Anisotropy of Ultrasonic Parameters in Fresh Rat Skeletal Muscle *In Vitro*

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Abstract— **The anisotropy of frequency dependent backscatter coefficient, attenuation, and speed of sound is assessed in fresh rat skeletal muscle within 5 hours *post mortem*. Excised rat semimembranosus and soleus muscles are measured in 37 °C Tyrode solution, with the muscle fibers at 90° and 45° orientations to the incident sound beam. Reflected and through transmission signals from either a 6 or 10 MHz focussed transducer give frequency dependent information in the 4-14 MHz range. The attenuation coefficient in each muscle is consistently 60±25% lower for propagation perpendicular to the fibers than at 45°, whereas speed of sound shows a much milder anisotropy (slightly faster for the 90° orientation) which is inconclusive within experimental error. The largest anisotropy is seen in the backscatter coefficient, most notably in the semimembranosus where the magnitude at 90° is over an order of magnitude greater than at 45°, with the frequency dependence in both cases giving a power law close to 2.**

I. Introduction

The variation of acoustic properties with direction of the incident sound field relative to the orientation of the medium being studied is known as acoustic *anisotropy*. Anisotropy in ultrasonic parameters characterizing biological tissue has been reported for a number of tissues with oriented structures — myocardium [1], tendon [2, 3], skeletal muscle [3, 4, 5], kidney [6] — but the great majority of this work has been done on fixed, frozen, or *post-rigor* samples, and the few studies using fresh tissue have measured only speed of sound or attenuation [4, 7].

The aim of this study is to characterize the anisotropy of frequency dependent backscatter coefficient $\sigma_b(f)$, attenuation $\alpha(f)$, and speed of sound c, in freshly excised skeletal muscle. Samples are measured in 37 °C Tyrode solution to create conditions as similar as possible to *in vivo*, which should be more clinically relevant.

II. Methods

A. Muscle specimen preparation

Two types of rat skeletal muscle were used is this study, the *semimembranosus* and *soleus*, both characterized by having straight and parallel muscle fibers. Each muscle was excised from an adult rat within 15 minutes of euthanization (for another experiment) and its ends fastened to a measurement holder such that the fibers are at a ~10-20% stretch from relaxation. The sample is immediately immersed in 37 °C Tyrode solution [8] where it remains for all measurements. By removing a second (same) muscle from the animal, and holding it in similar conditions during the course of the experiment, we ascertained that the measured sample does not go into rigor until approximately 4-5 hours after death, when the measurements are finished. Four fresh muscles of each type have been measured to date. For comparison, several of the muscles were measured one day later in *post-rigor*.

B. Acquisition of data

The muscle sample, transducer, and needle hydrophone were all immersed in a temperature controlled (37±0.5 °C) Tyrode bath in an arrangement that allowed both transmission through, and reflection from, the same spatial locations in the muscle. The sample was held at the focus of either a 6 MHz or 10 MHz (Panametrics V309, V311) spherically focussed transducer (both 1.27 cm diameter, 5 cm radius of curvature) which was excited by a Panametrics 5900 pulser/receiver. The reflected signal, and the through transmission signal from the 0.6 mm diameter needle hydrophone (Medicoteknisk Institut 1094) just behind the sample, are both received and amplified by the pulser/receiver and displayed on a LeCroy 9354TM digitizing oscilloscope, set at 250 MS/s.

The muscle holder is moved within the sound field by a Daedal micropositioning system. Two orientations with respect to the muscle fibers were measured: 90° (perpendicular) and 45°. (The effect of oblique incidence of the sound beam on the muscle surface in Tyrode was calculated for the 45° case to give a pressure transmission coefficient of greater than 0.998, in comparison to the normal incidence value of 0.999, and so is ignored.) At each orientation, a 5x5 grid of spatial samples each separated by 700 μm (greater than both transducers' -6 dB spot size) was measured, with the muscle moved axially as necessary to ensure that the focal point of the transducer was at its center. Three RF-waveforms were cap-

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tured for each spatial point: (a) a backscattered signal of 500 temporal averages encompassing the entire muscle depth and both surfaces (to be gated off-line), which is used to find $\sigma_b(f)$ as well as the thickness of the sample at that spot, (b) a transmission through muscle signal, (c) and then moving the muscle out of the beam path, a transmission signal through the Tyrode solution alone. The latter two RF-signals, each averaged 100 times, are used to find $\alpha(f)$ and c. At the end of the experiment, a reference reflection signal for the σ_b measurement is collected from a flat plexiglas plate positioned where the axial center of the muscle had been.

C. Analysis of data

Attenuation measurements require knowledge of the sample thickness, but since it is difficult to physically measure fresh tissue thickness, we use a technique employing the pulse times-of-flight (TOF) to the hydrophone with and without the specimen in the beam path (T_m and T_w respectively), and the TOF back to the transducer from the muscle surfaces (t_1 front, t_2 back). Correcting for the transmitted pulse duration, we can calculate the speed of sound in the muscle (c_m) by [9]:

$$c_m = c_w \left[1 + \frac{2(T_w - T_m)}{t_2 - t_1}\right] \qquad (1)$$

where c_w is the measured speed of sound in the Tyrode solution (1538 m/s at 37 °C). Then $c_m\ (t_2 - t_1)/2$ is the thickness of the muscle at that point. See Fig. 1 for an example of the backscattered signal with the $t_{1,2}$ surfaces determined by an algorithm which looks for the first and last points which are greater than 5 times the baseline amplitude. With thicknesses known, attenuation is then calculated by the standard insertion loss technique.

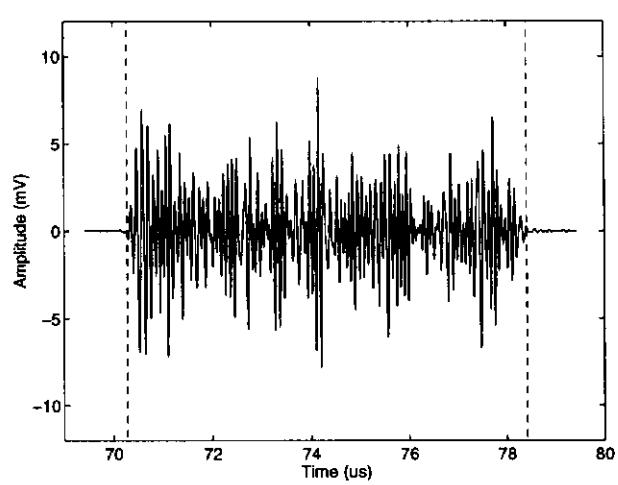

Figure 1: The backscattered RF-signal from a rat semimembranosus muscle. Dashed vertical lines are determined from an algorithm to find muscle surfaces $t_{1,2}$.

Backscatter coefficient is determined according to the method of Insana and Hall [10, 11]

$$\sigma_b(f) = \frac{0.36 R_1^2 \gamma^2}{A_o\, \Delta z}\, e^{+4(\alpha_m - \alpha_o)(R_s + \Delta z/2)}\, \frac{\langle |S(f)|^2 \rangle}{|R(f)|^2} \qquad (2)$$

where R_1 is the average distance between the transducer and the near surface of the sampled volume, A_o is the area of the transducer aperture, Δz is the axial length of the sampled volume, R_s is the distance from the muscle front face to the near surface of the sampled volume, α_m and α_o are the frequency dependent attenuation coefficients of the sample (measured in each case) and Tyrode solution (assumed similar to water) respectively, $\langle |S(f)|^2 \rangle$ is the spatially averaged power spectral estimate from the laterally sampled, diffraction corrected, Hanning gated backscattered waveforms, and $|R(f)|^2$ is the reference power spectral estimate obtained from the reflection against the plexiglas plane reflector — with amplitude reflection coefficient γ=0.35 — evaluated at axial distance $R_1 + \Delta z/2$.

The window depth for the semimembranosus muscles was 5 μs (4 mm), starting 1 μs below the front surface (to avoid surface reflections), and for the thinner soleus muscles was necessarily smaller, 3 μs (2.4 mm) at 0.5 μs below the front surface, to ensure the same sampling volume on all samples of the same muscle type.

III. Results

Attenuation in each muscle shows a distinct difference between measurement with the muscle fibers at 90° and 45° to the incident sound beam. An example of $\alpha(f)$ for

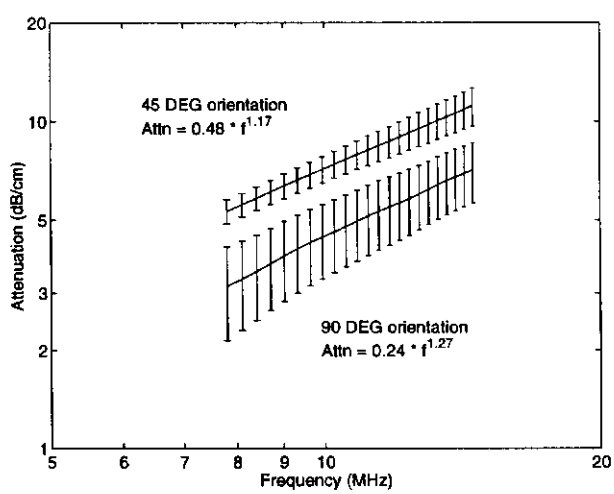

Figure 2: Example of attenuation in a single muscle at 45° and 90°. Data is shown over the -5 dB bandwidth of the received hydrophone signal for the 10 MHz transducer, although the fit (not shown) is performed over -3 dB. Error bars are standard deviation of the 25 spatial samples in each case.

a single muscle (semimembranosus #1) is shown in Fig. 2 where the lines are the spatial averages and the error bars the standard deviation of the 25 spatial samples. The average $\alpha(f)$ values for all samples are shown in Fig. 3, where paired lines of the same symbol are both orientations of the same muscle (45° is always higher than 90°). Although the two orientations among all

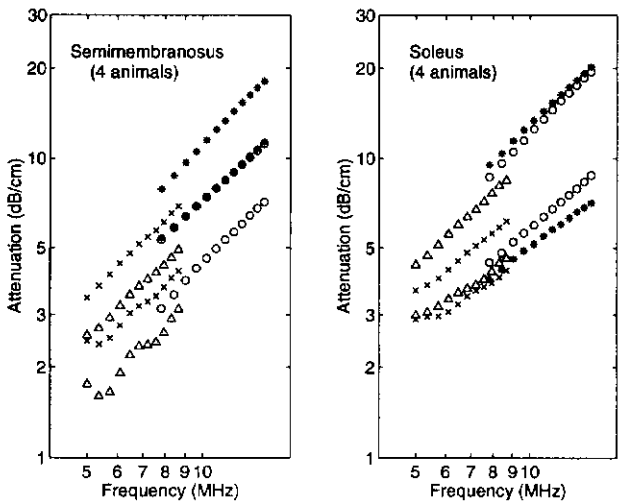

Figure 3: Attenuation measurements at 45° and 90° for the 8 rat skeletal muscles. Different symbols represent individual muscles. The higher valued data of each pair is the 45° orientation.

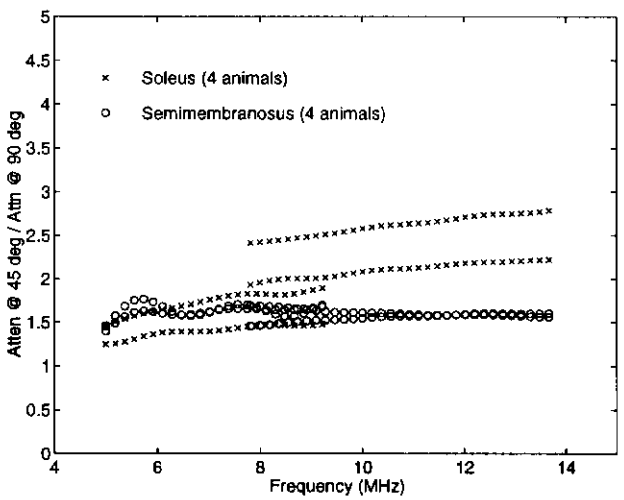

Figure 4: Ratio of $\alpha(f)$ measured at 45° versus measured at 90° for all 8 muscles.

samples are not entirely separate, the ratio within each muscle stays fairly consistent (see Fig.4), especially for the semimembranosus in which the attenuation for the 45° orientation is seen to be consistently ~60% higher than that for 90°.

The averaged speed of sound values are given in Table 1. The difference between the two orientations in

Table 1: Average speeds of sound ± standard deviation (m/s) in N freshly excised and post-rigor rat skeletal muscle at 37 °C. Measurements were taken with either a 6 or 10 MHz center frequency transducer. Standard deviation of the spatially averaged speed within one muscle is typically ~4 m/s, but can be up to ~7 m/s.

Fresh semimembranosus (N=4)	speed (m/s)	Post-rigor semimemb. (N=2)	speed (m/s)
90°	1590 ± 5	90°	1573 ± 6
45°	1580 ± 5	45°	1570 ± 3
Fresh soleus (N=4)	**speed (m/s)**	**Post-rigor soleus** (N=2)	**speed (m/s)**
90°	1582 ± 4	90°	1565 ± 1
45°	1576 ± 4	45°	1561 ± 1

fresh muscle is only on the order of 0.5%, but the error bars here, due to the method of measuring thickness at each sampled location, have values close to the difference between orientations and so little conclusion can be drawn. The observation, however, that c at 90° is faster than at 45° contrasts with previous work [3, 7] that shows faster propagation parallel to muscle fibers. For comparison between fresh and post-rigor muscle, two of the same muscle samples for each type were measured one day later. The speed of sound in each case decreases by close to 1%, indicating some softening.

The backscatter coefficient as a function of frequency is plotted in Fig. 5 for the 4 semimembranosus muscles, and in Fig. 6 for the 4 soleus. A factor of ~25 is clearly visible as the difference in orientations among all of the semimembranosus muscles. The soleus $\sigma_b(f)$ values, while averaging an order of magnitude difference between 45° and 90°, are less distinctly separated when

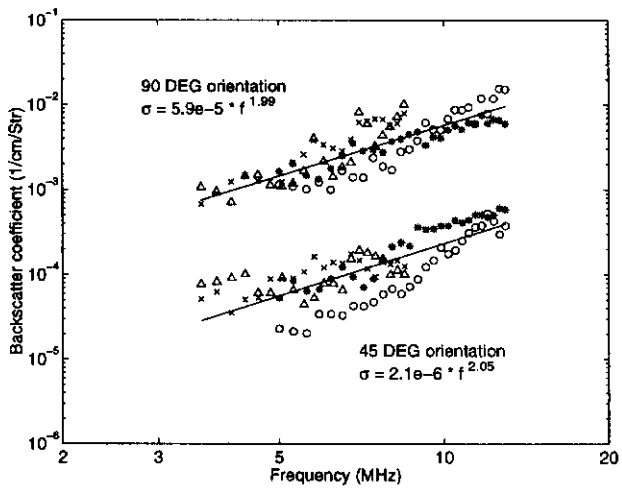

Figure 5: Backscatter coefficient at 45° and 90° for the 4 semimembranosus muscles. Symbols represent individual muscles. Lines are power law fits to the 4 muscles at the given orientation.

comparing all 4 muscles. Note that $\sigma_b(f)$ values taken by the same method on a homogeneous, isotropic tissue phantom show no difference at all, within experimental uncertainty.

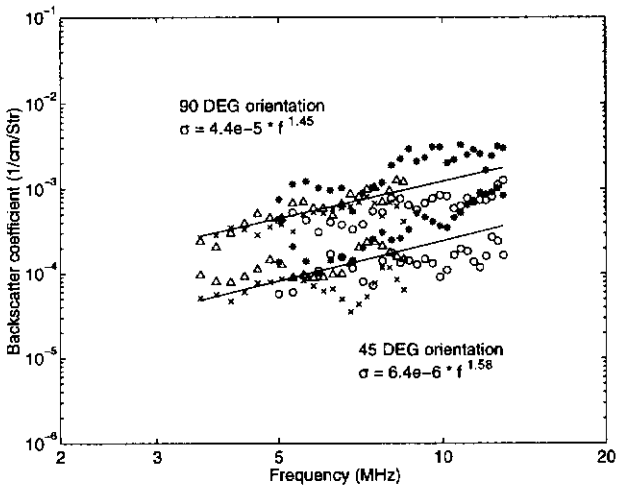

Figure 6: Backscatter coefficient at 45° and 90° for the 4 soleus muscles. Symbols represent individual muscles. Lines are power law fits to the 4 muscles at the given orientation.

IV. Discussion

Distinct differences are observed in the ultrasonic parameters of rat skeletal muscle held with muscle fibers at 90° and 45° to the sound beam. The backscatter coefficient between animals is consistent, and the two measured orientations are separated by over an order of magnitude, most notably in the semimembranosus. (Although the semimembranosus allowed for a longer time window, when its data was gated to the same length as that used for the smaller soleus, the $\sigma_b(f)$ values did not change significantly.)

Speeds of sound in both muscles are seen to be slightly faster for the 90° orientation, contrary to the suggestions of previous work [3, 7], but the experimental error here makes the differences inconclusive. Attenuation values within each muscle show a distinctive increase as the muscle fibers are turned from 90° to 45° with respect to the incident sound. The ratio of α_{45}/α_{90} for the semimembranosus is consistent at ~1.6 across the frequencies measured here. The spread of values (1.2-2.8) for this ratio in the soleus may be due to the larger amount of fascia on the surface of this muscle.

Anisotropy in rat skeletal muscle is quantified here in characterizing its ultrasonic parameters. The mechanism(s) of anisotropy must be related to orientation of the elastic tissue substructure, however the physical cause(s) are yet to be understood.

Acknowledgements

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