ON THE ULTRASONIC PROPERTIES OF TENDON

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Abstract—The strong dependence of tendon echogenicity on insonation angle is explored by analyzing echo spectra. Combining echo spectra with high-resolution images from several modalities reveals that fluid spaces surrounding fascicles and bundles are likely sources of ultrasonic scatter. Mathematical models of tendon structure are proposed to explain how the anisotropic microstructure of tendon gives rise to angle-dependent echogenicity. Echo spectra from spontaneously damaged equine tendon samples were compared with normal equine tendon and found to exhibit a dramatic decrease in anisotropic properties that appears to be related to the spatial organization and type of collagen generated during repair. Variation in echo spectra with insonation angle is a robust indicator of mechanical damage. (E-mail: wjhornof@ucdavis.edu) © 2003 World Federation for Ultrasound in Medicine & Biology.

Key Words: Anisotropy, Attenuation, Backscatter, Fascicles, Rupture, Signal models.

INTRODUCTION

Sonograms of normal tendon clearly show B-mode echogenicity to be highly dependent on insonation angle. Angle-dependent ultrasonic backscatter is a consequence of the anisotropic microstructure, in which submillimeter-diameter fascicles are uniformly oriented along the long axis of the tendon. Tendon appears most echogenic when the direction of sound wave propagation is normally incident to the long axis of the fascicles (Fig. 1a and c). However, small deviations from normal incidence result in significantly lower echo amplitude (Fig. 1b and d). We are investigating the relationship between tendon microstructure and the angle-dependent ultrasonic backscatter with an eye toward opportunities for detection of subclinical focal damage. Our investigation combines measurements and mathematical models of ultrasonic echo spectra with optical, magnetic resonance (MR), ultrasound (US) and electron microscopy images to help us understand how a minor disruption of normal tendon microstructure results in significant changes in echo amplitude. If detected early, minor structural damage can be successfully treated with conservative approaches, including treatment with anti-inflammatory medication, rest and ice with compression and/or massage. If left untreated, however, minor damage can es-
Healthy tendons contain densely packed collagen fibers (type I, 86% dry weight) that are organized into a hierarchy of structure that includes molecules, fibrils, fibers and fascicles. These structures are relatively uniform in cross-sectional diameter, spacing and orientation. Although the fascicles are regularly parallel, they have a crimped wavy appearance in the relaxed state on a scale smaller than 100 μm (Fung 1993). Published values on the size of bundle groupings vary, depending on species and location in the body, but the hierarchy and size range are generally as shown in Fig. 2 (Jozsa and Kannus 1997). The broken type I collagen fiber in damaged tendon heals over time by adding type III collagen and elastin scar tissue that is less spatially organized and prone to re-injury. The microstructure of tendons and ligaments found throughout the body and among species is similar, although ligaments generally have more elastin and higher type III collagen content.

Ultrasonic spectroscopy has been used to describe features of soft tissue microstructure throughout the body (Campbell and Waag 1983; Lizzi et al. 1997). Echo spectra are particularly sensitive to the aligned orientation of asymmetrical structures. Examples include myocytes in myocardium (Mottley and Miller 1988a, 1988b; Rose et al. 1995), fibers in skeletal muscle (Topp and O’Brien 2000) and aorta (Recchia et al. 1995), nephrons in kidney (Insana et al. 1991) and fibers in tendon (Tuthill et al. 1999). Given the high concentration of collagen in tendon fascicles responsible for the ultrasonic scattering, the spatial alignment of fascicles and the clinical observation of angle-dependent echogenicity, ultrasonic spectroscopy appears to be a promising tool for the noninvasive study of tendon structure.

The mean cross-sectional diameter of fascicles is smaller than the wavelength at frequencies less than 15 MHz and, thus, they are not resolved using conventional imaging techniques. However, if conditions are appropriate, ultrasonic spectroscopy provides accurate information about the mean size, number and orientation of fascicles with size below the resolution limit for imaging. The tissue properties required for accurate estimation of microanatomical features include 1. weakly stationary random scattering structure, such as is found in muscle (Levinson 1987) and kidney (Insana 1995) tissues; 2. a relatively dense concentration of scatterers, viz., more than 10 scatterers per ultrasonic pulse volume; and 3. prior knowledge of the scattering functions to select experimental parameters for the measurement. Prior knowledge includes the assumptions that 4. the tendon microstructure interacts weakly with the incident sound beam and 5. the distribution of correlation lengths in the random medium is known and relatively narrow (e.g., unimodal normal distribution of fascicle cross-sectional diameter with ratio of SD to mean of less than 0.25). When these conditions hold, the distribution of sizes is narrow and, for a large measurement band width (greater than 80% of the carrier frequency at −20 dB), the normalized echo spectrum is determined by the size, number density and orientation of scatterers whose diameter \( D \neq 0.8\lambda_0/\pi \) (Chaturvedi and Insana 1996), where \( \lambda_0 \) is the wavelength at the center frequency. For example, in tendon, a 3.5-MHz transducer with a 2.8-MHz band width produces an echo spectrum that is most sensitive to 112-μm diameter tendon structures. We did not estimate scatterer sizes in this study, although size is a key parameter determining features of echo spectra.

Ultrasonic spectroscopy is often considered to be a super-resolution technique because echo spectra describe statistical moments of microstructural distributions in the scan plane. These are not the narrow-band methods common in x-ray crystallography used to determine electron densities (these electron densities describe the size, shape and orientation of molecules). Rather, with broadband transmission, we localize a spatial region where the alignment of structures produces strong coherent scattering of amplitude that depends on the angle of incidence. Consequently, we do not expect to see strong peaks in the echo spectra or scalloping from resonances. Instead, we look for changes in how the backscattered energy is distributed over frequency as the incident angle varies to specify the degree of structural anisotropy.

This paper begins with a description of the spectral measurement and one-dimensional (1-D) signal models that link the underlying structure to measured echo spectra. Normal tendon spectra are presented that have been...
compensated for attenuation losses and the instrumenta-
response. These are matched to model spectra and
compared with measurements in damaged tendon and
known isotropic phantom media. We conclude by com-
paring US results with those from several imaging tech-
niques.

METHODS

Backscatter measurements

Equipment. The measurement apparatus (Fig. 3) com-
bines a scanning tank and transducer-positioning device.
The tank was designed to rotate the tendon sample by 360°
along its long axis at angle 0° with respect to the tendon. The transducer rotation
radius and focal length are equal.

Tendon samples and measurement procedures. Tendon
samples from horses submitted to the necropsy ser-
vise of the University of California, Davis, Veterinary
Medical Teaching Hospital for reasons other than tendon
injury, were obtained after humane euthanasia. Normal-
appearing deep digital flexor (DDF) and superficial di-
gital flexor (SDF) tendons were excised from the acces-
sory carpal bone to the distal pastern region. All samples
were collected from carcasses placed in a cooler imme-
diately after euthanasia, and within 3 days of euthanasia.
Tendon samples were carefully scanned sonographically
(ATAL HDI 5000, L.12–5 probe) to ensure that the sam-
ple was free of sonographically detectable lesions. If
spectroscopy measurements could not be made immedi-
ately, samples were frozen after excision and thawed at
room temperature before measurement. A damaged ten-
don from a 24-year-old Connemara gelding that was
euthanized for severe peritonitis was obtained. This
horse had severely damaged the SDF tendon 3 years
previously, resulting in a very large lesion involving
almost the entire cross-section of the tendon.

Each excised tendon sample was attached to the
rotator in Fig. 3 by gluing the ends (Superglue®) to
wooden dowels that were bolted to the gears. The dowels
were carefully adjusted so that the sample rotated about
its center. The tendon was taut but unstrained and sub-
merged in a water bath during data collection that took
approximately 1 h. Pure water was used instead of saline
after it was observed that no detectable swelling oc-
curred. The pivot for transducer rotation was designed to
be coincident with the center of the tendon axis and the
focal length of the transducer. Thus, the tendon axis
remained at the transducer focal length for all measure-
ments so that, at scanning angle 0 = 0°, the beam was
perpendicular to the tendon axis.

Echo data were recorded digitally at five positions
along the tendon axis, separated by 0 = 13 mm, one
transducer aperture width. At each axial position x_i (Fig.
3), where 1 = i = L and L = 5, 36 echo waveforms were
recorded following 10° rotations of the tendon, 0° < i < 360°
where 1 = j = J and J = 36. 10° rotations are
known to provide spatially uncorrelated waveforms (Hall
et al. 1993). Each N-point echo segment is labeled
\( g_{n,m,l} \), where 1 = n = N. The recording time t is
given by the product of the sample number and the
sampling interval, t = uT, where uT = 20 ns.

Each time series was multiplied by a rectangular
window function centered at time \( t_0 = n_0uT \) (i.e.,
\( w[n] = rect[(n - n_0uT)/N] \)), precisely to locate the analysis region.
One power spectral density estimate S (periodogram)
was computed at each tendon location x_i and scanning
angle 0 = 0 by averaging over the set of J uncorrelated
waveform segments using the equation:

\[
S[k, n_0, N, m, \ell] = \frac{1}{J} \sum_{j=1}^{J} G[k]G^*[k].
\]
where

\[ G[k] = \sum_{n=0}^{N-1} w[n - n_0] g[n, m, \ell] \exp(-i2\pi kn/N), \]  

and \( G^* \) is the complex conjugate of \( G \). The duration of each windowed time series is \( T = N\Delta t = 8 \mu s \). It is centered about the \( \varphi \) rotation axis and excludes scattering from tendon surfaces. Spectra are a function of frequency \( \omega = 2\pi k/N \), as indicated by the index \( k \), range time \( t_0 \) as indicated by the index \( n_0 \), echo segment duration \( T \) as indicated by the number of samples \( N \), position along the tendon \( x_\ell \) as indicated by the index \( \ell \) and scanning angle \( \theta_m \) as indicated by the index \( m \).

We measured power spectra over the range of scanning angles \( 0^\circ \leq \theta_m \leq 20^\circ \) for tendons and for graphite-in-agar cylinders (Hall et al. 1993) cast into a shape similar to that of the tendon. The largest graphite powder particle was smaller than 50 \( \mu m \). The random isotropic structure of the phantom contrasts with the anisotropic structure of the tendons. Phantom spectra are determined largely by the system response of the instrumentation. \( S' = S \times A \) identifies spectra that have been corrected for attenuation of the incident beam as described below and the normalized power spectrum \( S_N = S'/c^2S'_p \) is the ratio of the attenuation-corrected echo spectrum from a tendon divided by that from the phantom. Normalized spectra describe tendon properties independent of the instrumentation, assuming a linear-time invariant (LTI) system over a range corresponding to \( T \).

**Preliminary data.** Preliminary spectra at several scanning angles obtained from normal tendon without attenuation correction or normalization and phantom spectra are shown in Fig. 4. Echo power is enhanced between 3 and 10 MHz for the tendon compared with the phantom. Subjectively, no differences were noted between spectra from the DDF and the SDF tendons. For subsequent measurements, the DDF tendon was used because its cylindrical shape facilitated placement in the tendon holder. A typical normalized power spectrum value \( S_N \) was plotted against the tendon rotation angle \( \varphi \) to test for independence of backscatter on \( \varphi \). It can be seen in Fig. 4c that, even though the backscatter power fluctuates, the fluctuation has no regular dependence on \( \varphi \). The following section describes an echo data simulation developed to explore tendon properties that could result in the spectral features observed in Fig. 4.

**Echo simulation**

An LTI echo signal model was adopted to simulate ultrasonic interactions with tendon. Several scattering geometries were suggested after examination of the high-resolution tendon images shown below. Echo signal samples \( g[n] \) are given by the continuous-to-discrete temporal convolution equation:

\[ g[n] = \int_{-\infty}^{\infty} h(n\Delta t - 2z/c) f(z) + e[n], \]  

where \( f \) represents the scattering medium (the second spatial derivative of acoustic impedance) as a continuous function of position \( z \) along the beam axis. Parentheses indicate functions of a continuous variable and square brackets indicate functions of a discrete variable. \( h \) is the pulse-echo impulse response of the instrumentation, \( e \) is white-Gaussian signal-independent noise (WGN) and \( c \) is the longitudinal speed of sound. This is a commonly used 1-D linear-system representation of echo formation (Macovski 1983). The LTI pulse-echo impulse response is modeled as a Gaussian-modulated sinusoid,

\[ h(t) = \exp(-(t - t_0)^2/2\sigma_h^2) \sin(\omega_0 t). \]  

We specified the carrier frequency of the transmitted pulse as \( \omega_0 \) and the pulse duration as \( \sigma_h \). Fixing the fractional temporal bandwidth at 0.6, the relation \( \sigma_h = 2\pi \times 0.312/\omega_0 \) (an expression for a Gaussian spectrum at the \( -6\) dB bandwidth where the bandwidth is 60% of the carrier frequency) specifies the Gaussian impulse response using a single parameter, the carrier frequency \( \omega_0 \).
The regular component of the object function for model \( H_9021 \) was reduced from that used in simulations to enhance the basic Gaussian pairs plus WGN. Scaling varies between plots and functions plus WGN; (d) model 4: sum of variably spaced Gaussian functions plus WGN; (c) model 3: sum of two different variable Gaussian functions plus WGN; (b) model 2: sum of variably sized Gaussian functions plus WGN; (a) model 1: periodic step-function plus random f(z), used to model echo spectra from tendon obtained at normal incidence. (a) Model 1: periodic square wave to simulate an ensemble of \( \text{J} \) uncorrelated waveforms, each \( 10 \) μs in duration and obtained at normal incidence, \( \theta = 0^\circ \), and the scattering structure in each object model is the incoherent sum of larger regular structures representing various aspects of tendon fascicles and a WGN process representing a broad range of structure sizes.

Model 1 uses a periodic square wave to simulate regularly positioned fascicles. Model 4 uses pairs of rectangular pulses to represent fascicle surfaces. The separation width \( (W_s) \) between paired elements was a normal random variable of mean \( \Phi \) and variance \( \Phi \), \( W_s = N(\Phi, \Phi^2) \). Model 2 is one method for realizing scattering continua with a Gaussian spatial autocorrelation function (Insana and Brown 1993). The size of these scatterers is given by the equivalent-width definition in one dimension:

\[
D = \int_0^\infty dz f(z) f(z) = (A/\sqrt{2\pi}\sigma_0)\exp\left(-\left(z-z_0\right)^2/2\sigma_0^2\right),
\]

where \( \sigma_0 = (W_s/2)/1.26 \) (determined from \( D = 2.51 \sigma_0 \)) and \( A_s \) is the scattering amplitude. The quasiregular structure component in model 3 consists of shifted Gaussians, as in model 2, but now two groups of regular structures at different spatial scales are included. One structure is approximately 4 times larger in size than the other. Model 3 is an attempt to provide scattering from both individual fibers and fascicles (Fig. 2).

The object function in eqn (2) was computed by applying scattering and attenuation filters to the weighted sum of regular f_r and WGN random f_w scattering functions. This is most easily achieved in the frequency domain. Let \( F_r = \mathcal{F}^{-1}\{f_r\} \) represent the Fourier transform of \( f_r \) and \( f_r = \mathcal{F}^{-1}\{F_r\} \) its inverse. Then the object function is:

\[
f(z) = \mathcal{F}^{-1}\{(F_r + \beta F_w)(\omega/2\pi)^2\exp(-\omega^2a_0^2)}
\]

where \( \beta \) is a weighting constant, \( a_0 \) is the attenuation coefficient for tendon and \( z_0 \) is the radial distance through a cross-section of the tendon. The second multiplicative factor in the braces accounts for Rayleigh scattering effects and the third factor accounts for attenuation losses (see below for details). These are, respectively, high-pass and low-pass filters.

We also examined the possibility that the shape of tendon echo spectra could be determined entirely by small random (Rayleigh) scatterers. For this simulation, we set \( F_r \) in eqn (7) to zero, and adjusted \( \beta \) to match the measured spectra in Fig. 4. This is model 5, which would suggest that large non-Rayleigh scatterers play no role in determining the spectrum. If that were the case, then the shape of the tendon spectrum would be primarily determined by the instrument response and attenuation.

Models 2 to 4 express microanatomical features modeled from histologic observations. We will show below that these three models yield spectra similar to that measured for tendon. Scatterer sizes in the range 15 μm to 1000 μm were considered. Equation (3) was used to simulate an ensemble of \( J = 30 \) uncorrelated waveforms, each 10 μs in duration and obtained at normal incidence \( \theta = 0^\circ \) for the four object functions illustrated in Fig. 5. We assumed noise-free measurements (i.e., \( e[n] = 0 \)). Power spectra were computed while varying the model parameters \( \Psi \), \( \Phi \) and \( \beta \) about values observed from histology to match the spectral shape of the preliminary data for tendon in Fig. 4. The carrier frequency of the pulse was set to \( \omega_c/2\pi = 15 \) MHz.

Model 1 was discarded because it produced simulated spectra with square-wave harmonics not seen in the
measured tendon spectrum of Fig. 4b. Model 2 produced spectra more similar to those measured. The mean $\Phi$ and SD $\psi$ of the Gaussian structure size $D$ that give the spectrum in Fig. 6a are 130 $\mu$m and 39 $\mu$m, respectively, and $\beta$, the ratio of WGN to Gaussian structure amplitude, is 0.025. Model 3, with two scales of Gaussian structures ($D_1, D_2$), produced the spectrum in Fig. 6b for means of 130 $\mu$m and 500 $\mu$m and corresponding SDs of 40 $\mu$m and 150 $\mu$m. $\beta = 0.013$ in this model because random scatterers $f_{sc}$ were added individually to each Gaussian structure. The peak near 1.5 MHz in Fig. 6b, if it exists in the experimental data, would not be measured because of waveform filters applied before recording. The interfascicular space seen histologically (consisting of fluid, cells, etc.; see the Results section) is approximately 10% of the fascicle size. Model 4 gave the spectrum in Fig. 6c, when 10- $\mu$m size Gaussian pairs were separated by 130 $\mu$m, mimicking scattering from the front and back surfaces of 130- $\mu$m structures. Also, $\beta = 0.025$. The spectrum from a random medium with no attenuation is shown in Fig. 6a–c for comparison. Finally, model 5 is shown in Fig. 6d for $\beta = 0.200$.

Comparing models 2 through 5 in Fig. 6 with the tendon spectrum in Fig. 4b at normal incidence, it appears that a continuous scattering medium (models 2 and 3) with large regular scatterers provides the closest qualitative agreement with the tendon spectrum in Fig. 4.

**Attenuation and speed of sound measurements**

A standard narrow-band through-transmission substitution technique (Madsen et al. 1982) was applied to measure attenuation coefficients and sound speeds at 22°C between 2.5 to 13 MHz. Before sample measurements, the system was calibrated by measuring a castor-oil sample under identical conditions. System parameters were adjusted until measured attenuation values for castor oil fell within 5% of published values (Dunn et al. 1969).

Slices of fresh tendon with dimensions 15 mm $\times$ 50 mm $\times$ 5 mm thick were cut from the center, so that the collagen fiber direction was in the plane of the slice. Samples were positioned directly in front of the receiving transducer, so that sound propagated through the 5-mm thickness and the beam axis was oriented normal to the fiber orientation. Tendon slices were considered to be normal to the beam axis when the amplitude of the received signal after transmission through the sample, $A_1$, was maximized. The reasoning was that any orientation other than normal incidence would decrease $A_1$. Ten measurements were acquired with a sample in place and averaged at each measurement frequency by translating samples in a plane perpendicular to the beam axis. The amplitude of the received signal in water with the sample removed was $A_0$ and the sample thickness was $d$ in cm. The frequency-dependent attenuation coefficient $\alpha$ measured in dB cm$^{-1}$ is given by:

$$\alpha(\omega) = \frac{20}{d} \log \frac{A_0(\omega)}{A_1(\omega)}.$$  \hspace{1cm} (8)

Sample sound speed was estimated by measuring the average phase shift $\chi$ (measured in $\mu$s) of the received signal that occurred by placing the sample in the sound beam. From knowledge of the speed of sound in water at the measurement temperature $c_0$, the longitudinal speed of sound is

$$c(\omega) = \frac{dc_0}{d + c_0\chi}.$$  \hspace{1cm} (9)

Attenuation coefficients and sound speed were also measured in the phantom material using a 2.54-cm long 7.6-cm diameter test cylinder poured directly from the same graphite-agar material used for the random backscatter phantom. The cross-sectional surfaces of the test cylinder were covered by SaranWrap® to protect the submerged phantom sample. As with the tendon, 10 independent measurements were acquired and averaged at each frequency after translating the sample between measurements.

Attenuation coefficients were plotted as a function
of frequency and a least squares linear fit was obtained. The regression coefficients, slope $a_0$ and intercept $a_1$ were used to compute $a'(\omega) = a_0\omega/2\pi + a_1/20 \log e$ for each sample. $a'(\omega)$ is used in the simulations described above and to compensate for attenuation losses in measurements described below.

**RESULTS**

**Attenuation and speed of sound results**

Measured attenuation coefficients for tendon and phantom samples are shown in Fig. 7. Our values for equine DDF tendons are much lower than those measured for porcine flexor tendons of the forelimb by Takiuchi et al. (2001). We believe the differences are not species-related. The lower values are reasonable, given our ability to image through tendon at frequencies above 10 MHz on systems with limited dynamic range. The greater heterogeneity of damaged tendon is reflected by increased measurement variability, as seen by larger error bars. Regression analysis was applied to data at frequencies between 2.5 and 13 MHz for one normal tendon sample, one damaged tendon sample and the agar phantom (Table 1).

Sound speeds were measured for the same three samples over the same frequency range (Table 2). Because the speed of sound was relatively constant in each of the samples, there is no strong evidence that suggests that these are dispersive media. The average speed in the normal tendon closely agrees with the value measured by Miles (1996) for equine digital flexor tendon. The larger sound speed and lower attenuation in damaged tendon may reflect differences in collagen types.

**Backscatter results, normalized**

Backscatter spectra were measured using three broadband transducers with the same aperture geometry and overlapping frequency bands, but different resonant frequencies, 7.5 MHz, 10 MHz and 15 MHz. Figure 8 shows normalized echo spectra for the same region of DDF tendon using the three transducers. It is reasonable to assume system independence for normalized spectra within the noise limits of the measurement band width.

Backscatter spectra from the 10-MHz transducer acquired from a single tendon location $x$ and from the phantom are plotted in Fig. 9. Frequency channels in normalized spectra that deviate significantly from 0 dB indicate effects of the anisotropic structure. Figure 9b suggests that the enhanced backscatter near normal inci-

Table 1. Regression coefficients for attenuation measured between 2.5 and 13 MHz for tendon samples and the agar phantom

<table>
<thead>
<tr>
<th>Slope, $a_0$ (dB cm$^{-1}$ MHz$^{-1}$)</th>
<th>Intercept, $a_1$ (dB cm$^{-1}$ MHz$^{-1}$)</th>
<th>Correlation coefficient, $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.42</td>
<td>3.90</td>
</tr>
<tr>
<td>Damaged</td>
<td>1.33</td>
<td>0.703</td>
</tr>
<tr>
<td>Agar phantom</td>
<td>0.450</td>
<td>0.820</td>
</tr>
</tbody>
</table>

![Fig. 7](image-url)

(a) Measured attenuation coefficients for one healthy and one damaged tendon sample; both are measured perpendicular to the fiber direction. (b) Measured attenuation coefficients for the agar phantom. Error bars indicate one SE from 10 measurements at each frequency.

![Fig. 8](image-url)

Fig. 8. Normalized power spectra from the same healthy tendon using three transducers at $\theta = 0^\circ$, $8^\circ$.
The quantity \( p \) is the \( t \)-test probability for testing the hypothesis that the mean power spectra at different angles are equal; \( N \) is the number of measurements included; \( N' \) is the minimum number of measurements required to give a probability of \( p < 0.05 \) for type I and type II errors of 5%.

Table 3. Statistical analysis of the inter- and intratendon variability with changes in beam angle averaged over a 4 to 10 MHz band width

<table>
<thead>
<tr>
<th>Change in beam angle, ( \Delta \theta )</th>
<th>Percent change ( \pm 1 ) SE</th>
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<tr>
<td>Intratendon 0° to 4°</td>
<td>(-37.05 \pm 0.57)</td>
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<tr>
<td>4° to 8°</td>
<td>(-56.5 \pm 1.59)</td>
<td>5</td>
<td>0.02</td>
<td>4</td>
</tr>
<tr>
<td>Intertendon 0° to 4°</td>
<td>(-22.2 \pm 0.76)</td>
<td>5</td>
<td>0.005</td>
<td>2</td>
</tr>
<tr>
<td>4° to 8°</td>
<td>(-51.7 \pm 1.6)</td>
<td>5</td>
<td>0.01</td>
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Fig. 9. (a) Raw power spectra \( S \) from healthy tendon are measured at scanning angle 0° \( \leq \theta \leq 16° \) and from the phantom at normal incidence 0°; (b) corresponding normalized spectra, \( S_N \).

Statistical significance

To state confidently that the spectral changes observed are statistically significant, we examined angular dependence with respect to intra- and intertendon measurement variability. The data for this analysis were obtained from five healthy DDF tendons where, for each tendon, we made measurements at five spatially nonoverlapping locations, \( x \), \( 1 \leq \ell \leq 5 \). Measurement variability is expressed in terms of the standard error of power spectral estimates.

In Fig. 10a, the intratendon variability is summarized by plotting the mean \( \pm 1 \) SE values for \( S_N \) computed from data obtained at five locations in one DDF tendon. Changes in spectral values averaged over the measurement band width are listed in Table 3. The plots suggest that changes in \( S_N \) with \( \theta \) clearly exceed the intratendon measurement uncertainty. A \( t \)-test analysis (Table 3) confirms that the differences are statistically significant. The intertendon results (Fig. 10b and Table 3) also show that the between-tendon variability is small compared with the dependence on \( \theta \). The inter- vs. intratendon variability is presented in Table 4 by the mean spectral power density and the SE at each \( \theta \). This shows that there is not a statistically significant (\( p \approx 0.05\% \)) difference between taking samples intertendon compared with intratendon, with the exception of data at the \( q = 8° \) being significant only at the \( p \approx 0.25\% \) level.

Combining all 25 measurements (5 sections per tendon \( \times 5 \) tendons) the overall standard error averaged over frequencies in the measurement band width and \( \theta \) is 0.38 dB (Fig. 10c). Considering data at each angle \( \theta \), the mean spectral power density and the SE are shown in Table 4.

Backscatter results, damaged tendon

Backscatter spectra acquired from a severely damaged equine tendon are shown in Fig. 11a and b. Normalized spectra were compensated for frequency-dependent attenuation losses using attenuation parameters measured for this specific sample. Because the only samples of spontaneously damaged tendon that could be obtained were from horses with chronic injury, we assume these spectra are those of scar tissue. Like healthy tendon, these spectra have enhanced low-frequency power relative to the phantom, indicating that tendon scatterers are larger than phantom scatterers. Yet, spectra for this damaged tendon clearly show a loss of dependence on scanning angle. This finding suggests that the scatterers involved in repair of damaged tendon are similar to those in healthy tendon, except that the regular spatial organization is more random, leaving a more isotropic structure.
Independent verification of tendon structure/scatterer size

We employed several imaging modalities in an attempt directly to observe tendon fascicles and associated structures. These were x-ray computerized tomography (CT) (50-µm resolution); high-field magnetic resonance imaging (MRI), 7 T FLASH protocol, time of repetition (TR) 118 ms, echo time (TE) 12.2 ms, matrix size (MTX) 512, number of excitations (NEX) 400, 29 mm pixel size; scanning electron microscopy (SEM), fresh tendon cut, fixed in 2.0% paraformaldehyde and 2.5% glutaraldehyde, dehydrated with ethanol series, dried critical point dryer, Philips XL30 scanning electron microscope; transmission light microscopy (stained with reticulin); and high-frequency US B-mode imaging.

No contrast for structures at any scale was apparent for CT. MRI revealed no cross-sectional structures smaller than approximately 300 µm (see Fig. 12a); the 1 mm tertiary bundles were clearly visible. SEM (Fig. 12b) most clearly showed the cross-sectional hierarchy of structures described in the literature: these are tertiary bundles (1 mm), fascicles or fibers (50 to 300 µm) and fibrils (0.1 to 0.4 µm, not shown at this image resolution). Light microscopy revealed similar structures (e.g., Figs. 12c and d, although it was difficult to observe structures smaller than 200 µm with this approach because of low contrast and because sectioning adds artefacts that can look similar to anatomy. US B-mode images at 12 MHz, 20 MHz and 30 MHz were formed using a single-element transducer and a mechanical scanning technique. The tendon sample was scanned both parallel and perpendicular to the fiber direction. Figure 12e and f show images formed using the 30-MHz transducer as it was scanned perpendicular and parallel to

Table 4. Statistical analysis of the inter- vs. the intratendon variability averaged over a 4 to 10 MHz band width

<table>
<thead>
<tr>
<th>Beam angle, θ</th>
<th>Mean power ± 1 SE</th>
<th>N</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>x₀, 1 ≤ 1 ≤ 5 from 1 tendon and x₁, 1 = 1 from 5 tendons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0°</td>
<td>19.14 ± 0.45</td>
<td>9</td>
<td>0.02</td>
</tr>
<tr>
<td>4°</td>
<td>14.68 ± 0.67</td>
<td>9</td>
<td>0.05</td>
</tr>
<tr>
<td>8°</td>
<td>6.13 ± 0.76</td>
<td>9</td>
<td>0.25</td>
</tr>
<tr>
<td>x₀, 1 ≤ 1 ≤ 5 from each of 5 tendons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0°</td>
<td>19.09 ± 0.48</td>
<td>25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4°</td>
<td>13.48 ± 0.25</td>
<td>25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8°</td>
<td>6.21 ± 0.18</td>
<td>25</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The quantity p is the t-test probability of a type I or type II error for testing the hypothesis that the mean power spectra at each angle are equal; N is the number of measurements included.

Fig. 11. Power spectra from tendon with major focal damage: (a) raw spectra S and (b) normalized spectra $S_N$.

Fig. 12. Normal tendon was imaged using four different modalities: (a) MRI; (b) SEM; (c),(d) light microscopy; and 300-MHz US scanned (e) across and (f) along the tendon fibers.
fiber direction. The axial and lateral pulse dimensions were approximately 80 μm and 250 μm, respectively.

The white lines in the MR image on the scale 1.0 mm indicate tertiary bundles within normal tendon (Fig. 12a). Finer white lines on a submillimeter scale indicate fascicle boundaries. They appear white because of the fluid in the intervening spaces. The major circular bundle in the SEM image of Fig. 12b is a fascicle and the interfiber and intersubfascicle spaces appear as dark cracks. The spaces between fascicles and fibers also appear in light microscopy images (Fig. 12c and d) as lines that are weakly stained. Similar structures appear in the US images, particularly when imaged parallel to the fibers as in Fig. 12f. There are bright parallel lines spaced approximately 1-mm apart and finer lines in between. The fluid in the interfascicular and interfiber spaces seems to provide contrast for all of these modalities. They provide regions of lower acoustic impedance likely to scatter sound in the measurement band width applied. In combination, these images show that there is a quasiregular structure to tendon that strongly interacts with US.

**DISCUSSION**

The data provided in this report offer new insights into the sources of ultrasonic scattering in tendon and how structural anisotropy influences the appearance of tendon sonograms. At frequencies between 2 and 20 MHz, sound waves traveling through tendon are weakly scattered by collagen fibers aligned along the long axis. In cross-section, these fibers are not resolved by the pulse-echo imaging system, but their geometry nevertheless influences the frequency spectrum of the echo signal.

Viewing tendon in a cross-sectional plane, scattering is most likely to occur at the interfiber and intersubfascicular spaces. These sites are randomly positioned with a mean spacing of between 30 and 200 μm. To see this, draw a line through the SEM image in Fig. 12b and histogram the distance between the dark spaces. With minor exceptions, the spaces between fascicles and bundles are randomly positioned and spatially uncorrelated in the cross-sectional plane of the tendon. Consequently, backscattered spectra recorded in the plane (i.e., those acquired while rotating the tendon about the angle φ in Fig. 3) behave as if produced entirely by incoherent scattering (the average spectrum is independent of φ).

We know of no a priori reason for there to be a systematic variation in echo spectra with tendon rotation angle, φ. Nevertheless, we tested for the variation by observation of backscattered power as φ was changed in 10° increments from 0° to 360°. The independence of backscatter on φ is a rationale for averaging over rotation angle to reduce measurement noise without loss of information. Although there is no obvious trend shown in Fig. 4, the fluctuations cannot be explained entirely by uncertainty of values in the band width. A systematic variation with φ at two orthogonal measurements was suggested by Miles (1996). However, we do not find conclusive evidence for either conclusion. We decided to present spectra that are averaged over all 36 rotation angles, but we wish to note that there could be a weak systematic variation that is overlooked in these results.

Viewing the tendon in a plane along the fibers, we find a different situation. The interfiber and interfascicular spaces that scatter sound are spatially correlated over long distances in this plane because the fibers are much longer than their diameter. Spatially correlated scatterers add a strong coherent scattering component to the echo signal (Insana and Brown 1993) that varies in strength depending on the angle of insonation, θ. Of course, the transmitted sound pulse is 3-D and, therefore, generates echo signals with both coherent and incoherent scattering components. We clearly see the change in coherent scattering if we compare spectra acquired while rotating about θ. The greatest coherent scattering strength occurs at normal incidence, θ = 0. Increasing θ effectively reduces the number of fibers that contribute to the coherent scattering component of the echo signal.

The extent of tendon damage is defined by the number of ruptured fibers. Ruptured fibers and the more isotropic scar tissue that occur during repair both lower the spatial coherence of the scattering surfaces along the tendon axis which, in turn, reduces the coherent scattering component. Therefore, a sonogram of focal tendon damage appears to be hypoechoic at normal incidence and the lesion echogenicity changes very little with scanning angle. We saw that the echo spectrum from a region in tendon with severe damage exhibited no θ dependence.

One outstanding question is why tendon spectra, normal (Fig. 9) and damaged (Fig. 11), show higher backscattered power at frequencies less than 8 MHz than that from the random graphite-gel phantom. One tenable cause is that fibers/fascicles interact with sound waves as large scatterers. It is well known that “small” structures (i.e., those much smaller than the wavelength of sound) (Rayleigh scatterers), reflect much more energy at higher frequencies than at lower frequencies. “Large structures” (i.e., those of size on the order of the wavelength or larger) reflect energy more evenly across the measurement band width. Therefore, tendon spectra that are dominated by scattering from fibers/fascicles may be expected to reflect more energy at low frequencies than spectra from the phantom that consists entirely of Rayleigh scatterers. The relatively flat frequency response of normalized tendon spectra (Figs. 9 and 11) is consistent with the large-scatterer explanation. Also contributing to
the apparent enhancement of tendon spectra at low-frequency is the high attenuation that acts as a low-pass filter. The simulated model spectra summarized in Fig. 6 suggest that scattering from large regular structures, $f_s$, is likely. However, because the simulations assume normal incidence, the full range of coherent scattering effects has not yet been explored.

**CONCLUSIONS**

The anisotropic structure of tendon produces an ultrasonic image that is very sensitive to scanning angle. We measured a 1.5-dB decrease in backscattered power per degree deviation from normal incidence and explained the effect as being due to the angle-dependence of coherent scattering from the aligned fibers/fascicles. Our analysis of images from several modalities able to resolve the tendon microstructure, including SEM, MRI, US and light microscopy, suggest that the spaces between fibers and fascicles are most likely responsible for generating the echo signal from tendon. Simulations using several structural geometries showed that paired scatterers from fiber surfaces added to a weak random structure and gave spectra similar to that measured from healthy tendon at normal incidence to the fibers. Similar spectra were also observed in a chronic tendon tear. It is, therefore, unlikely that designing instrumentation to analyze changes in the frequency content of echo spectra will prove useful in a clinical setting. However, the dependence on scan angle all but disappears in the tendon scar tissue that forms after tendon is damaged. Designing real-time B mode instrumentation to detect this change in anisotropy could be used to provide greater contrast between healthy tendon and tendon repair. It is not known whether this change in acoustic property of chronic lesions also occurs in acute injury. It would be reasonable to suspect that the fibers ruptured in an acute injury would recoil and also lose their anisotropic properties. If this does occur, detection of those changes could be used better to characterize and define acute tendon injury, as well. These results are for equine DDF tendon. However, tendon structure is relatively similar between species and throughout the body; therefore, our findings have broad application.

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**REFERENCES**


