

● *Original Contribution*

## A COMPARISON OF THREE-DIMENSIONAL ULTRASOUND, TWO-DIMENSIONAL ULTRASOUND AND DISSECTIONS FOR DETERMINATION OF LESION VOLUME IN TENDONS

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(Received 12 October 2005, revised 13 February 2006, in final form 23 February 2006)

**Abstract**—The purpose of this work was to evaluate the accuracy and precision of a freehand three-dimensional (3-D) ultrasonography system in the determination of lesion volume in tendons. The accuracy and precision of a 3-D ultrasonography system was assessed by performing repeated measurements on a phantom of known volume. Volume measurements of tendon lesions performed with 3-D ultrasonography were compared with measurements based on a series of two-dimensional (2-D) ultrasound (US) scans and to direct measurements from dissections. A novel method for the creation of tendon lesions *in vitro* was developed. 3-D US showed excellent precision and accuracy in measurements of the phantom (mean measured volume = 3.76 mL, calculated volume = 3.77 mL, coefficient of variation (CoV) = 0.54%) and good repeatability in the determination of tendon lesions (repeatability coefficient = 0.00047). All three methods examined were repeatable (repeatability coefficient for 2-D US = 0.00032, repeatability coefficient for dissections = 0.00076). However, each of the methods produced different results and no constant relationship could be found between any of the measurement methods. Both 3-D and 2-D US proved to be repeatable techniques for the measurement of the volume of a tendon lesion. Even if they produced different results, each of them can be repeatedly used individually. It was not possible to define which one provided the most accurate value as a result of difficulties encountered in lesion identification on histology, and therefore the lack of a gold standard. (E-mail: [mferrari@rvc.ac.uk](mailto:mferrari@rvc.ac.uk)) © 2006 World Federation for Ultrasound in Medicine & Biology.

**Key Words:** 3-D ultrasonography, Superficial digital flexor tendonitis, Tendon lesion.

### INTRODUCTION

Achilles tendon injuries are among the more common serious conditions seen by sports medicine physicians (Schepisis et al. 2002). Evaluation of these injuries is needed to inform prognosis, treatment and healing. The two imaging techniques that best demonstrate abnormalities within the Achilles tendon are ultrasonography and magnetic resonance imaging (MRI) (Schepisis et al. 2002). MRI is widely used but expensive and not readily available at short notice for repeated assessments. Ultrasonography is much less expensive than MRI and the equipment is widely available (Laine et al. 1991; Schepisis et al. 2002). It is, however, very examiner-dependent, and reliability appears to correlate with the experience of the examiner. Quantification of soft tissue damage for

evaluation of healing over time is particularly difficult with existing methods (Schepisis et al. 2002).

Quantitative evaluation of lesions using two-dimensional (2-D) ultrasound (US) relies on calculation of single-plane cross-sectional areas. This leads to the evaluation of only one section of the lesion, usually the biggest area of injury. This may result in difficulties and imprecision in monitoring the healing process, especially if the lesion is irregular in shape because repeated measurements over time may not target precisely the same section, particularly if the lesion changes shape during healing. With conventional 2-D ultrasonography, the operator must mentally integrate multiple images to reconstruct three-dimensional (3-D) anatomy. Although this is conceptually possible for the experienced practitioner, it is a subjective evaluation and does not provide a quantifiable record (Downey et al. 2000; Kurjak et al. 2000). Visualising tendons and their lesions in 3-D is a prerequisite to performing volume measurements, which can be used to quantitatively monitor the healing process. 3-D

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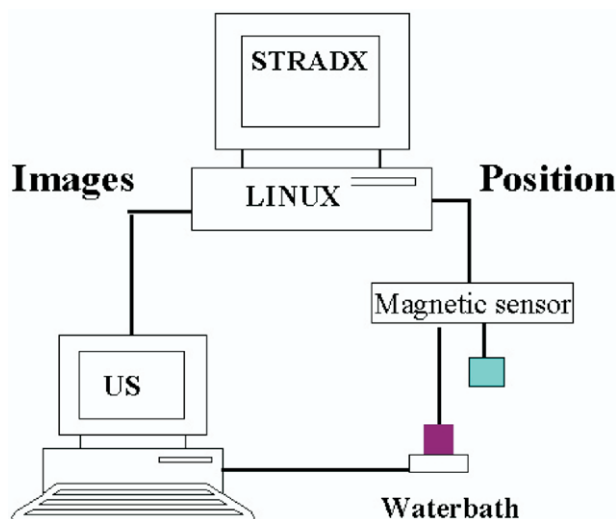


Fig. 1. Schematic drawing of a free-hand, real-time 3-D ultrasonography set-up. US images are acquired with a standard US machine and transmitted to a computer *via* an image capture card. The movement of the US transducer is recorded by a six-degrees-of-freedom magnetic tracking device. Image and position information is integrated by STRADX, a dedicated, Linux-based computer program.

images are highly illustrative and thus could be useful for explaining pathology to patients and for teaching purposes.

A freehand real-time 3-D US system uses a stack of conventional 2-D ultrasound images in conjunction with a tracking device to determine position and orientation of the US transducer in space. Dedicated software is used to integrate the position/orientation information provided by the position sensor with the stack of images from the US machine (Prager et al. 1999).

Here we consider a tracked free-hand 3-D real-time ultrasonography system that uses a conventional 2-D US machine in conjunction with a six-degrees-of-freedom magnetic sensor to determine the position and orientation of the transducer in space during scanning (Fig. 1). The major sources of error in 3-D reconstruction are the scanning procedure itself and the postscanning segmentation process that outlines the area of interest. Although the raw image can be readily displayed as 2-D slices, 3-D analysis and visualisation requires explicitly defined object boundaries. To create a 3-D object from a stack of images, the area of interest needs to be identified within the images and its boundary outlined. The pixel detection process is called image segmentation, which identifies the attributes of pixels and defines the boundaries for pixels that belong to the same group. Quantitative analysis for area, perimeter, volume and length can be obtained easily when object boundaries are defined and the object is rendered in 3-D.

The precision and accuracy of the system for the determination of lesion volume in tendons was examined. The hypothesis that 3-D US is superior to 2-D US in estimating volume of tendon lesions is tested.

## MATERIAL AND METHODS

### Preparation

Six animal cadaver tendons were used in this study. Equine digital flexor tendons were chosen because they have a similar cross-sectional area (CSA) and ultrasonographic appearance to Achilles tendons in human athletes and are more readily available than human tendons. Thirty centimeter-long pieces of tendon were harvested from the metacarpal region of six equine right front legs, where the tendons are of uniform CSA. The horses had been euthanised for reasons unrelated to this study or to musculoskeletal pathology.

### Creation of lesions

Lesions were induced in each of the tendons using 0.1 mL of collagenase solution (5 mg/mL, cat no. C-2139, Sigma Aldrich, Gillingham, UK).

Collagenase is a metalloendoproteinase which cleaves collagen into smaller peptide fragments. The injection of collagenase in the centre of the SDFT mass results in rapid fiber destruction, cellular necrosis, vascular damage, hemorrhage and inflammation, simulating many aspects of a naturally occurred traumatic lesion (Williams et al. 1984).

Tendons were preheated in a water bath at 37°C and the collagenase solution was injected with a 23G × 1-inch needle from one end of the tendon, along the direction of the fibers.

Tendons were then incubated in a water bath at 37°C for 17 h.

Development of the lesion was monitored ultrasonographically in a single tendon (Fig. 2).

### Ultrasound examination

Each tendon was placed in a water bath set to room temperature. In both 2-D and 3-D US data collection a 7.5-MHz linear transducer was used (Ausonics, Impact VFI, BCF technology, Livingston, UK).

### 3-D ultrasonography

A six-degrees-of-freedom magnetic tracking system (Minibird, Ascension Technology, Burlington, MA, USA) was attached to the US transducer. The magnetic tracking system consists of a transmitter, which generates a magnetic field, and a sensor which was attached to the US transducer with electrical tape. Metal objects cause distortion of the magnetic field, resulting in errors in position determination, thus the transmitter was

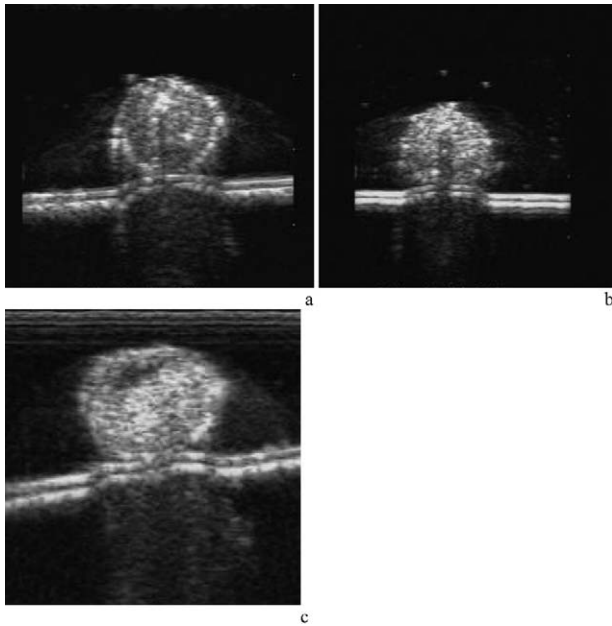


Fig. 2. Creation of the lesion. Appearance of tendon lesion at (a) 1 h; (b) 2 h; and (c) 17 h after injection.

mounted on a wooden and plastic frame and the water bath was put on a wooden table. A Linux-based, dedicated software (STRADX, Medical Imaging Group, Cambridge University Engineering Department) was used to integrate the US images with the positional information from the magnetic tracking device to compute 3-D US images in real time (Fig. 1). US images were transferred from the US machine to the computer via a Linux-supported video acquisition card (Hauppauge WinTVGo, Hauppauge ComputerWorks, Inc., Hauppauge, NY, USA). Before scanning, the system was calibrated, following the calibration protocol as recommended by the software developers (Prager *et al.* 2003).

**Phantom US scans.** A plastic rod with a diameter of 0.5 cm and length of 19.2 cm (volume = 3.77 mL) was used as a phantom. The phantom was scanned 11 times in the transverse plane along its long axis. The images were then segmented manually and the volume of the phantom calculated by the software. The scans were performed in a water bath.

**Tendon US scans.** Each tendon was scanned in the transverse plane along its long axis. This process was repeated three times per tendon by the same operator. The tendon and the lesion within the tendon were then segmented and surface rendering performed. This enabled visualisation of the shape and position of the lesion within the tendon and the subsequent determination of its volume (Fig. 3, 4). Segmentation and subsequent volume measurement was repeated 10 times on each of the scans.

### 2-D ultrasonography

Each tendon was divided into 4-cm-long segments. Each segment was scanned in transverse and longitudinal planes. US data were recorded on a digital video camera (Digital PC100, Sony, Tokyo, Japan) at 25 Hz. Images were downloaded to a computer using Pinnacle Studio (version 7.01.3, Pinnacle Systems Inc., Mountain View, CA, USA). Cross-sectional area of the lesion in each frame and length of the lesion in each segment was measured using Image Tool (v. 3.0 UCTHSA, University of Texas Health Sciences Center, San Antonio, TX, USA). Volume was calculated as the product of the average of largest and smallest cross-sectional area in each segment times the length of the lesion from the longitudinal scan and then summing the result of each segment (Fig. 5). Segmentation and subsequent volume measurement was repeated 10 times for each tendon lesion.

**Dissection.** Tendons were cut into 3-mm-thick transverse slices and scanned with a flatbed scanner (SnapScan 1236, AGFA-Gevaert, Brentford, UK). A cross-sectional area of the lesion was measured using Image Tool, and the volume was calculated by multiplying each cross-sectional area by the slice thickness (3 mm) and summing the results. The measurement process was repeated 10 times for each tendon.

### Data analysis

Data resulting from the 10 scans of the plastic rod (phantom) were used to test the accuracy and precision of the 3-D US scanning technique.

The coefficient of variation (CoV, standard deviation as proportion of the mean) was used as a measure of precision, whereas accuracy was evaluated by comparing the calculated volume to the measured volume.

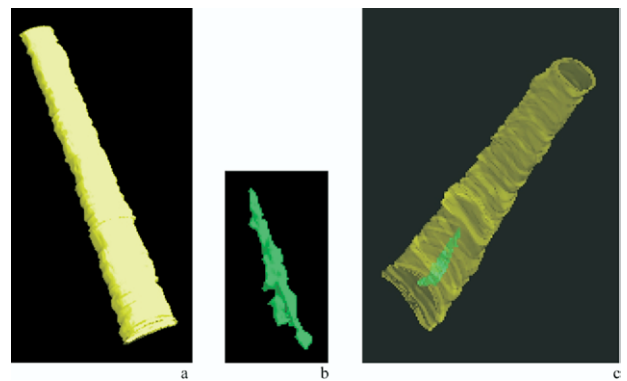


Fig. 3. 3-D visualisation of (a) a reconstructed tendon (palmarodistal aspect); (b) an irregularly shaped lesion within this tendon; and (c) the same tendon partially shaded with the lesion inside (dorsoproximal aspect).

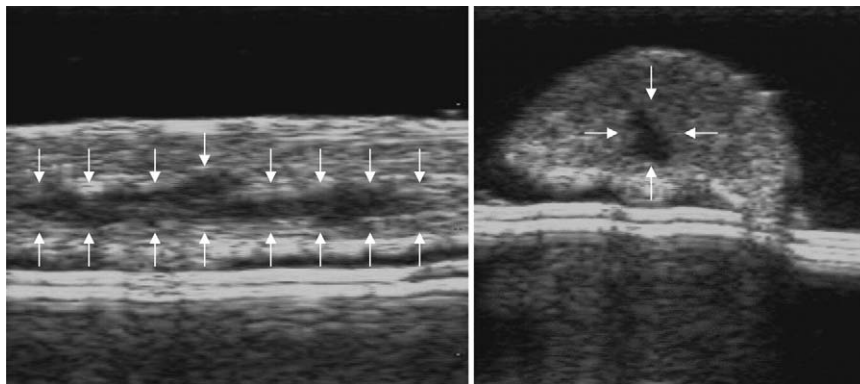


Fig. 4. B-mode longitudinal and transverse US images correspondent to the 3-D reconstructions shown in Fig. 3. A hypoechoic lesion is delimited by arrows.

Data resulting from the three scans performed on each of the six tendons were used to evaluate the precision of the 3-D US technique while scanning tendons by calculating the CoV.

Precision of the volume calculations resulting from 3-D US, 2-D US and dissections was assessed by repeating the volume calculations 10 times on the same scan for each of the six tendon lesions. This assessed the

ability of the operator to identify lesion boundaries (“segment”). As measures of repeatability the CoV, the average variance and the within-lesion standard deviation ( $s_w$ ) were calculated. The repeatability coefficient ( $2.77 \times s_w$ ) was determined for each method (British Standards Institution 1975) after assessing the assumption that the data were normally distributed.

The average measurements obtained by the three

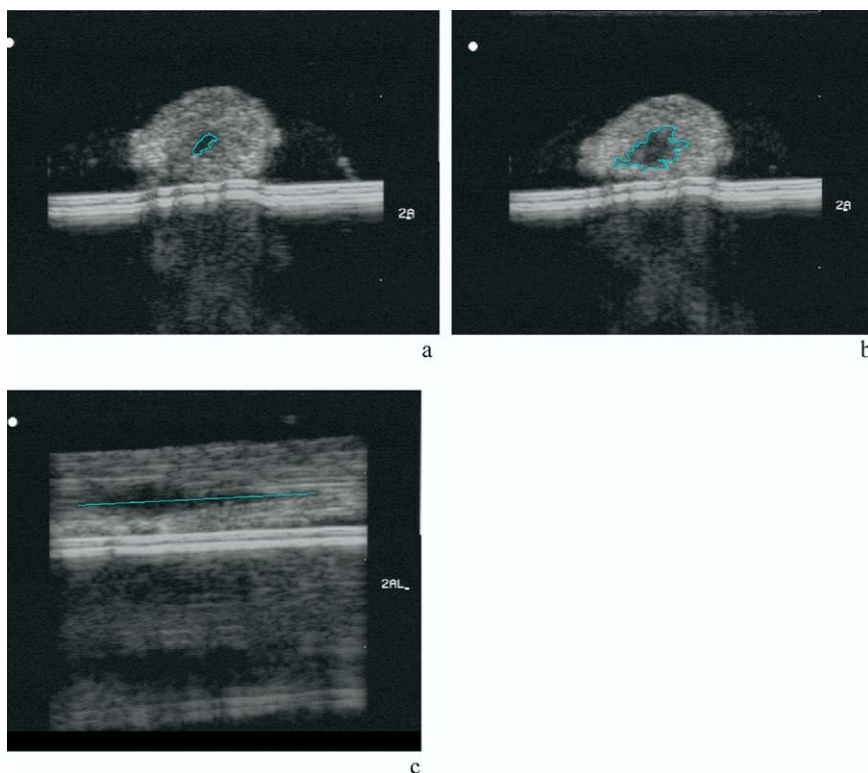


Fig. 5. Volume calculation using 2-D US. The (a) smallest and (b) largest cross-sectional areas of each lesion were identified by the operator and segmented. The average of the resulting values was then multiplied by the length of the lesion, as seen from the longitudinal scan (c).

Table 1. Repeatability of 3-D, 2-D US and dissections in the determination of tendon lesion volume

	3-D ultrasonography	2-D ultrasonography	Dissection
Range of the repeated measurements (mL) for each lesion	0.03–0.06	0.02–0.04	0.03–0.20
Range of CoV (%)	4.1–12.6	2.6–13.3	5.6–18.4
Average of variances (mL <sup>2</sup> )	0.00017	0.00015	0.00076
Within-tendon standard deviation (mL)	0.013	0.011	0.026
Repeatability coefficient (mL)	0.00047	0.00032	0.00210

different methods were compared by determining the 95% limits of agreement between each pair of methods (Bland and Altman 1986). The distribution of the differences between the methods was evaluated by plotting histograms and calculating the Kolmogorov-Smirnov statistics. The relationship between the differences and the mean was evaluated by plotting scatterplots. Once the assumptions of normality and independency of the mean for the differences were satisfied, the mean and standard deviation of these differences ( $s_d$ ) were determined. The 95% limits of agreement were calculated as mean difference  $\pm 1.96 \times$  standard deviation of the differences (Bland and Altman 1986).

Statistical analysis was done in SPSS 13.0 for Windows.

## RESULTS

### Creation of lesions

Collagenase solutions of different concentrations and volumes were tested before the start of the study. Injection of 0.1 mL of a 5-mg/mL solution was found to render the most satisfactory results in terms of localisation and extent of the lesion, resembling the appearance of lesions under clinical circumstances. Higher volumes of collagenase led to surface leakage; lower concentrations of collagenase (1 mg/mL) were ineffective. Development of the lesion was monitored in a single tendon. After one hour, a hyperechoic area appeared at the site of injection, which persisted for four hours, to be replaced by a hypoechoic area after 17 h (Fig. 2).

### 3-D ultrasonography

*Phantom measurements.* Accuracy and repeatability of 3-D US scanning procedure on phantom measurements were assessed.

The mean volume measured from 11 successive scans was 3.76 mL (calculated volume 3.77 mL) with a range of 3.74 to 3.79 mL, a standard deviation of  $\pm 0.02$  mL and a CoV of 0.54%.

### Repeatability of volume measurements on tendon lesions

Based on 3-D US measurements, the six lesions ranged in volume from 0.079 to 0.204 mL with a mean

lesion volume of 0.157 mL and a standard deviation of 0.048 mL. Segmentation was repeated ten times for each lesion and the range of the repeated measurements for each lesion was between 0.03 and 0.06 mL independent of the size of the lesion. The CoV ranged from 4.1 to 12.6%, and the average of the standard deviations from the 10 segmentations was  $\pm 0.012$  mL. The within-tendon standard deviation was 0.013 mL, resulting in a repeatability coefficient of 0.00047 mL (Table 1). The least repeatable measurements were obtained from tendons 4 and 5.

The scanning procedure was repeated three times for each tendon. The range of measurement variation for each lesion was between 0.02 mL and 0.05 mL, and the repeatability coefficient 0.00048 mL.

### 2-D ultrasonography

Based on 2-D US measurements, the six lesions ranged in volume from 0.065 to 0.279 mL with a mean lesion volume of 0.141 mL and a standard deviation of 0.0033 mL. Segmentation was repeated 10 times for each lesion, and the range of the repeated measurements for each lesion was between 0.02 and 0.04 mL, independent of the size of the lesion. The CoV ranged from 2.6 to 13.3% and the average of the standard deviations from the 10 segmentations was  $\pm 0.01$  mL, and the within-tendon standard deviation was 0.012 mL, resulting in a repeatability coefficient of 0.00032 mL (Table 1). In both 2-D and 3-D US techniques, the tendons with the lowest

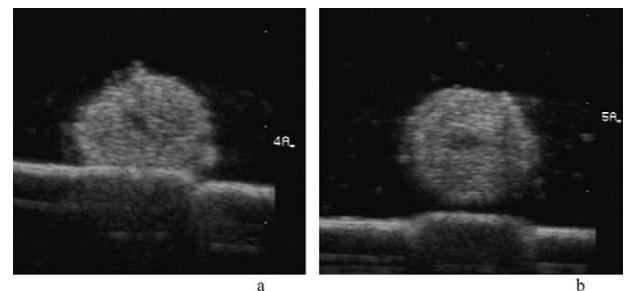


Fig. 6. Lesions in tendon 4 (a) and 5 (b) were the least defined on US. Segmentation was therefore difficult and affected the repeatability of the method.

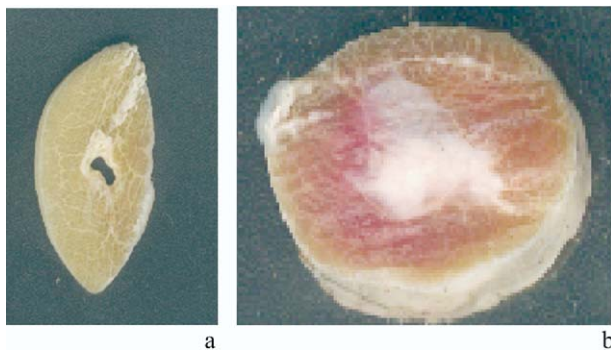


Fig. 7. Some lesions presented as (a) complete loss of tendon tissue; or (b) like an area of white discoloration. This area had a soft consistency.

repeatability of measurements were tendons 4 and 5. Tendons 4 and 5 presented with the least well defined lesions (see Fig. 6). Segmentation was therefore difficult and affected the repeatability of the method.

#### Dissection

Some lesions presented as complete loss of tendon tissue or like an area of white discoloration. This area had a soft consistency (Fig. 7). The extent of discoloration was often hard to establish. The loss of tissue corresponded to the anechoic areas seen on US, whereas the discolored area did not seem to match the hypoechoic area seen on US.

From direct measurements on the sections, the six lesions ranged in volume from 0.095 to 0.324 mL, with a mean lesion volume of 0.182 mL and a standard deviation of 0.0846 mL. Measurements were repeated 10 times on each lesion and the range of the repeated measurements for each lesion was between 0.03 and 0.20 mL, regardless of the size of the lesion. The CoV ranged from 5.6 to 18.4%; the average of the standard deviations from the 10 measurements was  $\pm 0.021$  mL. The within-tendon standard deviation was 0.028 mL, resulting in a repeatability coefficient of 0.00210 mL (Table 1). In this case, the tendons with the lowest repeatability of measurements were tendons 2 and 3.

#### Comparison of 3-D ultrasonography, 2-D ultrasonography and dissections for the determination of tendon lesion volume

The differences between 3-D and 2-D ultrasonography data had a mean of 0.015 mL and a standard deviation of 0.105 mL. Hence, the 95% limits of agreement were  $0.015 \pm 1.96 \cdot 0.105 = -0.19$  and  $0.015 + 1.96 \cdot 0.105 = 0.22$ . Hence a measurement by 3-D ultrasonography could be anywhere between  $-0.19$  mL less than a measurement by 2-D ultrasonography and 0.22

mL greater. The width of the 95% limits of agreement was 0.41 mL.

Comparison between 3-D ultrasonography and dissections resulted in a mean difference of  $-0.022$  mL with a standard deviation of 0.094 mL. The 95% limits of agreement are  $-0.21$  mL and 0.16 mL, with a width of 0.37 mL.

The mean difference between 2-D ultrasonography and dissections was  $-0.037$  mL with a standard deviation of 0.133 mL. The 95% limits of agreement are  $-0.30$  and 0.22, resulting in a width 0.52 mL.

In four of the six tendons 2-D ultrasonography rendered a smaller measurement than the other two methods; in the case of tendon two, however, it resulted in a volume that was almost double the average of the other two methods. Direct measurements on dissections resulted in the largest volume measurement in three of the six tendons. The measurement of tendon 3 was more than double the average of the other two methods. 3-D US measurements were between the other two methods in three of the six tendons, larger than the other two methods in two tendons and smaller in one tendon. There was no constant relationship between any of the measurement methods (Fig. 8).

## DISCUSSION

Tendon lesion volume as a proportion of whole tendon volume is a potential parameter of functional deficit that could be used in the initial diagnosis and following monitoring of tendon pathology. Volume can be calculated in 3-D reconstructions, whereas it can only be approximated in 2-D US, *i.e.*, the lesion is considered to be a cylinder. 3-D reconstruction would also allow the practitioner to visually monitor the evolution of the lesion in its entirety rather than considering separate slices.

The 3-D US freehand method can be performed with every transducer in clinical use.

Although methods for creating collagenase-induced lesions have been described in the literature for *in vivo* studies (Williams et al. 1984; Gaughan et al. 1991; Micklethwaite et al. 2001), no method was available for *in vitro* studies. We therefore developed a method for creating lesions in normal cadaveric tendon to provide material for the study. It was easier to create lesions in the deep digital flexor tendon (DDFT) than in the superficial digital flexor tendon (SDFT) because of its round shape and bigger size. Even with the optimal amount and concentration of collagenase, difficulties were encountered with the SDFT in the areas where it was particularly thin. We solved this by heating the waterbath to body temperature until the lesion was achieved, then reducing the waterbath to room temperature to prevent further changes.

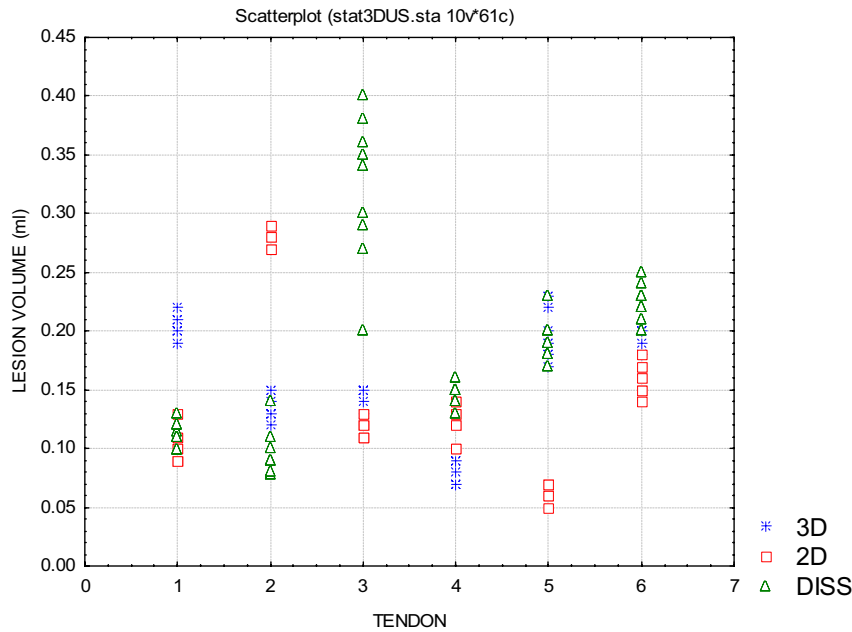


Fig. 8. The graph shows the volume of the lesions measured in each of the six tendons with the three methods. Measurements were performed 10 times on each of the lesions with every method. Stars = 3-D US; boxes = 2-D US; triangles = dissections. Equal values are represented by overlapping markers.

The 3-D US scanning technique was very repeatable and accurate when performed on the phantom, but less repeatable yet still within acceptable limits when performed on tendons. This was mainly because of difficulties during the segmentation process. Tendon lesions were not always well defined and thus results were affected by the ability of the operator to identify the lesion. It was easy to segment a well-defined anechoic lesion, whereas it was hard to segment a diffuse hypoechoic area. Because the lesions were relatively small, an error in segmentation even in a single frame, depending on the number of scans per volume, could significantly affect the resulting volume calculation. The STRADX software has an interpolation function, which decreases the number of frames required for segmentation. However, segmentation of more frames results in higher accuracy in irregularly shaped objects and, thus, a trade-off exists between accuracy and postprocessing time. Semiautomatic segmentation based on pixel brightness resulted in a distorted image reconstruction. This was the result of the inability of the system to pick up the slight differences in echogenicity (and hence pixel brightness) between tendon lesion and surrounding tendon tissue. This was especially true of lesions that presented as hypoechoic areas with less well-defined borders. Due to these software limitations, segmentation was performed manually on each frame in the data set, thus postprocessing was time consuming and could limit the usefulness of this method of 3-D ultrasonography in a clinical setting.

However, advances in automated segmentation may well lead to the development of a more user-friendly software in the near future.

Volume measurements based on 2-D ultrasonography rely on segmentation of the lesion before measurement; thus, the same segmentation-related problems were encountered as described above for 3-D ultrasonography. Volume determination using 2-D ultrasonography also relies on the geometric assumptions that the lesions approximate to cylinders or truncated cones. This is an additional source of error that could affect volume measurement, particularly during monitoring of the healing process, which could follow a nongeometric pattern.

Determination of lesion volume from dissections was also complicated by difficulties in segmentation. Although some lesions presented as complete loss of tendon tissue, others presented as discoloration of the tendon tissue; most of the tendons presented as a combination of both with a central loss of tissue and a surrounding halo of discolored tissue. The extent of the discoloration was often hard to establish, thus segmentation was difficult and dissections were found to be the least repeatable method. Although the loss of tissue corresponded to the anechoic areas seen on US, the discolored area did not seem to match the hypoechoic area seen ultrasonographically.

Both 3-D and 2-D US proved to be repeatable techniques for the measurement of the volume of a

tendon lesion. Even if they produced different results, each can be reliably used individually.

It was not possible to define which one produced the most accurate value as a result of difficulties encountered in lesion identification on histology and, therefore, the lack of a gold standard.

It is our opinion that 3-D US would be superior to 2-D US in monitoring the evolution of a lesion over time for the reasons above. However, more studies will be needed to test the technique in a clinical setting.

*Acknowledgments*—Medical Imaging Group, Department of Engineering, University of Cambridge for the STRADX program.

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