Cancellous bone fast and slow waves obtained with Bayesian probability theory correlate with porosity from computed tomography

Joseph J. Hoffman, Amber M. Nelson, Mark R. Holland, and James G. Miller^{a)}

Department of Physics, Washington University in St. Louis, St. Louis, Missouri 63139

(Received 23 January 2012; revised 20 June 2012; accepted 27 June 2012)

A Bayesian probability theory approach for separating overlapping ultrasonic fast and slow waves in cancellous bone has been previously introduced. The goals of this study were to investigate whether the fast and slow waves obtained from Bayesian separation of an apparently single mode signal individually correlate with porosity and to isolate the fast and slow waves from medial-lateral insonification of the calcaneus. The Bayesian technique was applied to trabecular bone data from eight human calcanei insonified in the medial-lateral direction. The phase velocity, slope of attenuation (nBUA), and amplitude were determined for both the fast and slow waves. The porosity was assessed by micro-computed tomography (microCT) and ranged from 78.7% to 94.1%. The method successfully separated the fast and slow waves from medial-lateral insonification of the calcaneus. The phase velocity for both the fast and slow wave modes showed an inverse correlation with porosity ($R^2 = 0.73$ and $R^2 = 0.86$, respectively). The slope of attenuation for both wave modes also had a negative correlation with porosity (fast wave: $R^2 = 0.73$, slow wave: $R^2 = 0.53$). The fast wave amplitude decreased with increasing porosity ($R^2 = 0.66$). Conversely, the slow wave amplitude modestly increased with increasing porosity ($R^2 = 0.39$).

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PACS number(s): 43.80.Qf, 43.60.Pt, 43.35.Cg, 43.20.Hq [CCC]

Pages: 1830–1837

I. INTRODUCTION

Osteoporosis is a skeletal disease that is characterized by a decrease in bone mass leading to an increase in bone fragility and fracture risk. The current gold standard for assessing fracture risk and diagnosing osteoporosis is dual energy x-ray absorptiometry (DXA), which measures bone mineral density. However, DXA does not have the ability to assess the structural properties of the bone, which is a key component in bone strength. Quantitative ultrasound has the potential to improve the estimation of bone fragility; however, more understanding is needed of the basic physics underlying wave propagation in cancellous bone before that potential is realized.

Cancellous bone supports the propagation of two ultrasonic wave modes, so-called fast wave and slow wave modes. We have previously demonstrated that analysis of this mixed-mode wave can produce misleading results, including apparent negative dispersion, if these two modes overlap in time.^{1,2} To address this issue, we have introduced a Bayesian probability theory approach for isolating the fast and slow waves.³ This method has been validated with simulated data⁴ and with experimental data from phantoms.⁵ It has been applied to data from cancellous bone, and the resulting separated fast and slow waves are consistent and plausible.⁵ However, in the case of temporally overlapped fast and slow waves, no conventional analysis methods are capable of independently measuring the two waves' properties. Therefore, no

appropriate gold standard for comparison with the Bayesian method is available.

This problem is especially significant because many clinical ultrasonic assessments of bone are performed by transmission throughout the calcaneus in the medial-lateral direction.⁶ In this orientation, the sound beam propagates perpendicular to the predominant trabecular orientation. Previous experimental and theoretical work^{7–11} has demonstrated that the speeds of sound of the fast and slow waves are most similar in this configuration, and this similarity of speeds directly leads to fast and slow waves with significant temporal overlap.

The objective of the current study was to use a Bayesian probability theory approach to identify the fast and slow waves obtained from through-transmission measurements in the medial-lateral direction of *ex vivo* human cancellous bone sections and to compare the properties of these waves to independently acquired anatomical data from the bone samples. This comparison was designed to determine whether or not the Bayesian-derived wave parameters correspond to physical properties of the samples. A follow-on objective was to show, for the first time, fully separated fast and slow waves from the medial-lateral insonification of the calcaneus.

II. METHODS

A. Bone samples

Ultrasound and micro-computed tomography (microCT) data were acquired from the trabecular portion of eight human calcaneal bones collected from donors at autopsy. The cortical shell was removed from each sample, and the

^{a)}Author to whom correspondence should be addressed. Electronic mail: james.g.miller@wustl.edu

TABLE I. Physical characteristics of defatted calcaneus samples. Direct thickness measurements are reported as mean plus or minus the standard deviation of five independent manual measurements.

Sample	Mass (g)	Direct thickness measurement (mm)		
		Medial-lateral	Ventral-dorsal	Proximal-distal
1	1.50	11.52 ± 0.18	26.94 ± 1.12	22.14 ± 0.13
2	4.24	11.74 ± 0.22	31.68 ± 1.13	22.90 ± 0.35
3	3.62	11.36 ± 0.26	29.62 ± 1.10	22.04 ± 0.35
4	4.14	8.09 ± 0.52	44.04 ± 1.07	24.91 ± 0.43
5	4.61	15.45 ± 0.55	32.66 ± 1.64	25.83 ± 0.20
6	5.23	12.68 ± 0.15	29.79 ± 1.07	28.72 ± 0.23
7	1.26	12.44 ± 0.81	29.30 ± 0.27	24.41 ± 0.66
8	1.89	11.52 ± 0.40	30.84 ± 0.87	22.01 ± 0.43

remaining cancellous bone was cut into a rectangular parallelepiped. Dimensions of each sample can be found in Table I.

Each sample was defatted using a modified version of the method outlined by Ding *et al.*¹² Visible marrow was removed by rinsing the samples with a water jet. Subsequently, each sample was soaked in a one-to-one mixture of acetone and ethanol for 48 h. The samples were then rinsed again with water and dried in a vacuum desiccator. Defatting the samples resulted in a loss of approximately 20%–50% of the initial mass. Success of the defatting procedure was confirmed by visual inspection of subsequently acquired microCT images.

The apparent mass densities of the defatted samples were calculated using the parameters in Table I and are shown in Table II.

B. X-ray microCT measurements

Bone porosity of each sample was measured using x-ray microCT. MicroCT data were acquired with a vivaCT 40 (Scanco Medical, Brüttisellen, Switzerland). The system was configured to provide resolution of 17.5 μm in all three rectangular spatial directions. The grayscale threshold for distinguishing voxels corresponding to trabeculae was selected manually by choosing a value that provided the optimal

TABLE II. Derived parameters of defatted calcaneus bone samples. Apparent density was determined by dividing the mass of the dehydrated samples (mass column of Table I) by the product of the mean linear dimensions (direct thickness measurement columns in Table I). The micro-computed tomography system provides a bone volume per total volume (BV/TV) measurement. Porosity is $(1 - \text{BV/TV})$.

Sample	Apparent density (g/cm ³)	(BV/TV)
1	0.22	0.086
2	0.50	0.171
3	0.49	0.213
4	0.47	0.198
5	0.35	0.162
6	0.48	0.165
7	0.14	0.059
8	0.24	0.116

compromise when applied to the least dense sample and the densest sample. Once chosen, the threshold remained constant for the entire analysis. The bone volume per total volume (BV/TV) values obtained from these measurements are shown in Table II. Porosity is defined as $(1 - \text{BV/TV})$. These porosity values fall within the typical range (80%–95%) reported for the calcaneus.¹³

C. Ultrasonic data acquisition

Through-transmission ultrasonic data were acquired using a pair of matched broadband immersion transducers with 500 kHz center frequency (v391, Olympus NDT, Waltham, MA). The transducers are cylindrically focused with a focal length of 1.5 in. (3.8 cm) and have a diameter of 1.125 in. (2.9 cm). In a room temperature water bath, the transducers were aligned coaxially with a separation distance of twice the focal length. Proper alignment was achieved by employing a procedure of adjusting the alignment to maximize the amplitude of a water path signal in the time domain and subsequently adjusting the alignment to maximize the signal's bandwidth in the frequency domain. This process was iterated until the optimal alignment was obtained. The transmitting transducer was excited by a single 500 kHz sine wave pulse with an amplitude of approximately 125 V peak-to-peak (P-P). The received signal was passed through a 1.6 MHz low pass filter, amplified, and digitized at 50 MHz at 8-bits by a digital oscilloscope (5052B, Tektronix, Inc., Beaverton, OR). After time-averaging 128 consecutive signals, the resulting waveform was saved to disk.

To acquire cancellous bone data the sample was inserted into the ultrasonic propagation path equidistant from the transmitting and receiving transducers. After acquisition from each bone sample, the specimen was removed and a water path reference trace was recorded with all settings unchanged. Representative radiofrequency traces from a cancellous bone sample and from a water path are shown in Fig. 1.

D. Bayesian analysis

A Bayesian probability theory approach for estimating the fast and slow wave parameters from acquired ultrasonic signals has been previously introduced by our laboratory.^{3–5}

FIG. 1. Ultrasonic rf signals having propagated through a water path (gray circles) and the same path with a cancellous bone sample inserted (black circles).

1. The model

In this method, the propagating ultrasonic wave is assumed to be the superposition of two wave modes. These modes correspond to the fast and slow wave modes predicted by Biot^{14,15} and subsequently observed experimentally by Hosokawa and Otani.¹⁶ In our work, this situation is modeled as

$$\text{output}(f) = \text{input}(f) \cdot [H_{\text{fast}}(f) + H_{\text{slow}}(f)] + n, \quad (1)$$

where f is frequency, $\text{output}(f)$ is the frequency domain representation of the signal having propagated through the sample, $\text{input}(f)$ is the frequency domain representation of the signal incident upon the sample, $H_{\text{fast}}(f)$ and $H_{\text{slow}}(f)$ are the transfer functions of the fast and slow wave modes, respectively, and n is additive noise. In the current work, the signal acquired through a water only path was used as the source for $\text{input}(f)$. The transfer functions are given by

$$H_{\text{fast}}(f) = A_{\text{fast}} \exp(-\beta_{\text{fast}}fd) \exp\left(\frac{i2\pi fd}{c_{\text{fast}}(f)}\right), \quad (2)$$

$$H_{\text{slow}}(f) = A_{\text{slow}} \exp(-\beta_{\text{slow}}fd) \exp\left(\frac{i2\pi fd}{c_{\text{slow}}(f)}\right), \quad (3)$$

where c_{fast} and c_{slow} are the phase velocities, β_{fast} and β_{slow} are the slopes of the attenuation coefficients plotted as functions of frequency (nBUA), d is the thickness of the sample, and A_{fast} and A_{slow} determine the amplitude of each mode. Over the limited bandwidth (350 kHz to 630 kHz) employed, a linear fit to the attenuation coefficient plotted as a function of frequency is appropriate. At higher frequencies, this assumption is not appropriate.¹⁷ The significance of A_{fast} and A_{slow} is addressed further in Sec. IV. The relationship between the phase velocity and the slope of attenuation is constrained to ensure consistency with the Kramers-Kronig relationships. The form used is appropriate for media that exhibit attenuation coefficients that rise linearly with frequency,¹⁸

$$c_{\text{fast}}(f) = c_{\text{fast}}(f_0) + [c_{\text{fast}}(f_0)]^2 \frac{\beta_{\text{fast}}}{\pi^2} \ln\left(\frac{f}{f_0}\right), \quad (4)$$

$$c_{\text{slow}}(f) = c_{\text{slow}}(f_0) + [c_{\text{slow}}(f_0)]^2 \frac{\beta_{\text{slow}}}{\pi^2} \ln\left(\frac{f}{f_0}\right). \quad (5)$$

In these expressions, f_0 is a reference frequency chosen within the experimental bandwidth. For the current study, f_0 was set to 500 kHz.

The units of β as it is written in Eqs. (2) and (3) are the natural units of nepers per frequency per distance [Np/(cm MHz), for example]. Because nBUA is more commonly reported in units of decibels per frequency per distance [dB/(cm MHz), for example], we convert β to units of dB/(cm MHz) for the remainder of this manuscript. The conversion factor is $20/\ln 10 \approx 8.69$.

2. Parameter estimation

Bayesian parameter estimation was used to identify the most likely propagation parameters given the model above.

The six parameters estimated were the phase velocities ($c_{\text{fast}}, c_{\text{slow}}$), the slopes of attenuation ($\beta_{\text{fast}}, \beta_{\text{slow}}$), and the amplitudes ($A_{\text{fast}}, A_{\text{slow}}$). These terms were combined into a parameter vector, $\Theta = \{c_{\text{fast}}, c_{\text{slow}}, \beta_{\text{fast}}, \beta_{\text{slow}}, A_{\text{fast}}, A_{\text{slow}}\}$, the probability of which can be expressed using Bayes' theorem as

$$P(\Theta|D, I) = \frac{P(D|\Theta, I) P(\Theta|I)}{P(D, I)}, \quad (6)$$

where D is the acquired experimental data and I is the relevant background information, including the model.

In a parameter estimation problem the relevant quantity is the relative probability of $P(\Theta|D, I)$, the joint posterior probability, rather than the absolute probability. The term in the denominator of the right side of Eq. (6) can therefore be omitted because it does not involve the parameter vector, Θ . The joint posterior probability is proportional to the product of the likelihood, $P(D|\Theta, I)$, and the joint prior probability, $P(\Theta|I)$.

In the current work, the data term D is the ultrasonic signal that propagated through the cancellous bone sample, and the likelihood function is the probability of acquiring this signal given a set of parameters Θ and model and background information I . The likelihood was assigned using a Gaussian prior to describe the noise, and the dependence on the standard deviation of the Gaussian was removed by marginalization.¹⁹

The joint prior probability can be factored into a product of the prior probabilities of the individual parameters because the six parameters are assumed to be logically independent. The individual prior probabilities were assigned as truncated Gaussian distributions with mean, standard deviation, upper bound, and lower bound as given in Table III. The priors were chosen to be general enough that the outcomes of the calculations were not dependent upon the specific values chosen.

To determine the most likely value of each parameter, a marginalization procedure was applied to the joint posterior probability such that the dependence on the other five parameters was removed. For example, the marginal posterior probability of c_{fast} was calculated as

TABLE III. Prior probability distributions used for Bayesian calculations. Each prior distribution is a bounded Gaussian described by the four parameters in this table. For each parameter, the upper and lower bounds were chosen to be wide enough so as to affect the final result only minimally. The mean was chosen to be the midpoint between the upper and lower bounds, and the standard deviation was chosen to be one half of the difference between the upper and lower bound.

Parameter	Lower bound	Upper bound	Mean	Standard deviation
A_{fast}	0	1.0	0.5	0.5
A_{slow}	0	1.0	0.5	0.5
$v_{\text{fast}} \left(\frac{\text{m}}{\text{s}}\right)$	1500	3000	2250	750
$v_{\text{slow}} \left(\frac{\text{m}}{\text{s}}\right)$	800	2300	1550	750
$\beta_{\text{fast}} \left(\frac{\text{dB}}{\text{cm-MHz}}\right)$	0	86.9	43.4	43.4
$\beta_{\text{slow}} \left(\frac{\text{dB}}{\text{cm-MHz}}\right)$	0	86.9	43.4	43.4

$$P(c_{\text{fast}}|D,I) = \int \int \int \int P(\Theta|D,I) dc_{\text{slow}} d\beta_{\text{fast}} d\beta_{\text{slow}} \times dA_{\text{fast}} dA_{\text{slow}}. \quad (7)$$

The peak values of the resulting marginal posterior probability distributions for the six parameters were used for the results in Sec. III below.

3. Markov chain Monte Carlo

Because of the difficulty in analytically evaluating the five-dimensional integral of Eq. (7), a Markov chain Monte Carlo simulation with simulated annealing was employed to estimate an approximate solution. The specifics of the implementation have been described previously.⁵ Further details on Bayesian probability theory are given by Jaynes and Bretthorst,²⁰ Sivia and Skilling,²¹ and Bretthorst *et al.*²² For each of the eight sample traces, the algorithm was run six times.

III. RESULTS

For all eight cancellous bone samples, the Bayesian algorithm converged on a set of parameters that produced a model function that closely resembled the acquired data. The top panel of Fig. 2 shows the model function and acquired data for one of the samples. The bottom panel shows the residual—the difference between the model and the data. To quantify the quality of agreement between the model and the data, the ratio of the absolute maximum value of the residual to the absolute maximum value of the data was calculated for each sample. For the eight samples, the residual absolute maximum to data absolute maximum ratio ranged from 0.014 to 0.055 and had a mean of 0.031. For reference, the residual maximum to data maximum ratio for the sample shown in Fig. 2 is 0.027. These values indicate good agreement between the data and models.

A representative model wave (the same model as in Fig. 2) is shown along with the separated fast and slow

FIG. 2. Experimental data from human calcaneus, Bayesian model, and residual. The top panel shows the experimental data that are input into the algorithm. The Bayesian model is generated as described in Sec. II D. The bottom plot shows the residual, defined as the difference between the experimental data and the Bayesian model, on a substantially expanded vertical scale.

waves in Fig. 3. Visual inspection of this radio-frequency signal would suggest that only one wave is present. The same is true of the radio-frequency waveforms corresponding to the other seven samples—the signal appears to be a single wave. Separation of the two modes reveals that propagation speeds of the fast and slow waves are similar enough that even though the amplitudes of the two waves are comparable, no obvious interference effects are visible in the received radio frequency (rf) signal.

A. Phase velocity

For the eight samples measured, the mean fast wave phase velocity at 500 kHz was 1675 m/s, and the velocities ranged from 1518 m/s to 1754 m/s. The slow wave velocity of the samples averaged 1527 m/s and ranged from 1452 m/s to 1574 m/s. This range of speeds of sound is similar to the range seen in clinical measurements of the speed of sound in the calcaneus based on analysis of the unseparated wave.^{23,24} The difference between the fast and the slow wave speeds is smaller in this study than in other studies that have reported the speed of sound for both waves.^{7,16,25–27} This result is expected because the fast and slow wave speeds are most similar when propagating perpendicular to the predominant fiber direction.^{7–11,16,28} The solid lines are linear best fit lines. The *R*-squared value for the fit is shown in each panel.

Figure 4 shows the fast wave velocity (left panel) and slow wave velocity (right panel) plotted as a function of the porosity of the sample, which was determined by μ CT. As the porosity of the sample increases, the fast and slow wave speeds decreases. As indicated by the *R*-squared values in Fig. 4, the trend is robust. A similar relation between the speed of the unseparated wave and the porosity is exploited in current bone sonometers, where the speed of sound is positively related to bone quality.

B. Slope of attenuation

The slope of attenuation β is the slope of the attenuation coefficient plotted as a function of frequency. In the current model, the attenuation coefficient rises linearly with frequency. Figure 5 shows the fast wave slope of attenuation β_{fast} and the slow wave slope of attenuation β_{slow} plotted

FIG. 3. A representative model waveform (same waveform as in Fig. 2) and the fast and slow waves that comprise it.

FIG. 4. Fast wave speed (left panel) and slow wave speed (right panel) plotted against sample porosity. Porosity was determined by μ CT. Each value shown is the mean of six runs. The vertical bar on each point represents the standard deviation of these six runs.

versus porosity for the eight samples in this study. For both the fast wave and the slow wave, the slope of attenuation decreases with increasing porosity. This trend again parallels what is known from bone sonometry—that increasing bone mass per volume (decreasing porosity) causes an increase in the slope of attenuation.

C. Fast and slow wave amplitude

The transfer functions described in Eqs. (2) and (3) each contain a term (A_{fast} or A_{slow}) that determines the frequency independent amplitude of the fast wave or the slow wave, respectively. As seen in Table III these parameters are constrained to lie between 0 and 1, indicating that the amplitude of these modes must be less than or equal to the amplitude of the signal incident on the bone (represented in this calculation by the water path only signal). The physical interpretation of these parameters is addressed in detail in Sec. IV.

One of the roles the A_{fast} and A_{slow} parameters play is dividing the incident wave into the two waves that propagate through the bone—the fast wave and the slow wave. It might therefore be expected that samples supporting a fast wave of larger amplitude would support a slow wave of relatively smaller amplitude. The left panel of Fig. 6 shows that for bone samples of increasing porosity, the fast wave amplitude decreases. The opposite trend is seen in the right panel of Fig. 6, where the slow wave amplitude increases as the porosity increases.

IV. DISCUSSION

A. Fast and slow waves identified by the Bayesian method

The results presented above indicate that the fast and slow waves extracted by the Bayesian probability theory method correlate strongly with porosity, an anatomical parameter with direct relevance to clinical assessment of bone health. Previous work demonstrated the plausibility of the Bayesian-derived fast and slow waves. The current study shows, for the first time, that waves isolated by this method individually reflect bone anatomy.

It has been shown previously that the fast wave is least likely to be observed when the sound propagates perpendicular to the predominant trabecular orientation,^{7–11,28} which in the calcaneus corresponds to medial-lateral insonification. The current work suggests that the fast and slow waves are present in the medial-lateral orientation and propagate with comparable velocities such that they do not separate enough in the time domain to be directly observed. This study demonstrates that with a Bayesian probability theory approach, the fast and slow waves can be identified and isolated even in cases of substantial temporal overlap. To our knowledge, this work represents the first identification of fast and slow waves from medial-lateral insonification of the calcaneus. This comprehensive separation of the fast and slow waves in the case of perpendicular propagation is of particular significance because medial-lateral insonification of the calcaneus

FIG. 5. Slope of attenuation for the fast wave (left panel) and slow wave (right panel) plotted against sample porosity. Porosity was determined by μ CT. Each value shown is the mean of six runs. The vertical bar on each point represents the standard deviation of these six runs. The solid lines are linear best fit lines. The R -squared value for the fit is shown in each panel.

FIG. 6. Fast wave amplitude (left panel) and slow wave amplitude (right panel) plotted against sample porosity. Porosity was determined by μ CT. Each value shown is the mean of six runs. The vertical bar on each point represents the standard deviation of these six runs. The solid lines are linear best fit lines. The R -squared value for the fit is shown in each panel.

is widely employed in bone sonometry to assess osteoporotic fracture risk. Commonly, analyses of this clinical data assume the presence of only a single wave. With the technique described here, future devices might make use of information from both waves. This technique could also be applied to data acquired in other directions of propagation.

B. Interpretation of fast and slow waves

The nature of the correlation of the propagation parameters with porosity lends insight into the interpretation of fast and slow waves in cancellous bone. Biot theory predicts a fast wave generated by the hard trabecular framework and the soft interstitial marrow moving in phase and a slow wave generated by the trabecular network and marrow moving out of phase by π radians. In this theoretical framework, changing porosity leads to a change in speed for both the fast and slow waves. This trend predicted by Biot theory is corroborated by Fig. 4.

The results of this study do not support an alternate interpretation of the fast and slow wave phenomenon that claims the fast wave travels predominately, if not exclusively, through the trabecular framework, and the slow wave travels predominantly through the marrow. This interpretation would seem to predict that the fast wave speed would be independent of porosity, in contrast to the trend observed above. The results of this study, therefore support the notion that the fast and slow waves result not from simply traveling through two different media, but arise rather from the interaction between the two media, as predicted by Biot theory.

C. Fast and slow wave amplitudes

Of the three sets of parameters—the phase velocities, the slopes of attenuation, and the wave amplitudes—the fast and slow wave amplitudes (A_{fast} and A_{slow}) are the most challenging to interpret. A pulse propagating through a cancellous bone sample incurs two types of loss. The first is loss with propagation distance in the bulk of the sample, which is determined by the attenuation coefficient and varies with frequency. Typically at frequencies below 1 MHz (such as those employed in this study), the attenuation coefficient increases linearly with frequency. The second type of loss is insertion loss, which occurs only at the boundaries, and is

therefore independent of sample thickness. Because the pulse is assumed to be incident normal to the faces of the sample, the specular reflections at the boundary are approximately independent of frequency. In the model described by Eqs. (2) and (3), the term $\exp(-\beta fd)$ explicitly accounts for the frequency and distance-dependent bulk loss. The frequency-independent insertion loss is accounted for by the amplitude term A . However, the amplitude terms A_{fast} and A_{slow} also determine how much energy from the incident pulse gets put into each of the two modes. Because the A_{fast} and A_{slow} parameters determined by the Bayesian method contain information about both the insertion loss and distribution of energy into the two wave modes, straightforward interpretation is not possible. Further complicating the situation is the fact that insertion loss and distribution into the two modes occur at the same location and time—at the boundary.

An equivalent way to view the situation is to consider an incoming pulse incident on the interface between the water and the bone sample. When the pulse strikes the boundary, three waves result. There will be one reflected wave, the energy contained in which will correspond to the insertion loss. There will be two transmitted waves, the fast wave and the slow wave, with amplitudes A_{fast} and A_{slow} , respectively. Because some of the incoming wave's energy is lost to the reflected wave, it is not possible to write down a simple relationship between A_{fast} and A_{slow} . More generally, the individual values of A_{fast} and A_{slow} cannot be interpreted without knowledge of the reflected wave's amplitude. However, the ratio of A_{fast} to A_{slow} does provide information about the relative distribution of energy into the two transmitted modes.

The ratio of A_{fast} to A_{slow} plotted versus porosity is shown in Fig. 7. The correlation between this ratio and porosity is very high, indicating that the distribution of energy into the two modes depends strongly on the porosity. This finding shows that in a sample with more bone volume per total volume, the fast wave amplitude relative to the slow wave amplitude is much greater than in a bone with

FIG. 7. The ratio of the fast wave amplitude to the slow wave amplitude plotted against porosity. Porosity was determined by μ CT. Each value shown is the mean of six runs. The vertical bar on each point represents the standard deviation of these six runs. The solid line is a linear best fit line. The R -squared value for the fit is shown.

relatively less bone volume per total volume. This result is consistent with previous work,^{16,29} and further supports the view that the fast and slow waves identified by the Bayesian algorithm are valid.

The relative amplitudes of the fast and slow waves depend strongly on the path length traveled and on the bone mineral density. Much of the literature reporting on fast and slow wave modes is derived from studies of large animal tissue for which the bone mineral density, and therefore the ultrasonic attenuation, is substantially larger than for human bone. This, in combination with the relatively modest path lengths in the current study, may account for the relatively larger fractional size of the fast wave amplitude.

D. Comparison with Biot theory

The fast and slow waves found in this study seem consistent with the essence of Biot theory. However, direct comparison is difficult because of the number of adjustable parameters contained in the theory. A number of questions also exist regarding which form of Biot theory is most applicable to the samples described in this work. Depending on the exact form of Biot theory one uses, different variation in the fast and slow wave speeds with porosity can be predicted.¹⁰ The trends reported in the current study in Fig. 4 are plausible in this context.

Because, until the current work, the fast and slow waves could not be identified when wave propagation is perpendicular to the predominant trabecular orientation (medial-lateral in the calcaneus), it remains unknown how well Biot theory applies in this situation.

E. Limitations

The results of this study and their future application are limited by a number of practical considerations. A strength of the propagation model employed is its simplicity, but this simplicity comes at the cost of not being able to include every feature of the physical situation. Features such as diffraction, surface irregularities, and phase cancellation at the face of the receiving transducer have been assumed to have only modest effects. Because the results are so consistent, these assumptions are likely to be valid, but remain essentially untested.

The use of carefully prepared samples was essential for this controlled laboratory experiment, but knowledge obtained from these samples might not directly correspond to the situation *in vivo*. The samples used in this study had no cortical shell, had the marrow replaced with water, had the sides carefully flattened, and were measured at room temperature. In the clinical setting, the heel does have a cortical shell, is filled with marrow, has non-planar sides, and remains at body temperature. Clinical measurements of the calcaneus also include propagation through the surrounding soft tissue.

V. CONCLUSION

The results of this study suggest that the Bayesian probability theory approach for separating the fast and slow

waves in cancellous bone yields wave parameters that correlate with porosity, an important parameter related to the diagnosis of osteoporosis. The results provide the first confirmation that the fast and slow waves generated by the Bayesian method are directly related to the anatomy of trabecular bone.

By virtue of the successful separation of the fast and slow waves from human calcaneal bones insonified in the medial-lateral direction, this study also represents, to our knowledge, the first reported complete separation of the fast and slow waves in this configuration.

ACKNOWLEDGMENTS

The authors wish to thank Christian C. Anderson and G. Larry Bretthorst for their invaluable assistance with Bayesian analysis, Tarpit Patel for his help making μ CT measurements, and Dan Loesche for his help acquiring tissue samples. This work was supported, in part, by NIH/NIAMS Grant Nos. R01AR057433 and P30AR057235.

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