provided written informed consent. We included in our study bilateral Achilles tendons of 56 healthy individuals (29 men and 27 women; median age, 42 years; range, 25-65 years). The volunteers were divided into four age groups according to ages: age group 1 (25-35 years), age group 2 (36-45 years), age group 3 (46-55 years), and age group 4 (56-65 years). Volunteers with a history of diabetes, Achilles tendon problems, systemic inflammatory disorders, long-term heavy loading of the tendon, or a condition that might influence our results, such as rheumatoid arthritis, spondyloarthropathies, or hypercholesterolemia, were excluded.

ARFI measurement

ARFI measurement was performed with a Siemens Acuson S2000™ ultrasound system (Siemens Medical Solutions, Mountain View, CA, USA). A linear array transducer (9L4) was equipped for B-mode ultrasound and ARFI elastography examinations. According to a previous study [18], the Achilles tendon was considered according to three regions: the proximal (musculotendinous junction), middle (2-6 cm above the insertion on the calcaneus), and distal (insertion on the calcaneus) sections. In our study, we selected the middle section of the tendon for ARFI measurement.

Each Achilles tendon was first examined by a musculoskeletal radiologist with the volunteer being in a prone position and the foot hanging over the edge of the examination bed in a relaxed position to avoid tendon stress. Then, the same tendon was examined with the volunteer being in a lateral position and the foot being in passive flexion to ensure tension in the Achilles tendon. Longitudinal images of each tendon were obtained by ultrasound and ARFI elastography. A quantitative assessment of the tissue stiffness was made with VTQ. In VTQ, an acoustic push pulse and detection pulses were used to calculate SWV, which increases with increasing tissue stiffness.

The probe was held perpendicular to the tendon while performing the examination to avoid anisotropy [18] and the probe was moved gently with no downward pressure. Because the Achilles tendon is relatively superficial, more medical ultrasonic coupling agent was used between the transducer and skin for examinations. When switching to the elasticity imaging mode to perform ARFI on B-mode ultrasound images, the target was found after the selected ROI was determined with a predefined size (5 × 5 mm) provided by the system. A stationary state was maintained for 3 s followed by image stabilization, framing, and recording of the image (saving to hard drive). ARFI measurement was recorded, and SWV value was expressed in meters per second and displayed on the screen. For each tendon, six measurements were performed. The mean value was calculated and used for analysis. The range for SWV was 0-9 m/s. In each volunteer, the procedure was repeated at approximately the same depth in the states of relaxation and tension.

Statistical analysis

Data from men and women in the same age group were recorded separately for analysis. Statistical analysis was performed using SPSS 14.0 software package (SPSS Inc, Chicago, IL, USA). Data were expressed as means ± standard deviation (SD). $P < 0.05$ was considered statistically significant. SWVs between men and women in the states of relaxation or tension and between relaxed and tense states of all individuals in the same age group were compared using t tests. Analysis of variance (ANOVA) was used to compare SWVs between any two age groups in the same state and in individuals of the same gender.

Results

SWVs of the Achilles tendon are not correlated with gender in the same age group

To perform SWV measurements, sites that were approximately 3 cm above the insertion on the calcaneus were selected as ROI. Data acquired from subjects of the four age groups were recorded separately according to genders. The results showed that SWVs of healthy tendons in different age groups varied within the range of 0.72-8.36 m/s for men and 0.77-8.34 m/s for women in the relaxed state, and within the range of 1.12-8.67 m/s for men and 1.06-8.72 m/s for women in the tense state (**Tables**
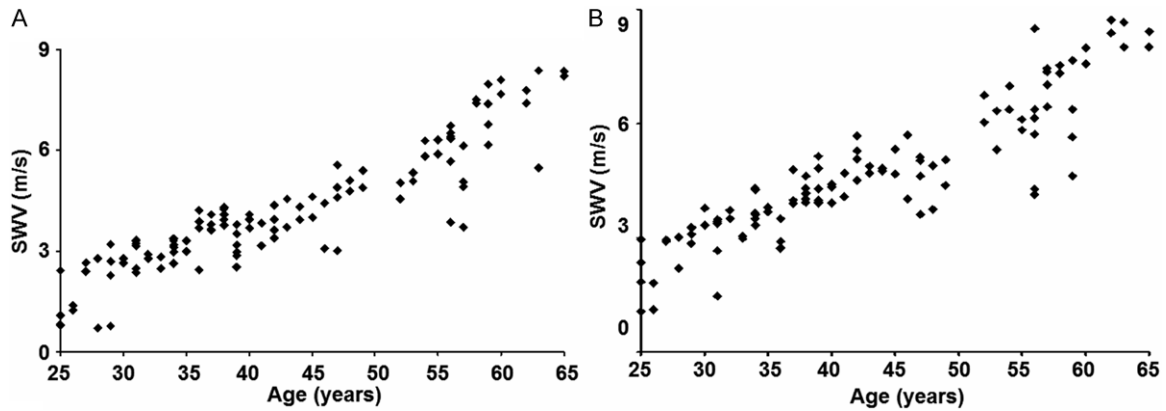
Figure 2. Distribution map of SWVs for healthy Achilles tendons of subjects at different ages in states of (A) relaxation and (B) tension. ARFI imaging (S2000, 9-MHz probe) was performed on the ROI located approximately 3 cm above the insertion on the calcaneus.

1-3). The values for SWVs in the state of relaxation or tension showed no significant difference between men and women in the same age group ($P > 0.05$). These data suggested that SWV values of Achilles tendon had no apparent correlation with gender.

SWVs of the Achilles tendon are different between any two age groups in either relaxed or tense state and between relaxed and tense states in the same age group

To further investigate the SWVs of the Achilles tendon, we compared SWVs between any two age groups in the same state and between relaxed and tense states in the same age group. SWVs of the four age groups varied within the range of 0.72-8.36 m/s in the relaxed state and within 1.06-8.72 m/s in the tense state (**Tables 1-3**). In the relaxed state, SWV values of healthy tendons of 30, 42, 51, and 60 years old was 1.39 m/s, 3.95 m/s, 4.88 m/s, and 7.43 m/s, respectively (**Figure 1**). ANOVA results showed significant differences in the SWVs of Achilles tendons between any two age groups in either relaxed or tense state ($P < 0.05$). SWVs of all tendons in the four age groups showed significant differences between the relaxed and tense states in age groups 1 and 2 ($P < 0.05$). However, SWV was 5.00 ± 0.90 m/s in the relaxed state and 5.33 ± 1.01 m/s in the tense state in age group 3, and 6.66 ± 1.34 m/s in the relaxed state and 7.02 ± 1.37 m/s in the tense state in age group 4, both showing no significant difference between the relaxed and tense states ($P = 0.308$ and

0.362, respectively). These data indicated that SWVs of the Achilles tendon were different between any two age groups in either relaxed or tense state and between relaxed and tense states in the same age group.

The elasticity of healthy tendon is decreased with increasing age

To understand the relationship of SWVs of the Achilles tendon in both relaxed and tense states with age, we constructed the distribution map of SWVs for healthy Achilles tendons of subjects at different ages in states of relaxation and tension. Between the ages of 25 and 65, increasing trend was observed in both relaxed and tense states, showing that the SWVs of the Achilles tendon in both relaxed and tense states were increased with age (**Figure 2A**, **2B**). These data suggested that the elasticity of healthy tendon was decreased by increasing age.

Discussion

There are some technical concerns related to SWV measurement of Achilles tendons in this study. First, ROI often covers part of the surrounding tissues because of its predefined size (5 × 5 mm), which is set by the system and might adversely affect the measurement. The predefined size of ROI box is fixed. However, due to the existence of individual differences in the thickness of the Achilles tendon, ROI sometimes includes not only tendon tissue, but also some adjacent soft tissues, which may introduce error. To reduce this error, we centered

the ROI on the tendon while minimizing the adjacent tissues that were included in ROI. In addition, six measurements were performed for each tendon. Second, anisotropy is a property of tendons that may impact SWV measurements. The ultrasound appearance of tendon varies with the angle of insonation of the incident ultrasound beam. Changing beam angulation allows the operator to overcome the potential pitfall of anisotropy in ultrasound assessment of peripheral tendons [19]. The normal appearance of tendon depends on the incident ultrasound beam being perpendicular to the tendon [20, 21]. Therefore, care was taken to make the transducer parallel to the longitudinal axis of tendon fibers with repeated adjustment of probe position to ensure that the sound beam was directed perpendicular to the tendon fibers in order to minimize anisotropy. Finally, the possible range for the SWV is 0-9 m/s. Values beyond this range are displayed as "x.xxm/s", which is either 0 m/s or > 9 m/s, with 0 m/s corresponding to the cystic portion and 9 m/s corresponding to the solid portion [22]. In other words, both extremely hard and soft tissues can exceed the instrument's ability to measure SWV. SWV of normal, healthy tendon would not exceed the instrument's ability to accurately record it. In our study, an indicator display of "x.xxm/s" appeared frequently when the examination depth was less than 1.0 cm or the operator made an inappropriate movement without maintaining the stationary state for 3 s. We presumed that measurement failure might indicate that the location of the ROI was too superficial or the operator had held the probe unstably rather than that the tendon stiffness was beyond the measurement range (0-9 m/s) of the instrument. In addition, SWV can only reveal the local stiffness of the tendon, which might cause sample errors in practice. In view of the above factors, further studies with more detailed methods of analysis are required to increase the reproducibility of SWV measurement. In clinical applications, SWV should be applied in combination with other techniques including conventional ultrasound.

The Achilles tendon (12-15 cm in length) is the largest tendon in the human body. We decided to perform our study on the Achilles tendon because of its superficial location and large size. It was necessary to study the elastographic changes of healthy Achilles tendons in individuals of different ages and in different func-

tional states. Adult tendons are composed of type I collagen fibers, which are approximately 150 nm in diameter and tightly packed with type III collagen proteoglycans and various elastic fibers [23]. As much as 95% of the collagen in healthy tendons is type I [24]. In degenerated and ageing tendons, type III collagen is more prevalent. As a result of normal ageing, the tendon is less elastic and more prone to rupture in older individuals [25, 26].

In our study, there were significant differences in the SWV of Achilles tendon between the states of relaxation and tension in age groups 1 and 2 ($P < 0.05$) and between any two age groups in relaxation state or tension state ($P < 0.05$). ARFI showed that there was increased stiffness of Achilles tendon with increasing age, which was consistent with the above theoretical basis. Furthermore, it is well known that tendon stiffness increases when the tendon is in the state of tension [27]. From the data acquired for age groups 1 and 2, we conclude that tendons were less elastic in the state of tension than in the state of relaxation. This conclusion is inconsistent with the statistical analysis of data acquired for age groups 3 and 4. We interpret these data to suggest that the reason for this discrepancy is the relatively small sample sizes in groups 3 and 4. Although men are statistically stronger than women and increasing muscular strength is related to decreasing elasticity, it appeared that gender did not significantly influence SWV values in this study. However, it is premature to definitively exclude the relationship between tendon elasticity and gender when ARFI technology was applied within an ROI of 5 × 5 mm. Because the basic data for SWV of Achilles tendon has not been reported previously, the data presented here can serve as an initial reference in the assessment of tendon stiffness.

We selected the middle portion of tendon (2-6 cm above the insertion on the calcaneus) for ARFI measurement, because it is a common site of rupture [28]. This may correspond closely to a significant reduction in the number of blood vessels observed in the central part of the tendon approximately 6-7 cm from its insertion [29]. Mechanically, the tendon is most likely to rupture in the region with the greatest development of stress, i.e., at its narrowest point. A previous study clearly showed a variation in the cross section along the tendon

length. The smallest cross-sectional area of the Achilles tendon is located toward its central part, coinciding with the region that is most commonly cited as the site of rupture [29].

In this study, the principle and value of ARFI were discussed and applied to study the strongest and thickest tendon of the human body, the Achilles tendon. The usefulness of ARFI can be expected to increase rapidly in the musculoskeletal field, as soon as we learn to interpret elastographic artifacts and to take advantage of the new information provided by ARFI. This study included only 56 healthy volunteers and thus represents only a preliminary study. Further studies are needed to confirm the value of this new technique in the elastographic assessment of tendons and differential diagnosis of tendon lesions.

In conclusion, SWV measurement using ARFI imaging provides quantitative and reproducible information about the tendon stiffness in real-time and represents an excellent supplement to B-mode ultrasound. Furthermore, our study confirms that the elasticity of healthy tendon decreases with increasing age (although tendon elasticity can also be reduced in disease and in individuals accustomed to long-term heavy loading). However, further studies are needed to demonstrate the usefulness and potential accuracy of ARFI.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bin Zhao, Shandong Medical Imaging Research Institute, Shandong University, 324 Jingwu Road, Jinan City 250021, Shandong Province, P.R. China. Tel: 86-139-53110988; E-mail: zhaobin0988@163.com

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