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TECHNICAL REPORT

# ShearWave elastography: repeatability for measurement of tendon stiffness

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## Introduction

Tendon injuries are common and a significant source of pain and disability. Tendon injuries are often treated with a variety of non-surgical (e.g., physical therapy) and surgical interventions, along with a reduction of normal physical activities as the tendon heals [1–3]. However, it is difficult to objectively determine when the tendon has healed sufficiently and has the functional capacity to return to normal activities. Previous research has documented the functional capacity of tendons by measuring their stiffness under in vitro [4, 5] and in vivo [6, 7] conditions, but the technologies used for measuring tendon stiffness are applicable only to in vitro conditions (e.g., mechanical testing systems) or require the highly invasive implantation of sensors (e.g., strain gauges) under in vivo conditions. Conventional imaging modalities (e.g., ultrasound, MRI) can monitor changes in the appearance of tendons over time, but these imaging modalities do not provide an objective, quantitative assessment of tendon healing. A technique that could reliably provide a non-invasive quantitative assessment of tendon mechanical properties in vivo would have significant clinical application as a tool for monitoring tendon changes as a result of injury, pathology, and/or treatment.

Shear-wave elastography is a relatively new technology that has the potential to assess the functional capacity of healing tendons in vivo. This technique applies an acoustic radiation force via an ultrasonic beam to the tissue(s), and then utilizes an ultrafast (up to 20 kHz) imaging sequence to measure the speed of the shear waves that result from this applied force. Shear-wave propagation increases as tissue stiffness increases, thus allowing for the indirect estimation of tissue stiffness from shear-wave speed. This technology has been used primarily as a diagnostic tool to identify fibrous masses in breast tissue [8] and has also been applied to the liver, arteries, and muscle [9–12], but its use in tendon is relatively new. Shear-wave elastography has been shown to have good reproducibility in breast imaging [8], but the repeatability for assessing tendon stiffness is not known. Therefore, the objective of this study was to determine the in vitro and in vivo repeatability of shear-wave elastography for estimating tendon stiffness. We hypothesized that in vitro repeatability would be better than in vivo repeatability, and that repeatability would be no worse than "moderate" (i.e., ICC≥0.41) under both in vitro and in vivo conditions.

## Materials and methods

*In vitro assessment* Four fresh-frozen rusine (deer) forelimb tendons were dissected free from surrounding bone and musculature. Following dissection, the tendon ends were sutured and the tendons were placed in a room-temperature saline bath

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(Fig. 1). The ultrasound probe (SuperLinear SL15-4, bandwidth 4–15 MHz, SuperSonic Imagine, Aix-en-Provence, France) was rigidly secured in a clamp, positioned within 2 cm of the tendon, and aligned with the imaging plane parallel to the tendon's long axis. The tendons were loaded with a series of weights (100 g, 200 g, 300 g, 400 g) via a pulley system (Fig. 1). One minute after application of each weight, shear-wave elastography images were obtained (Aixplorer, SuperSonic Imagine). Two trials were acquired for each loading condition. The weight was then removed and the next larger weight was applied and the image acquisition was repeated. Each image provided a local estimate of tendon stiffness at each pixel within the rectangular acquisition box, with the estimated stiffness value represented by a standard color map that was superimposed over the simultaneously acquired B-mode grayscale image (Fig. 2). Using the Aixplorer system's proprietary software, mean tendon stiffness was determined by averaging the local stiffness values within a circular region of interest that was manually sized and positioned relative to the tendon (Fig. 2). This process was performed by a single operator for three locations within each tendon, resulting in three estimates of stiffness for each trial.

*In vivo assessment* Following IRB approval and informed consent, 12 subjects (six male, six female, age: 32 ±— 9) enrolled in the study. These subjects were healthy volunteers recruited by word of mouth. The subjects had no history of preexisting injuries and were asked to maintain consistent activity levels on testing days. For each subject, images were acquired bilaterally for the Achilles, patellar, quadriceps, and flexor pollicis longus (FPL) tendons. For each tendon, images were acquired with subjects in a position that minimized passive tendon tension (e.g., maximum knee extension for the patellar and quadriceps tendons). Specifically, imaging of the patellar and quadriceps tendons was performed with the subject lying prone, with a rolled towel underneath the subject's knee so that the knee was flexed approximately 20°. Imaging of the Achilles tendon was performed with the patient standing on the opposite leg with the leg of interest's knee flexed 90°, lower leg supported by a stationary chair, and the foot hanging off the chair's edge. Finally, the FPL tendon was imaged with the subject seated and the arm placed palm up on an examination table. Again, the subject was instructed to relax the hand and wrist being imaged. Images were acquired using the same ultrasound probe as the in vitro experiments. For in vivo applications, the system's penetration mode was utilized with either the MSK acquisition mode (quadriceps tendon, FPL) or superficial MSK acquisition mode (Achilles and patellar tendons).

For each subject, we acquired two trials for each tendon at four separate testing sessions. The first two testing sessions were on the same day and separated by a minimum of 4 h, while the third and fourth testing sessions occurred 2 days later and were also separated by a minimum of 4 h. This resulted in a total of 64 measurements for each subject (two sides per subject, four tendons per side, four testing sessions per tendon, two trials per tendon per testing session). All images were acquired and all measurements were performed by a single operator who had been trained by a radiologist and representative from the manufacturer. For each image, stiffness was estimated in the same manner as described for the in vitro experiment (Fig. 3). For all tendons, the acquisition box was located a standardized distance from the tendon's bony insertion using the 0.5-cm increment lines on the B mode image. For the Achilles tendon, the image was acquired with the acquisition box 2 cm proximal to the Achilles' insertion on the calcaneus. For the patellar tendon (Fig, 3), the acquisition box was placed 1.5 cm distal to the bony insertion of the tendon onto the patella. For the quadriceps tendon, the acquisition box was placed 2 cm proximal to the bony insertion onto the patella. Finally, for the FPL tendon, the acquisition box was placed

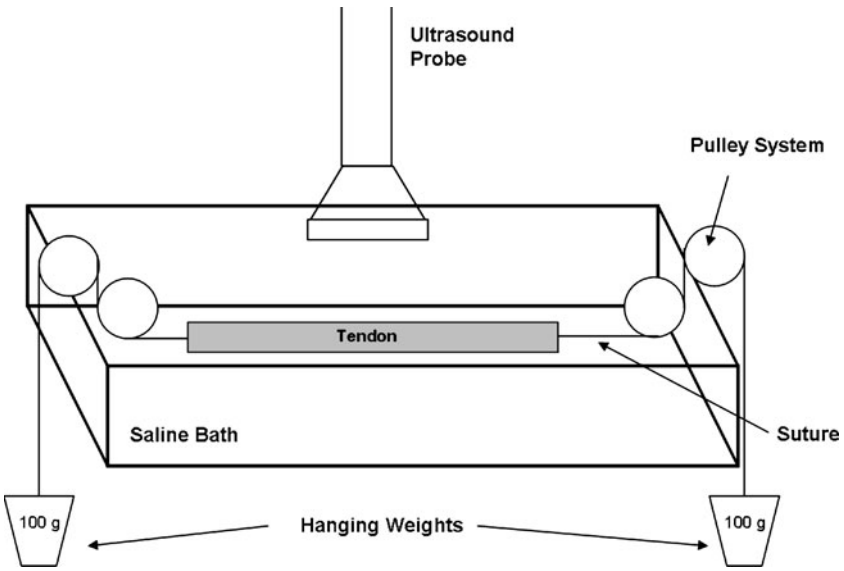


**Fig. 1** In vitro testing setup. The tendon is positioned horizontally in a saline bath with load applied through pulleys and hanging weights. The ultrasound probe is located parallel to the tendon just below the saline surface

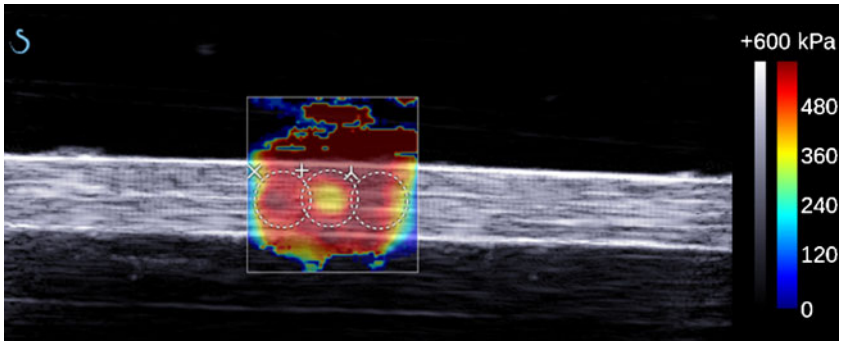


**Fig. 2** Representative in vitro image of a rusine tendon. Local tendon stiffness is estimated at each pixel within the square measurement region and represented by the color map. The average tendon stiffness is computed from the local stiffness values within each circular region of interest

1 cm distal to the insertion. Stiffness was calculated within a circular region of interest centered within the acquisition box.

*Statistics* To assess repeatability, the intra-class correlation coefficient (ICC) was determined for both the in vitro and in vivo conditions. For the in vitro condition, an ICC value was calculated across the two trials for each tendon and loading condition. This resulted in four ICC values for each tendon (one for each loading condition), which were then averaged (using Fisher's z-transform) across loads to determine an ICC for each tendon. Since these were all forelimb tendons similar in size, the averaged ICC values for each tendon were lastly averaged across tendons to obtain a global in vitro ICC. For the in vivo condition, an ICC was calculated for each combination of tendon, side, and testing time point. A mean ICC for each tendon on the right and left was then calculated by averaging the Fisher's z-transformed ICC values across the four time points and back transforming the average z. Finally, a global tendon ICC was determined by averaging the right and left ICC values for each tendon the same way. ICC values of 0–0.2 were considered poor, 0.21–0.4 fair, 0.41–0.6 moderate, 0.61–0.8 good and 0.81–1.0 very good [13].

## Results

*In vitro assessment* The average ICC value for each of the four forelimb tendons were 0.93, 0.64, 0.88, and 0.84, which ranged from "good" to "very good" repeatability (Table 1). The global ICC value was 0.85, which is considered "very good" repeatability.

*In vivo assessment* The average ICC values for each tendon varied across tendons as well as between the right and left sides of the same tendon (Table 2). The Achilles tendon had "poor" repeatability on the right side (0.17 ICC), but "good" repeatability on the left (0.62 ICC). The Achilles tendon global ICC was considered "moderate" at 0.42. Repeatability of stiffness estimates also varied between the right (0.45 ICC) and left (0.67 ICC) sides of the patellar tendon, but demonstrated better repeatability than the Achilles with a "moderate" global ICC of 0.57. Repeatability for the FPL tendon was considered "good" on both the right and left sides, with a global ICC of 0.73. The quadriceps tendon demonstrated the most consistent repeatability between right (0.72 ICC) and left (0.77 ICC) sides, as well as the highest global ICC (0.75).

In addition to differences between tendons and sides in the average ICC values, there was also considerable variability in the range of ICC values between tendons and sides. For the Achilles tendon, the ICC values ranged from fair (0.31) to very good (0.82) on the left, and from poor (0.002) to fair (0.37) on the right. ICC values for the patellar tendon ranged from moderate (0.40) to very good (0.90) on the left and from fair (0.23) to good (0.74) on the right. For the quadriceps tendon, the ICC values ranged from good (0.67) to very good (0.81) on the left and similarly from good (0.61) to very good

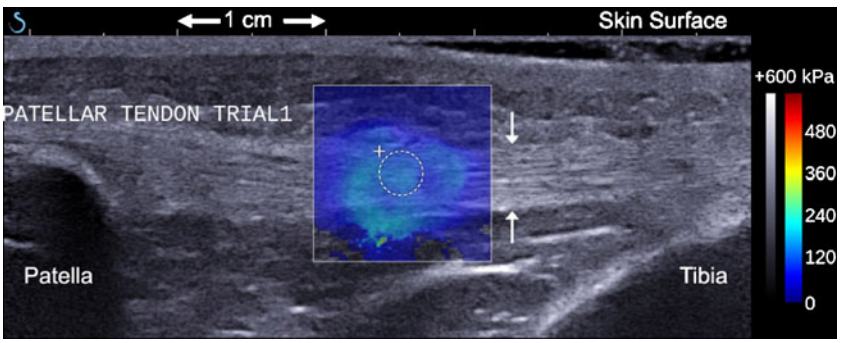


**Fig. 3** Representative in vivo image of a patellar tendon. Local and average tendon stiffness are determined as in the in vitro image. The superficial and deep surfaces of the patellar tendon are identified with arrows. The location of the tendon's insertion onto the patella is noted as well as the standardized distance from that bony landmark to the ROI

**Table 1** Each in vitro ICC value was calculated from two trials across three locations for each tendon and loading condition. Average and Global average ICC values represent those calculated using Fisher's z-transform

| Loading condition (g) | Rusine tendon # | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 100 | 0.99 | 0.35 | 0.79 | 0.80 |
| 200 | 0.95 | 0.52 | 0.95 | 0.87 |
| 300 | 0.97 | 0.93 | 0.96 | 0.79 |
| 400 | 0.35 | 0.42 | 0.60 | 0.89 |
| ICC range | 0.35–0.99 | 0.36–0.93 | 0.60–0.96 | 0.79–0.89 |
| Avg ICC | 0.93 | 0.64 | 0.88 | 0.84 |
| global avg ICC | 0.85 | | | |

(0.87) on the right. Finally, ICC values for the FPL ranged from moderate (0.43) to good (0.74) on the left and from good (0.61) to very good (0.92) on the right.

## Discussion

As we hypothesized, the repeatability of shear-wave elastography under in vitro testing conditions was better than in vivo testing conditions. Surprisingly, we also found that the in vivo repeatability varied between tendons. Specifically, the average repeatability of the quadriceps (0.75 ICC) and FPL (0.73 ICC) tendons were closer to that of the in vitro tests (0.85 ICC), while the Achilles (0.42 ICC) and patellar (0.57 ICC) tendons had appreciably lower measures of repeatability. Not only did the quadriceps and FPL tendons have the highest average ICC values, they had the smallest range of ICC values over the testing time points. Repeatability is influenced by a variety of factors, but the most likely explanation for low in vivo repeatability is that it can be difficult to maintain a consistent imaging location when both the probe and subject/tendon are allowed to move (as opposed to the in vitro testing conditions where the probe and tendon were in fixed positions). In addition, although every effort was made to maintain a consistent joint position across all four testing sessions, it is possible that subtle differences in joint position between testing sessions may have affected the passive stiffness of the Achilles and patellar tendons. Variations in subjects' activity levels prior to testing could also contribute to variability, although every effort was made to keep these consistent for each subject.

Although this technology was originally developed for use in breast imaging, it has been used in several areas recently including liver, muscle, and other soft tissues [10–12, 14, 15]. Arda and colleagues assessed the stiffness of a variety of soft tissues using shear wave elastography, including several muscles and the Achilles tendon [14]. Although they did not indicate the accuracy or repeatability of the measurement technique, they reported a coefficient of variation for the Achilles tendon of 49 %. This high variation could indeed stem from difficulties in maintaining a standardized testing position with such an anatomically complex tendon. The system used in the current study was also investigated by Kot and colleagues for effects of altering various technical settings [15]. They suggest that it is important to use the lightest possible transducer pressure, use a short acquisition time and measure the mean stiffness regardless of the ROI size. In the current study, care was taken to always use the lightest pressure on the transducer, all technical parameters (such as acquisition time) were kept

**Table 2** Each in vivo ICC value was calculated from two trials across 12 subjects for each tendon, side, and time point. Average and global average ICC values represent those calculated using Fisher's z-transform

| Testing day | Testing time point | Achilles | | Patellar | | Quadriceps | | FPL | |
|---|---|---|---|---|---|---|---|---|---|
| | | Left | Right | Left | Right | Left | Right | Left | Right |
| 1 | 1 | 0.54 | 0.19 | 0.90 | 0.45 | 0.67 | 0.67 | 0.74 | 0.92 |
| | 2 | 0.31 | 0.002 | 0.49 | 0.74 | 0.80 | 0.61 | 0.43 | 0.61 |
| 2 | 1 | 0.66 | 0.11 | 0.40 | 0.24 | 0.79 | 0.87 | 0.76 | 0.84 |
| | 2 | 0.82 | 0.37 | 0.67 | 0.23 | 0.81 | 0.67 | 0.54 | 0.71 |
| ICC range | | 0.31–0.82 | 0.002–0.37 | 0.40–0.90 | 0.23–0.74 | 0.67–0.81 | 0.61–0.87 | 0.43–0.76 | 0.61–0.92 |
| Avg ICC | | 0.62 | 0.17 | 0.67 | 0.45 | 0.77 | 0.72 | 0.64 | 0.80 |
| Global tendon Avg ICC | | 0.42 | | 0.57 | | 0.75 | | 0.73 | |

standard for every procedure, and the outcome measure used to determine ICC was the mean stiffness.

However, these studies have not attempted to investigate the repeatability of the system for these applications and it is therefore difficult to compare the results of the current study to previous studies. Investigations in breast tissue with this technology have been on-going for some time and repeatability of the system has been assessed for that tissue. Evans and colleagues assessed both intra-and inter-user repeatability in breast imaging and found good to very good repeatability [8]. However, the best repeatability (ICC 0.99) was obtained when the stiffness was measured by two different users on the same image. When two separate images were obtained from two different users, the ICC value dropped to 0.80, which is within the range of the in vitro values and in vivo values for the FPL and quadriceps tendon reported here.

While the repeatability was very good in vitro and good for the FPL and quadriceps tendon in vivo, the system was not as repeatable for the Achilles and patellar tendons. It is possible that inherent properties of these tendons may have contributed to the lower repeatability found in this study. Specifically, it is plausible that the underlying fiber architecture of these tendons (and in particular, the Achilles tendon which involves the blending and rotation of the medial gastrocnemius, lateral gastrocnemius, and soleus tendons at the calcaneal insertion site) may have contributed to the increased variability between testing sessions. While the patellar tendon does not share this complex fiber architecture, it also is different from other tendons in that it connects bone to bone and therefore a greater portion of the tendon is in proximity to bony insertion sites. It is possible that this anatomical feature may result in a tendon with a greater range of stiffness values and therefore make the tendon more susceptible to subtle changes in the imaging area that occur between trials or testing sessions. Given that there is significant clinical interest in both the Achilles and patellar tendons, future efforts should evaluate strategies for improving the repeatability of this measurement technique for these tendons.

An important limitation of this study was the use of only one operator who was not a trained sonographer. Although the manufacturer's literature states that the technique is "user-skill-independent", it is possible that repeatability may in fact be higher when performed by a trained sonographer. Therefore, the data reported here likely represent the lower bounds of repeatability. Furthermore, because of this limitation, the extent to which this approach may be useful in clinical practice remains unclear. However, the assessment of intra-user repeatability is a necessary and important first step before progressing to inter-user repeatability. Subjects were also not screened for pre-existing injuries other than being verbally asked an injury history. However, the study period for each subject was only 3 days and we do not expect any changes in stiffness during that period even if an injury was present.

Overall, this assessment of shear-wave elastography demonstrated fair repeatability for the Achilles tendon, moderate repeatability for the patellar tendon, and good repeatability for the quadriceps and FPL tendons. These data suggest that shear-wave elastography may be useful clinically to non-invasively assess changes in tendon stiffness over time as a method for monitoring the functional capacity of healing tendons such as the quadriceps or FPL, but for tendons where repeatability is moderate or low this technique may be better suited as a diagnostic imaging tool for identifying the location of specific pathologic lesions with tendons. In order for this approach to achieve widespread clinical acceptance for monitoring soft-tissue healing, future research efforts should focus on rigorously characterizing the effect of operator experience on measurement repeatability, as well as the accuracy and sensitivity of shear wave elastography for detecting subtle changes in tendon stiffness associated with injury and/or treatment.

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