Acoustic Properties of Bovine Cancellous Bone in the Frequency Range of 0.5-2 MHz

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Abstract

Most previous studies using ultrasound for osteoporosis diagnosis have employed ultrasound in a frequency range of 0.2-1 MHz. In this study, acoustic properties of the 12 defatted bovine cancellous bone specimens were investigated in vitro. Speed of sound (SOS) and broadband ultrasonic attenuation (BUA) were measured using three matched pairs of transducers with the center frequencies of 1, 2.25, and 3.5 MHz, respectively, in order to cover a broad frequency range of 0.5-2 MHz. The relative orientation between ultrasonic beam and bone specimens was the mediolateral (ML) direction of the bovine tibia. SOS showed significant linear positive correlations with apparent density for all three pairs of transducers of 1 MHz, 2.25 MHz, and 3.5 MHz, respectively. BUA showed relatively weak correlations with apparent density for the pairs of transducers of 1 MHz and 2.25 MHz. Furthermore, in the measurement with the pair of 3.5 MHz transducers, BUA was independent of apparent density. SOS and BUA were only weakly correlated with each other. The linear combination of SOS and BUA showed significant correlations with apparent density. These results suggest that the frequency range up to 1.5 MHz may be also useful in the osteoporosis diagnosis.

I. Introduction

Quantitative ultrasound (QUS) technique is now widely used for non-invasive assessment of osteoporosis [1]. Bone mineral density (BMD) is regarded as the most important parameter for the assessment of osteoporotic fracture risk. BMD can be measured at specific fracture-related skeletal sites by dual energy X-ray absorptionmetry (DEXA). Ultrasound has been found to measure bone characteristics of calcaneus, tibia, and patella. This technique has some advantages over DEXA. It is less expensive, relatively simple, portable, and does not apply ionizing radiation. Moreover, ultrasonic parameters may reflect BMD as well as structural characteristics of bone. Most commercial devices report values for SOS, BUA, or their combination in the os calcis. SOS is related in a predicted manner to elasticity and density of cancellous bone, whereas BUA is related to both density and structure.

The main objective of the present study is to investigate the relations of SOS, BUA, and the linear combination of SOS and BUA with apparent bone density over a broad frequency range up to 2 MHz, which has received comparatively little attention so far. Acoustic properties of the 12 defatted bovine cancellous bone specimens were investigated in vitro. SOS and BUA were measured using three matched pairs of transducers with the center frequencies of 1, 2.25, and 3.5 MHz, respectively, in order to cover a broad frequency range of 0.5-2 MHz.

II. Materials and Methods

Twelve cancellous bone specimens were obtained from the proximal end of bovine tibia. Using a rotary electric saw we cut the specimens to make parallel and plane surfaces. Their orientations were chosen so that the ultrasound could pass through the specimens along the ML direction of the tibia. They are the same orientations with those in vivo measurements commonly performed with commercial bone sonometers. The specimens were defatted by boiling the specimens for 1 hour in water. Defatting was assumed not to significantly affect ultrasonic measurements because the acoustic properties of defatted trabecular bone have showed just slightly different from those of bone with marrow left intact. The thickness of the specimens varied from 12 to 20 mm. To remove air bubbles, the specimens were degassed under vacuum. After removing air bubbles, the specimens were kept at room temperature prior to ultrasonic measurements.

Specimen density was assessed from separate measurements of mass and volume. Apparent density, the ratio of the defatted tissue mass to the total specimen volume, was employed. Mass was measured with a balance. Volume was determined by measuring the volume difference with and without the specimens using a mass cylinder. Thickness between two parallel planar surfaces was measured by calipers.

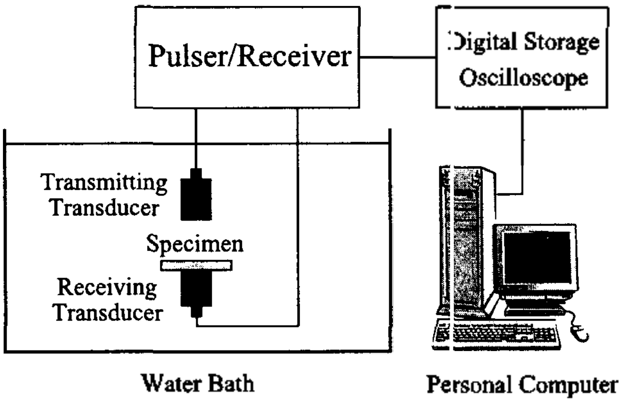

Figure 1. Schematic diagram of the experimental setup for the ultrasonic measurements.

A schematic diagram of the experimental setup for the ultrasonic measurements is shown in **Figure 1**. Ultrasonic measurements were performed in a water bath maintained at room temperature. The temperature was measured for each experiment and ranged between 16 °C and 19 °C. A 200 MHz computer controlled pulser/receiver (Panametrics 5900PR) was used. Three matched pairs of coaxially aligned transducers (Panametrics, 0.5" diameter) with the center frequencies of 1 MHz (V303), 2.25 MHz (V306), and 3.5 MHz (V382) were oriented vertically to make it possible to mount the specimens directly on the face of the receiving transducer. Received ultrasonic signals were acquired using a 500 MHz digital storage oscilloscope (LeCroy LT322) and stored on computer for off-line analysis. A through-transmission method was used to measure SOS and BUA. Using two opposing coaxially-aligned transducers, transmitted signals were recorded both with and without the bone specimen in the acoustic path. The bone specimens were large enough to cover the receiving transducer face.

To measure SOS, arrival times of received broadband pulses were measured with and without specimen in water path. SOS, c_s, can be estimated from

$$c_s = \frac{c_w}{1 - \frac{c_w \Delta t}{d}}, \tag{1}$$

where d is the thickness of the specimen and Δt is the difference in arrival times. The temperature-dependent speed of sound in distilled water, c_w, is given by [2]

$$\begin{aligned} c_w &= 1402.9 + 4.835 \times T \\ &\quad - 0.047016 \times T + 0.00012725 \times T^3, \end{aligned} \tag{2}$$

where T is the temperature in °C. Each arrival time was taken to the first zero crossing time. The procedure was repeated with each face of the specimen oriented to be the incident face. The two measurements obtained for each specimen were averaged to obtain a single value of SOS for each pair of transducers with the center frequencies of 1, 2.25, and 3.5 MHz.

BUA was analyzed using the same signals acquired for our SOS measurements. A fast Fourier transform (FFT) was used to obtain the power spectra of the transmitted signals with and without specimen in water path. The signal loss as a function of frequency was obtained by subtracting the power spectrum obtained through the specimen from the reference non-attenuating power spectrum obtained through water. A linear fit was performed over the bandwidth of interest, and the slope of the fitted line was divided by the thickness of the specimen to obtain a value of BUA in units of dB/cmMHz. This parameter is often referred to as "normalized broadband ultrasonic attenuation" (nBUA). Clinical systems generally do not normalize attenuation to calcaneal thickness. Due to the anatomical variations in the bone size of human subjects, it would seem reasonable to attempt to normalize for bone thickness. For the first pair of 1 MHz transducers, the usable frequency bandwidth was 0.5-1 MHz. It was 0.5-1.5 MHz for the second pair of 2.25 MHz transducers and 0.5-2 MHz for the third pair of 3.5 MHz transducers. These frequency bandwidths used in this study provided good signal to noise ratios. A relatively small bandwidth of 0.5-2 MHz for the pair of 3.5 MHz transducers was selected to reduce the effect of noise at low signal levels particularly at the upper frequency limit, due to the high attenuation of the denser bone specimens.

III. Results

In the present study, the relative orientation between the ultrasonic beam and the bone specimens was the ML direction of the bovine tibia where the trabeculae are aligned in the perpendicular direction to propagation at normal incidence. Although the experiments were carefully performed, the separation of the fast and slow waves was not observed from the signals transmitted through any bone specimens. The fast and slow waves completely overlap and are observed as if a single wave propagated. **Figure 2** shows a reference signal only through water and a typical transmitted signal through a bone specimen using the pair of transducers of 1 MHz. The bone signal arrives earlier due to the faster speed of sound in bone than that in water. The center frequency downshift due to frequency-dependent attenuation is also evident.

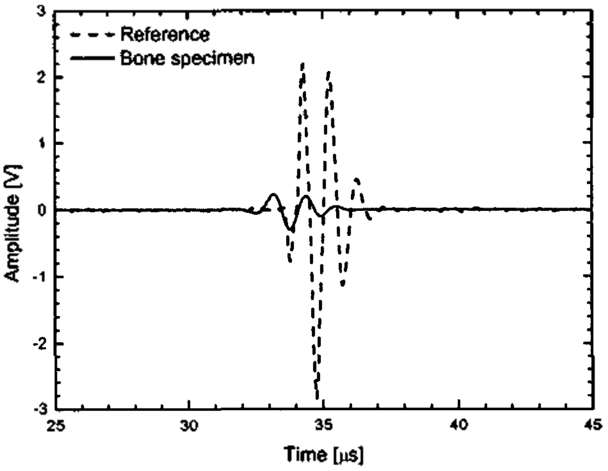

Figure 2. Reference signal only through water and a typical transmitted signal through a bone specimen using the pair of transducers with the center frequency of 1 MHz.

BUA in the frequency range up to 2 MHz has been measured with three different pairs of transducers in defatted bovine cancellous bone in vitro. **Figure 3** shows three attenuation curves obtained with three matched pairs of transducers for a representative specimen, and linear fits for the attenuation coefficient as a function of frequency over three different frequency bandwidths.

Figure 4 shows the correlations between SOS and apparent density. Separate linear fits were performed for each of three pairs of transducers. SOS shows significant linear positive correlations with apparent density for all three pairs of transducers of 1 MHz ($r = 0.61, p < 0.03$), 2.25 MHz ($r = 0.68$, $p < 0.01$), and 3.5 MHz ($r = 0.59, p < 0.04$), respectively. A p value is the probability that the correlation coefficient r is zero.

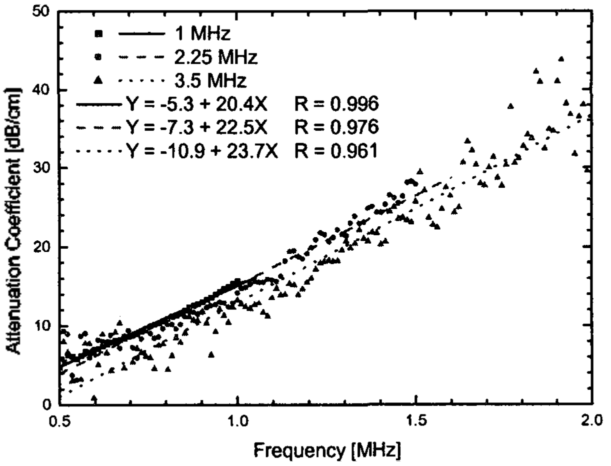

Figure 3. Attenuation curves for a representative specimen, and separate linear fits performed for the attenuation coefficient as a function of frequency over three different frequency bandwidths from different transducer pairs.

Figure 5 shows the correlations between BUA and apparent density. BUA shows moderate linear positive correlations with apparent density for the pairs of transducers of 1 MHz (bandwidth 0.5-1 MHz: $r = 0.55, p < 0.06$) and 2.25 MHz (bandwidth 0.5-1.5 MHz: $r = 0.45, p < 0.14$). However, in the measurement with the pair of 3.5 MHz transducers (bandwidth 0.5-2 MHz), BUA is independent of apparent density ($r = -0.03$, $p < 0.93$). The average correlation coefficient of the linear fit for the attenuation coefficient as a function of frequency is $r =$ 0.996 for the 1 MHz pair, $r = 0.976$ for the 2.25 MHz pair, and $r = 0.937$ for the 3.5 MHz pair.

Figure 6 shows the correlations between SOS and BUA. Separate linear fits were performed for each pair. SOS and BUA are only weakly correlated with each other (1 MHz: $r = 0.33, p < 0.28$; 2.25 MHz: $r = 0.37, p < 0.23$; 3.5 MHz: $r = 0.21$, $p < 0.50$).

The linear combination of SOS and BUA shows significant correlations with apparent density for all three pairs of transducers (1 MHz: $r = 0.71$; 2.25 MHz: $r = 0.71$; 3.5 MHz: $r = 0.61$). The multiple regression model for the prediction of apparent density is based on the following regression equation:

$$\text{apparent density [g/cm}^3\text{]} = A + B_1 \times \text{SOS [m/s]} + B_2 \times \text{BUA [dB/cmMHz]},$$

where A is the intercept and B's are the regression coefficients to be chosen according to the principle of least squares, representing the independent contributions of each independent variable to the prediction of the dependent variable.

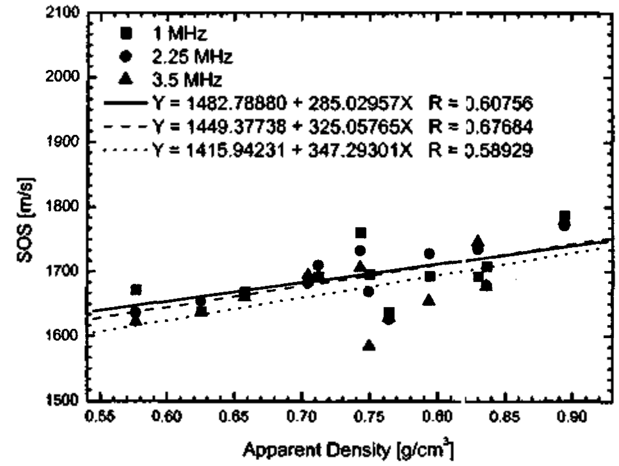

Figure 4. Correlations between SOS and apparent density.

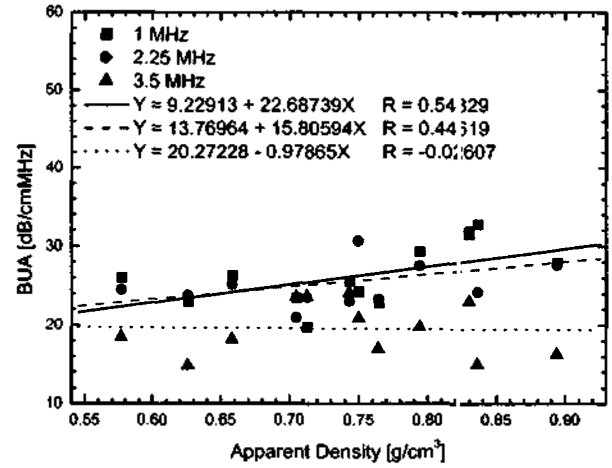

Figure 5. Correlations between BUA and apparent density.

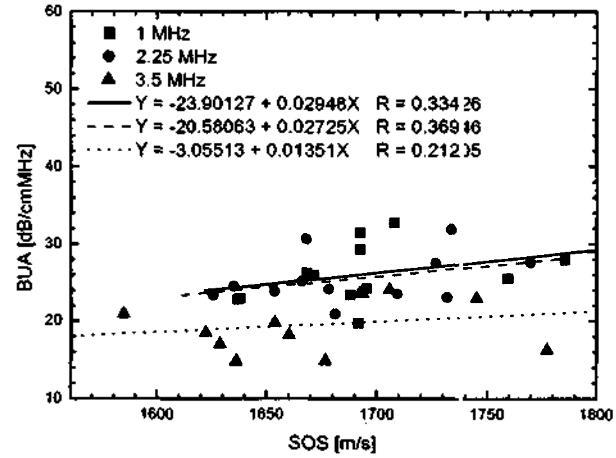

Figure 6. Correlations between SOS and BUA

IV. Conclusions

We investigated the relations of SOS, BUA, and the linear combination of SOS and BUA with the apparent density of bovine cancellous bone. Three matched pairs of transducers with the center frequencies of 1, 2.25, and 3.5 MHz were used in order to investigate the relations over a relatively broad frequency range of 0.5-2 MHz. The results demonstrate several facts. It is clear that for the range of bone densities studied, SOS was a more consistent and accurate predictor of apparent bone density than BUA. For all three pairs of transducers, SOS showed significant linear positive correlations with apparent density. The same facts cannot be stated for BUA. The correlation between BUA and apparent density was relatively weakened for high-density bovine cancellous bone. This is a typical characteristic of high-density cancellous bone. It was also found that SOS and BUA were only weakly correlated with each other. This correlation is different from the previous studies, which have reported a strong correlation between SOS and BUA in low-density human calcaneus [3]. Additionally, the linear combination of SOS and BUA appeared to offer a significant improvement in the accuracy for the prediction of apparent bone density. These results suggest that the frequency range up to 1.5 MHz may be also useful in the osteoporosis diagnosis.

Acknowledgements

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