

Influence of Marrow on Ultrasonic Velocity and Attenuation in Bovine Trabecular Bone

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Abstract. Measurements of ultrasonic velocity and specific differential attenuation (SDA) were obtained on 24 bovine trabecular bone specimens from the femoral condyles. The measurements were obtained using two pairs of ultrasonic transducers, one with a low nominal center frequency (500 kHz) and the other pair with a high nominal center frequency (1 MHz). The ultrasonic velocity and specific differential attenuation associated with the bone samples were determined both with and without marrow, i.e., replacing the marrow with water in the pores of the trabecular bone. Significant increases (2.1% and 2.9%) in the velocity of ultrasound were observed after removal of the marrow, for the low and high frequency transducer pairs, respectively. In contrast, significant decreases (−6.5% and −8.8%) in SDA were observed after removal of the marrow, for the low and high frequency transducer pairs, respectively. The bone densities (BD) of the samples were also determined using single photon absorptiometry (SPA). Correlations between ultrasonic parameters and bone densities for samples both with and without marrow were found to be similar. For example, for the 1 MHz transducer pair, the correlation between BD and velocity was $r = 0.86$ with marrow, and $r = 0.89$ without marrow. This study also compared the results obtained using a contact (no water bath) technique and an insertion (with a water bath) technique of ultrasonic measurements. For the high frequency transducer pair, the correlation coefficients between the two methods were $r = 0.99$ and $r = 0.93$, for the velocity and specific differential attenuation, respectively. Similar results were found for the low frequency transducer pair as well. In addition, approximately equal correlations between BD and ultrasonic velocity and SDA were also found, indicating that contact and insertion measurements provide essentially equivalent information.

Key words: Osteoporosis — Ultrasonic assessment — Specific differential attenuation — Ultrasonic velocity — Broadband ultrasound attenuation.

ease, especially osteoporosis, has been proposed [1, 2] as an alternative to radiation-based bone densitometry, as for example with dual energy X-ray absorptiometry (DXA). In contrast to current ionizing electromagnetic radiation-based densitometric methods, ultrasound is a mechanical wave and interacts with bone tissue in a fundamentally distinct manner. Therefore, ultrasound has the potential for providing information not only on bone mass but on architecture and overall bone “quality” as well [2].

Ultrasonic assessment of bone relies primarily on two fundamental measurements, namely, the velocity and attenuation of the ultrasonic wave. Velocity is a measure of the speed with which the ultrasound propagates through the bone tissue and is measured in meters per second (m/second). Attenuation, on the other hand, is a measure of the loss of energy after the ultrasonic wave has propagated through the bone tissue, and is measured in nepers or decibels (dB). Since for bone the attenuation is frequency dependent and may be reasonably well approximated by an affine function over a specified frequency range, it is usually quantified in terms of the average slope of the attenuation. This differential attenuation is measured in units of dBMHz^{-1} and has been termed broadband ultrasound attenuation (BUA). The differential attenuation, BUA, may be normalized with respect to tissue thickness to produce the specific differential attenuation, specific differential attenuation (SDA), and then is expressed in units of $\text{dBcm}^{-1}\text{MHz}^{-1}$.

Many *in vitro*, *in vivo*, and clinical studies have reported significant correlations between trabecular bone mineral density (BMD) and ultrasonic velocity and attenuation; see for example [3–10]. Nevertheless, there is as yet a relatively limited understanding of how ultrasound interacts with cancellous bone tissue. From a physical standpoint, this interaction is quite complex, and relatively few models have been set forth for describing it. One potentially useful modeling approach characterizes ultrasonic interactions with bone as propagation through a fluid-filled porous medium, and some degree of progress has been made using the principles of Biot theory [11–13]. This characterization leads analytically to the demonstration that ultrasound propagation through bone is dependent on several factors, including the properties of the fluid which saturates the pores of the cancellous bone tissue [11, 14]. Thus, a natural question to ask with respect to ultrasonic wave propagation in bone is how the presence of marrow affects the velocity and attenu-

Ultrasonic assessment of bone for managing metabolic dis-

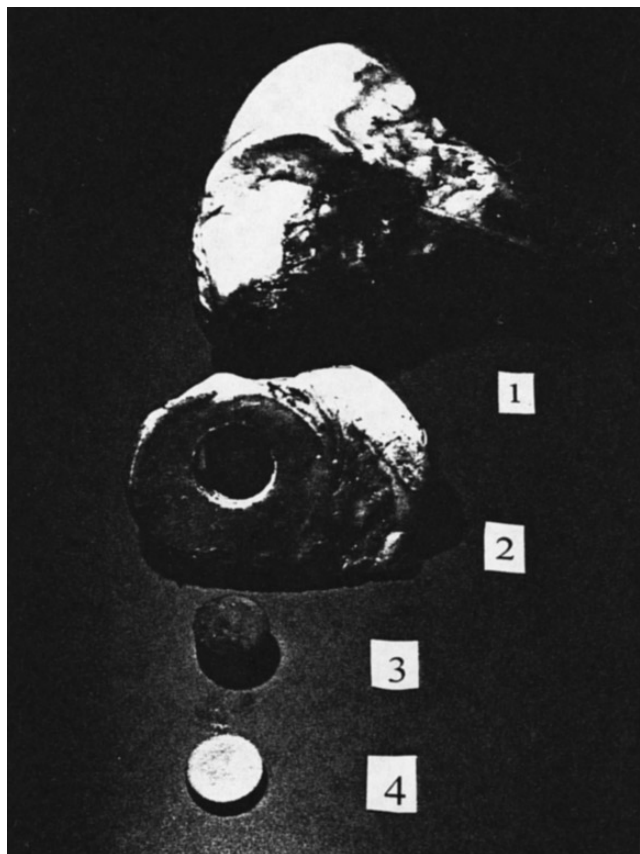


Fig. 1. Drilling and slicing of the trabecular bovine bone core: (1) distal femur, (2) condyle slice, (3) raw core, (4) final core.

ation measurements. Besides being potentially useful for clarifying the mechanisms of interaction of ultrasound with cancellous bone, this information may also prove useful for relating the results from clinical investigations to *in vitro* studies in which the marrow is often removed.

As a second part of this study, we also compared the measurements of ultrasonic attenuation and velocity on bovine cancellous bone samples using a standard insertion technique with those obtained using a contact method. Most clinical [16–23] and *in vitro* studies with human bone [24] and animal bone [25–27] have been carried out using insertion techniques, in which the bony member or bone sample is placed in a water bath between two ultrasonic transducers. For clinical measurements especially, the use of a water bath is inconvenient and also does not make transducer positioning as straightforward as in a contact approach. Thus, we wanted also to examine the effect of using contact measurements compared with measurements on the same bone samples using an insertion method.

Materials and Methods

Sample Preparation

Twelve fresh bovine femurs were acquired from a slaughterhouse within 12 hours of death, and a 2.7 cm diameter by 0.8 cm cylinder of trabecular bone was cut from each distal medial and lateral condyle (Fig. 1), respectively, using a drill with an attached drill

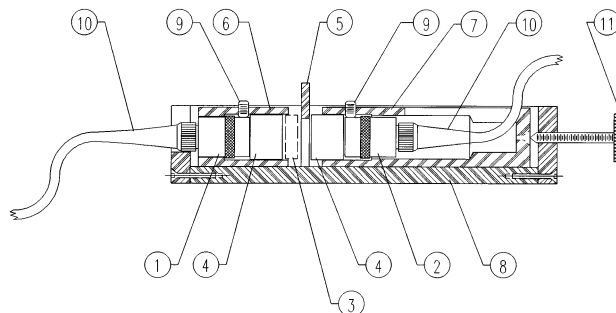


Fig. 2. The ultrasonic measurement apparatus: (1) receiver transducer, (2) transmitter transducer, (3) bone sample, (4) gel pad, (5) spacer, (6) receiver transducer holder, (7) transmitter transducer holder, (8) plate, (9) set screw, (10) transducer cable, (11) compression screw.

corer and a two-blade saw with constant irrigation. The orientation of the bone core was chosen arbitrarily but in such a manner as to allow for consistent positioning of the drill corer. The 24 trabecular bone cylinders were kept in 0.9% saline solution in a refrigerator at 4°C until the day of the BMD and ultrasonic measurements, which were carried out within 3–4 days. Defatting of the samples was carried out using an ethyl-methyl-isopropyl alcohol and acetone mixture with a 3:1 proportion and an ultrasound cleaner to accelerate the marrow removal process. The samples were kept in 0.9% saline solution in a refrigerator at 4°C until subsequent ultrasound testing (an additional 3–4 days) and maintained under vacuum to ensure total saturation of the samples' pores.

BMD Measurement

Each sample's BMD was evaluated using single photon absorptiometry (SPA) (Model 2780, Norland Corp., Fort Atkinson, WI, USA). These measurements were carried out on the samples before the marrow was removed. The scanner was calibrated using the manufacturer's bone standard on a weekly basis. The bone sample circular surface was divided into eight regions, and eight rectilinear scans with the sample submerged in water were taken encompassing the entire sample; the area of each region was used to calculate the total BMD area-weighted mean in g/cm^2 , which was then divided by the sample thickness d to get the bone density (BD) in units of g/cm^3 .

Ultrasonic Measurements

Two $\frac{3}{4}$ " diameter transducer pairs were used in this study, with nominal frequencies of 0.5 MHz and 1 MHz, respectively (Models #V318-SU and #V314-SU, respectively, Panametrics, Inc., Waltham, MA). For the contact measurements the transducers were spaced $d_t = 4.4$ cm apart (Fig. 2). For the water bath insertion measurements the transducers were separated by $d_t = 6.9$ cm in order to allow a sample holder to be inserted into the acoustic measurement path. This change in distance has already been shown not to have a significant effect on the velocity or attenuation values [30, 31]. In the contact method, two gel pads (Aquaflex, Parker Laboratories, NJ) were used and the transducers were placed in direct contact with the gel pad surface, using basic ultrasound gel for coupling.

For both insertion and contact mode measurements, the source transducer was excited by a pulser receiver card (Model SR-9000, Matec Inc., Hopkinton, MA) installed in a portable computer (PC-LCD 486DX2/66MHz, Computop Inc., La Mirada, CA). The acoustic signal measured by the receiving transducer was recorded on a digital oscilloscope card (Model Compuscope 220, Gage Applied Sciences, Inc., Montreal, Quebec, Canada) at a 40-MHz

Table 1. Influence of marrow on ultrasonic velocity and attenuation

		With marrow	Without marrow	Difference	Correlation coefficient
500 kHz	SDA (\pm SD)	31 (\pm 3.3)	29 (\pm 2.6)	-2	0.90 ($P < 0.0001$)
	Velocity (\pm SD)	1607 (\pm 53)	1642 (\pm 55)	+35*	0.96 ($P < 0.0001$)
1 MHz	SDA (\pm SD)	57 (\pm 7.3)	52 (\pm 6.3)	-5*	0.97 ($P < 0.0001$)
	Velocity (\pm SD)	1687 (\pm 67)	1735 (\pm 70)	+48*	0.97 ($P < 0.0001$)

* Denote statistical significance at the $P = 0.05$ level

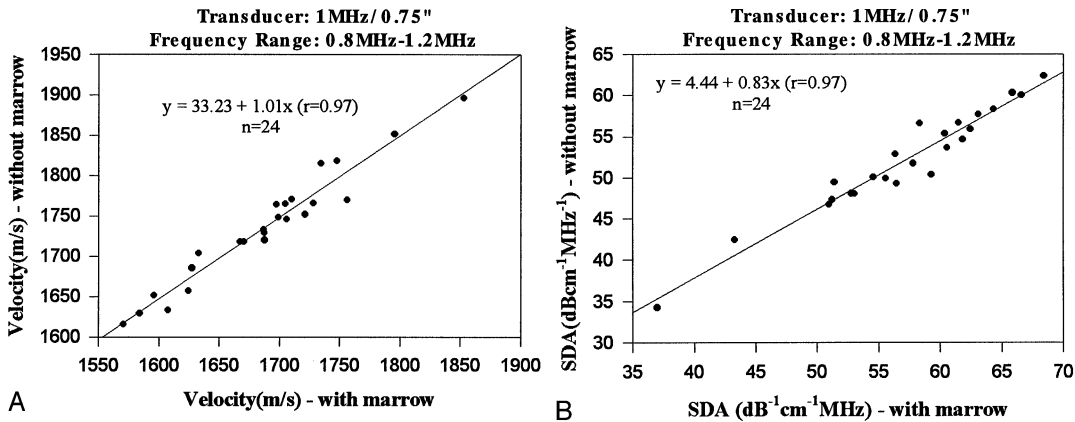


Fig. 3. (A) Ultrasonic velocities with and without marrow in the samples for the higher frequency (1 MHz) transducer pair for the 24 bovine trabecular samples. (B) Specific differential attenuations with and without marrow in the samples for the higher frequency (1 MHz) transducer pair for the 24 bovine trabecular samples.

sampling rate, and stored for subsequent analysis. For both the insertion and contact measurements, a waveform that propagated through water only was also collected and served as a reference to determine the velocity and attenuation of each bone sample. For the evaluation of the ultrasonic attenuation, the discrete Fourier transforms of the acoustic waveforms were evaluated (MATLAB 4.0, The MathWorks Inc., Natick, MA) and used to calculate each sample's acoustic transfer function [30]. An estimate of the specific differential attenuation in units of $\text{dBcm}^{-1} \text{MHz}^{-1}$ was obtained using a least-squares straight line fit over the frequency range 300–700 kHz for the 500 kHz transducer pair data, and 0.8–1.2 MHz for the 1 MHz transducer pair data. The bone sample ultrasonic activity, v_s , was evaluated using a pulse transit time technique appropriately adapted to the contact and insertion methods (see Appendix for the explicit equations used in the velocity calculations). Three independent sets of velocity and attenuation data from each sample were recorded and averaged to obtain the mean velocity and mean specific differential attenuation associated with each sample.

Statistical Analysis

The regression and statistical analyses were made with a statistical software package (SigmaStat, Jandel Scientific Software, San Rafael, CA). Statistical significance was chosen at the $P = 0.05$ level.

Summary of Protocol

The twenty-four bovine trabecular bone specimens were measured densitometrically (SPA) and ultrasonically (velocity and attenuation) in both insertion and contact modes, with their marrow intact. Following marrow removal, all 24 bone samples were again measured ultrasonically, in the insertion mode only.

Results

Influence of Marrow on Ultrasonic Attenuation and Velocity

Table 1 shows the mean ultrasonic velocity for the bone samples both with and without marrow for the 500 kHz and 1 MHz transducer pairs. As may be seen, replacement of marrow by water led to an increase in the mean ultrasonic velocity of 35 m/second (+2.1%) and 48 m/second (+2.9%) for the 500 kHz and 1 MHz transducer pairs, respectively. Table 1 also shows the mean ultrasonic specific differential attenuation for the bone samples both with and without marrow for the 500 kHz and 1 MHz transducer pairs. As may be seen, replacement of marrow by water led to a reduction in the mean SDA of $2.0 \text{ dBcm}^{-1} \text{MHz}^{-1}$ (-6.5%) and $5.0 \text{ dBcm}^{-1} \text{MHz}^{-1}$ (-8.8%) for the 500 kHz and 1 MHz transducer pairs, respectively. Table 1 also shows the linear correlation coefficients between the respective velocities and specific differential attenuations with and without marrow for both frequency ranges, obtained using the insertion technique.

Figure 3A and 3B show the ultrasonic velocity and attenuation (SDA), respectively, for bone samples with marrow versus values obtained for bone samples without marrow, for the higher frequency (1 MHz) transducer pair. A similar plot (not shown) exists for the lower frequency (500 kHz) data as well. Figure 3, together with Table 1, show that there was a high degree of linear correlation ($r \geq 0.9$) obtained for the four pairs of ultrasonic data associated with the marrow-filled and marrow-free samples.

Table 2. Comparison between insertion and contact ultrasonic techniques

		Insertion	Contact	Difference	Correlation coefficient
500 kHz	SDA (\pm SD)	31 (\pm 3.3)	32 (\pm 2.9)	+1	0.91 ($P < 0.0001$)
	Velocity (\pm SD)	1607 (\pm 53)	1612 (\pm 53)	+5	0.99 ($P < 0.0001$)
1 MHz	SDA (\pm SD)	57 (\pm 7.3)	56 (\pm 7.0)	-1	0.93 ($P < 0.0001$)
	Velocity (\pm SD)	1687 (\pm 67)	1699 (\pm 67)	+12	0.99 ($P < 0.0001$)

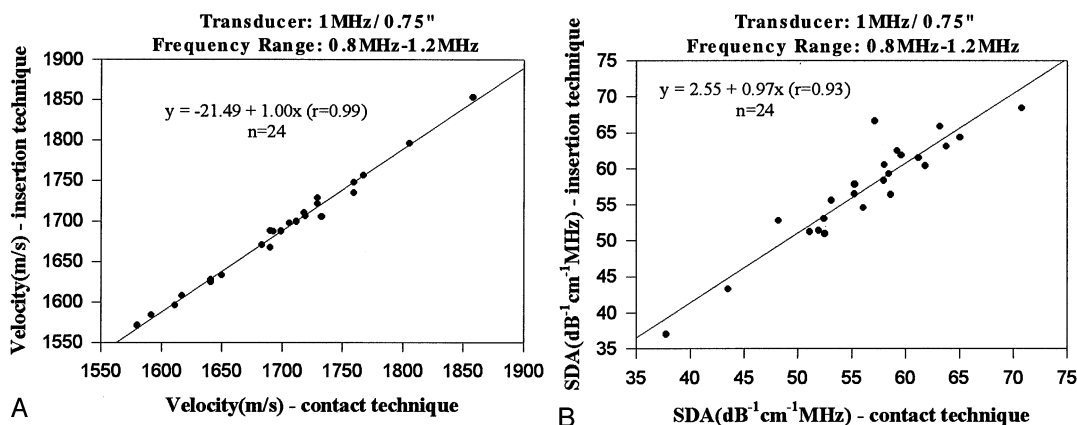


Fig. 4. (A) Ultrasonic velocities for the insertion and contact measurement techniques for the higher frequency (1 MHz) transducer pair for the 24 bovine trabecular samples. (B) Specific differential attenuations for the insertion and contact measurement techniques for the higher frequency (1 MHz) transducer pair for the 24 bovine trabecular samples.

Comparison Between Ultrasonic Insertion and Contact Techniques

Table 2 shows the mean ultrasonic velocities for the bone samples using an insertion and contact measurement technique for the 500 kHz and 1 MHz transducer pairs. As may be seen, there is essentially no significant difference in any of the ultrasonic parameters for the two measurement techniques. Table 2 also shows the linear correlation coefficients between the respective velocities and specific differential attenuations for the contact and insertion techniques. Figure 4A and 4B present the ultrasonic velocity and specific differential attenuation, respectively, made with the insertion and contact techniques for the 1 MHz transducer pair. Similar results were obtained also for the 500 kHz data.

Correlations with Bone Density and Precision of the Measurements

Table 3 summarizes the linear correlation coefficients between velocity and specific differential attenuation and BD for the low (300–700 kHz) and high frequency (0.8–1.2 MHz) ranges and for the contact and insertion techniques. As may be seen, there were relatively high and approximately equivalent correlations between velocity and bone density for the samples made with either the contact or insertion techniques, for both the low and high frequency ranges. For example, the low frequency transducers produced correlations of $r = 0.79$ and $r = 0.80$ between velocity and BD for the insertion and contact techniques, respectively. There was also qualitative agreement exhibited between SDA and BD, with respect to the contact and insertion techniques, for the low and high frequency ranges. In particular, the correlations were both weakly negative for

the low frequency case, and weakly positive for the high frequency measurements.

Finally, the average precisions (i.e., coefficients of variation) of the insertion technique were found to be 0.55% and 1.44% for the velocity and attenuation, respectively. The average precisions of the contact technique were 0.39% and 2.1% for the velocity and attenuation, respectively.

Discussion

Two subjects related to ultrasonic assessment of bone tissue have been studied. The first concerned the effects of marrow on the values of ultrasonic velocity and specific differential attenuation. Since the majority of *in vitro* ultrasonic studies on cancellous bone have been carried out without marrow although clinical data always includes the effects of marrow, it would be interesting to determine what contribution, if any, the marrow has on the ultrasonic parameters. Several basic results emerge from this part of the study. The first is that the correlations of ultrasonic velocity and specific differential attenuation with bone density remain essentially unaffected by removal of the marrow and its replacement with water. Thus, with respect to the presence or absence of marrow, the results from *in vitro* and clinical studies may be compared insofar as bone density estimation is concerned. Although the actual regression equations may be different, the overall information content in the two cases appears to be approximately equivalent. The second important aspect of these results relates to the actual changes produced by marrow removal on the ultrasonic parameters. The increase in ultrasonic velocity after marrow removal is consistent with the fact that the velocity of ultrasound in water is higher than the velocity of ultrasound in fat [32]. For example, the velocity in fat is 1450 m/second whereas the

Table 3. Linear correlations between bone density and ultrasonic velocity and attenuation

		Bone density		
		Insertion		Contact
		With marrow	Without marrow	With marrow
500 kHz	SDA	$r = -0.50$ ($P = 0.013$)	$r = -0.41$ ($P = 0.048$)	$r = -0.32$ ($P = 0.125$)
	Velocity	$r = 0.79$ ($P < 0.0001$)	$r = 0.86$ ($P < 0.0001$)	$r = 0.80$ ($P < 0.0001$)
1 MHz	SDA	$r = 0.22$ ($P = 0.307$)	$r = 0.16$ ($P = 0.436$)	$r = 0.28$ ($P = 0.180$)
	Velocity	$r = 0.86$ ($P < 0.0001$)	$r = 0.89$ ($P < 0.0001$)	$r = 0.86$ ($P < 0.0001$)

velocity in water at the temperature used in this experiment was 1495 m/second. This represents an increase of about 3.1%, which is not that far from the increases observed for the bone samples after marrow removal, namely, 2.1% and 2.8%, for the 500 kHz and 1 MHz transducers, respectively. Thus, it appears that the relative changes in ultrasonic velocity for a trabecular bone sample is strongly influenced by the relative changes in velocity associated with modifications of the material (decreased viscosity) in the trabecular pore spaces. In our case, assuming an average increase of 2.5% for the velocity after marrow removal, then about 80% ($2.5 \div 3.1$) of the total or potential increase in velocity is actually observed. Also of interest is the fact that the percent increase in velocity is greater for the higher frequency (1 MHz) transducer pair. This is somewhat unexpected in view of the fact that the ultrasonic velocity in water and in fat is not significantly affected by the change in frequency [32], and also that higher frequency ultrasound has been assumed to be more strongly dependent on the trabecular bone material *per se* [33]. Clearly these are questions that remain to be more fully explored.

In contrast to the ultrasonic velocity, the specific differential attenuation is decreased by removal of the marrow. This decrease can be attributed to the fact that the attenuation, i.e., the SDA, of the marrow is significantly greater than the attenuation due to water, which can be considered to be essentially equal to zero. A reasonable value for the marrow material can be assumed to be about $1 \text{ dBcm}^{-1} \text{ MHz}^{-1}$ [32]. The actual changes observed in the specific differential attenuation, namely, $-2.0 \text{ dBcm}^{-1} \text{ MHz}^{-1}$ and $-5.0 \text{ dBcm}^{-1} \text{ MHz}^{-1}$, for the 500 kHz and 1 MHz pairs, respectively, are thus much larger than the changes expected based on the differences in attenuation properties of the water and marrow. This too should be further investigated in order to better understand ultrasound-trabecular bone interactions. It should, however, be pointed out that direct comparisons between the *in vitro* results presented here and clinical studies should be carefully considered, since many other factors such as the effects of cortical bone and overlying soft tissue need to be taken into account. It should also be noted that one recent study did examine the influence of fat on ultrasonic measurements [15]. In this study, ultrasonic measurements were made on fat samples, phantoms and cadaver heels, as well as on adult volunteers. The overall effect of fat was found to reduce the ultrasound velocity and not to affect the acoustic attenuation. However, this study did not measure the ultrasonic parameters before and after removal of marrow, but rather attempted to correlate

total fat content with ultrasonic velocity and attenuation. In addition, the velocity as reported was not the actual velocity associated with the heel but rather a systematically biased one, due to the use of a fixed normalizing length constant.

The second subject studied in this report dealt with the comparison between contact and insertion modes of ultrasonic measurements. As pointed out earlier, contact measurements would be much preferred in clinical applications since water baths are inconvenient and can make repositioning of the foot somewhat problematic. Our results demonstrated two main elements. First, the overall correlations of velocity and attenuation with BD were not significantly affected by using a contact method in place of an insertion (water bath)-based approach. Second, the velocities and specific differential attenuations produced by the contact and insertion modes were, respectively, highly correlated. Thus, and as may have been expected, the same information appears to be present in the two different types of ultrasonic measurements. We should note, however, that we have not fully addressed the issues of precision and reproducibility, which will depend strongly on the specific experimental conditions used in the respective techniques [28].

In summary, we have reported on both the effects of marrow removal and a comparison of contact and insertion measurements in trabecular bone samples for estimating ultrasonic velocity and specific differential attenuation. Our results suggest that contact measurements should be considered in clinical applications as they appear to provide essentially equivalent information as compared with water bath approaches. The marrow aspect of this study allows the possibility of comparing clinical with *in vitro* data, as well as providing useful information for further elucidating the processes by which ultrasound interacts with cancellous bone tissue.

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Appendix

The estimation of velocity was carried out in the time domain, according to the principle of time of arrival of signal energy. The estimation of velocity can also be achieved in the frequency domain, through the use of phase unwrapping and linear models, as

described by Kaufman et al. [31]. However, since most prior studies have relied on the pulse transit time technique, we chose to use that method here as well.

The times of arrival of the reference and sample signals are defined to be the times at which the maximum absolute values of the respective signals occur. Using τ_s and τ_r to denote the times of arrival of the sample and reference signals, respectively, the ultrasonic velocity, v_s , in the bone sample using the insertion technique can be evaluated according to the following expression:

$$v_s = \frac{1}{\frac{1}{v_r} - \frac{(\tau_r - \tau_s)}{d}} \quad (\text{A-1})$$

where v_r is the velocity of ultrasound in the reference medium, in this case water ($v_r = 1495$ m/second) and d is the sample thickness.

The velocity calculation must be modified for the contact method in order to account properly for the gel pads in the sample measurement. First, an effective velocity, v_e , is calculated as follows:

$$v_e = v_r \frac{d_t}{d_t + v_r(\tau_s - \tau_r)} \quad (\text{A-2})$$

In eq. (A-2), d_t is the distance separating the ultrasound transducers, which remains fixed for the sample and reference measurements. The ultrasonic velocity, v_s , associated with the bone sample is then given by

$$v_s = v_e v_{\text{gel}} \frac{d}{v_{\text{gel}} d_t - v_e(d_t - d)} \quad (\text{A-3})$$

where v_{gel} is the velocity of ultrasound in the gel pad. This velocity was determined by a pure insertion measurement, and was found to be $v_{\text{gel}} = 1621.5$ m/second.

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