

BONES CHARACTERISATION WITH ULTRASOUND

J. LITNIEWSKI, A. NOWICKI

INSTITUTE OF FUNDAMENTAL TECHNOLOGICAL RESEARCH,
ULTRASONIC DEPARTMENT
POLISH ACADEMY OF SCIENCES

ul. Świętokrzyska 21, 00–049 Warsaw, Poland

and

A. SAWICKI

NATIONAL FOOD AND NUTRITION INSTITUTE AND WARSAW OSTEOPOROSIS CENTER,
Warsaw, Poland

Macroscopic, X-ray methods for bone quality assessment are mainly based on porous bone density measurements. The bone quality is a property that is difficult to define, as it is related to both density and structure of the bone. In recent years, several new ultrasonic diagnostic methods have been developed to examine bones “in vivo”. These methods are based on measurements of the velocity (SOS – speed of sound) and attenuation (BUA – broadband ultrasound attenuation) of waves penetrating porous bones. The large interest in these methods is a result of the fact that they provide information not only about the bone density but also about their structure without using ionizing energy. The principal element that determines the bone strength is the trabecular structure of a porous bone. In our project we measure acoustic properties of a single trabecula using a scanning acoustic microscope and we introduce the system for “in vivo” measurement of an overall properties of a calcaneus (a heel bone).

1. SCANNING ACOUSTIC MICROSCOPY MEASUREMENTS

Ultrasonic microscopy makes it possible to assess in vitro the quality of bones and to examine their structure. At high frequencies, the acoustic microscope ensures sufficient resolution for imaging the internal structure of a trabecular bone and of a single trabecula (Figs. 1 and 2).

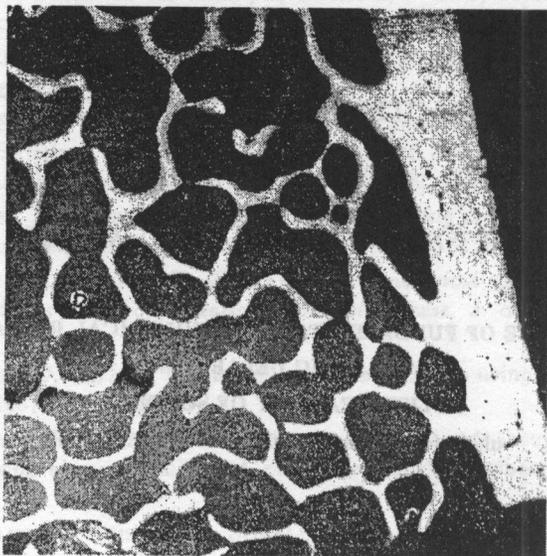


FIG. 1. The acoustic microscope image of the trabecular structure of cancellous bone.

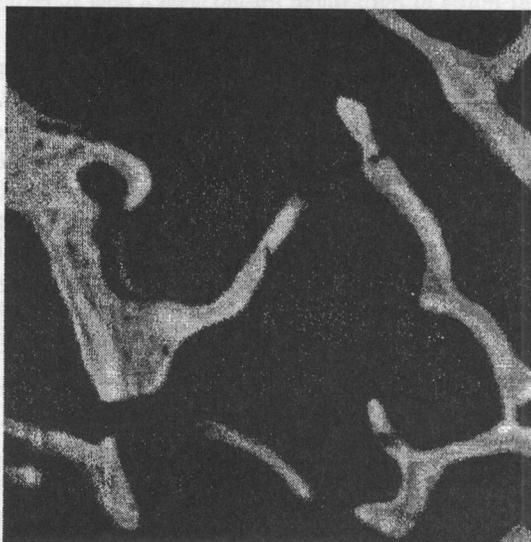


FIG. 2. The acoustical microscopic image of a cross-section of trabecular bone destroyed by osteoporosis.

The unique properties of the acoustic microscope make it possible to measure and to image acoustic properties of a single trabeculae, namely the acoustic impedance and velocity of longitudinal waves in selected areas of a porous bone, including samples from patients who suffer from metabolic bone diseases, such as osteoporosis, osteomalacia and osteoidosis.

The samples of a porous pelvic bone pre-submerged in methyl methacrylate were prepared at the Food and Nutrition Institute. Flat, 0.5 mm thick parallelepiped samples were sliced using a wire saw. In order to obtain a very smooth surface for microscopic examination, its upper layer was removed from it beforehand using a microtome. A sapphire sample with the impedance $z = 44$ MRayl was used as the impedance standard in defining the reference reflection. The value of the reference signal was obtained as the mean echo amplitude from the sapphire surface image (200×200 pixels) while keeping intact all the orientations of the microscope transmitter and receiver, such as those applied when bone samples are imaged.

Scanning Acoustic Microscope (SAM), built at our laboratory, was used for imaging and measurements. The microscope is working at the frequencies of 35, 100 and 200 MHz and make it possible to obtain surface and subsurface images. The images are stored in the computer memory, greatly facilitating their further processing.

Over the incidence angle range up to about 20 degrees, the reflection coefficient of a longitudinal wave on the water-bone boundary may be taken as constant. A 100 MHz head with a lens with a 20-degree half V-angle was used for the imaging. The application of a lens with a small V-angle allows for the normal wave incidence on the sample surface to be assumed in the model of wave and bone interaction.

2. IMPEDANCE AND VELOCITY DETERMINATION

The brightness of images of flat samples obtained using SAM focused on the surface being imaged mainly depends on the reflection coefficient at the water-sample boundary.

The dependence between the bone impedance Z_b , water impedance Z_w and that of the reference medium Z_r can be represented as:

$$Z_b(x, y) = \frac{Z_w[A_b(x, y)(Z_r - Z_w) + A_r(Z_r + Z_w)]}{A_b(x, y)(Z_r - Z_w) - A_r(Z_r - Z_w)}$$

where $A_b(x, y)$ is the echo amplitude at the point (x, y) of the bone image, and A_r is the amplitude of the reference signal (a reflected wave focused on the surface of the sapphire sample).

The microscopic trabecula images obtained were processed according to the formula given above providing impedance distribution images. In the selected areas the average impedance values were calculated using 50×50 impedance points.

In the same area, the longitudinal wave velocity was measured using the $V(z)$ method [1]. The $V(z)$ technique is used for surface waves velocity determination. In the case of a bone, the focused acoustic beam excites lateral longitudinal leaky wave, which propagates on the bone surface with the velocity of a longitudinal wave producing the characteristic oscillations of $V(z)$ curve. By spectral processing, the period of oscillations was determined and next used for velocity calculation.

We have examined samples of trabecular bone obtained by biopsy from patients with osteoporosis (12 cases), osteomalacia (10 cases) and osteoidosis (8 cases). In selected areas of each sample, average impedance and longitudinal wave velocity were measured. Then, the density of the bone sample was calculated (Table 1).

Table 1. Mean values of impedance, velocity and density found for single trabeculae obtained from patients with metabolic bone disease.

	impedance	velocity	density
osteoporosis	7.2 MRayl	3.9 km/s	1.8 g/cm ³
osteomalacia	4.4 MRayl	3.4 km/s	1.3 g/cm ³
osteoidosis	4.9 MRayl	3.2 km/s	1.5 g/cm ³

Osteoporotic trabecula can be clearly distinguished from other bone samples. They are characterized by relatively high velocity of ultrasounds, high impedance and density that are close to the values found for a cortical bone. Much lower values are found for samples from patients with osteoidosis and osteomalacia. Medical description of these metabolic bone diseases explains the results found experimentally. Osteoporosis is characterized by the lack of the mass of trabecular bone but the bone building the trabecula remains almost unchanged. In case of osteoidosis and osteomalacia, the mineralization process is disturbed what results in lowering acoustic velocity and density.

3. IN VIVO DETECTION OF BONE DISEASE WITH ULTRASOUND – COMPARISON WITH BONE DENSITOMETRY

There is an increasing interest in development of noninvasive diagnostic techniques for detection of osteoporosis and predicting the risk of a bone fracture. For several years ultrasound was recognized as a very promising method, successfully competing with well-established X-ray techniques. Both methods study the trabecular structure of a cancellous bone. The surface/mass ratio of this bone is 10-fold greater than that of the cortical bone. Since the turnover of the bone is a surface-based event, any disturbances of this process caused by osteoporosis or other metabolic bone disease are expressed earlier and more distinctly at cancellous sites than in the cortical bone.

Most of the publications concerning ultrasonic bone investigations describe the experiments performed in transmission mode. Also, the commercially available ultrasonic densitometers utilize the signals transmitted through the bone. Two acoustic properties of a trabecular bone are measured. The slope of the frequency-depended attenuation – known as broadband ultrasonic attenuation (BUA) and a speed of sound (SOS). Usually, the measurements are performed on a heel bone – calcaneus. This bone is well suited for ultrasonic investigations. It is composed mainly of a trabecular bone and it is easily accessible. The thickness of the cortical shell and overlying soft tissue is relatively low and the shape of the bone assures good penetration of ultrasound. Acoustic results were often verified by comparing the bone mineral density – BMD (g/cm^2) assessed by X-ray densitometry. It was shown [2, 3], that the bone density can be measured by ultrasound as well as with X-rays and that the bone fracture risk of elderly women can be predicted based on ultrasonic results.

In our in vivo experiments 0.3 MHz – 0.7 MHz frequency ultrasound was used. The corresponding wavelength (5mm-2mm) at the upper frequency limit approached characteristic dimensions of the trabecular structure (trabecula thickness 0.1 mm – 0.4 mm, trabecula spacing 0.5 mm – 2 mm). We believe that characterization of a bone by scattered signal and determination of a trabecular structure cross-section function introduces a new quality into ultrasonic assessment of bone status.

BUA (Broadband Ultrasonic Attenuation) results are well correlated with BMD (Bone Mineral Density). Since the scattering of acoustic waves by trabecular structure is a fundamental component of attenuation, the scattering should also correlate with BMD. This dependence was confirmed by P. LAUGIER *et al.* [4], who found a moderate correlation between the integrated backscattered coefficient and BMD.

In our approach we were searching for new indicators of trabecular bone properties. Two of them, the shift of an amplitude spectrum of a transmitted

wave and a TSC (Trabecular Structure Cross-section) function were found to be good candidates for further study.

4. INSTRUMENTATION

We built a system for *in vivo* heel examination (Fig. 3). The system consisting of a pair of wideband, flat, composite transducers (diameter = 25 mm) was operating at a central frequency equal to 0.58 MHz.

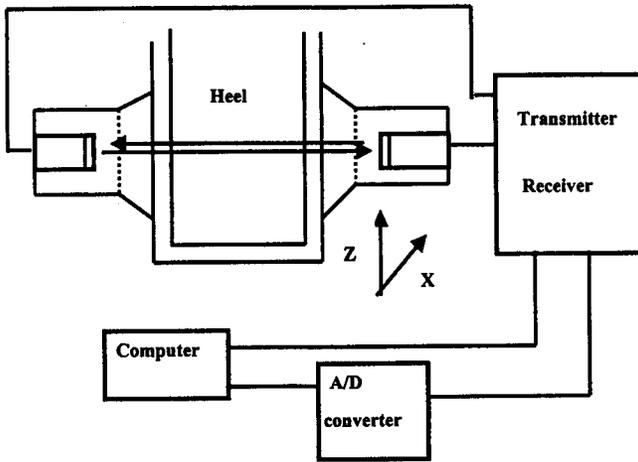


FIG. 3. Simplified block diagram of the system for ultrasonic *in vivo* examination of a heel bone.

Transducers were mounted in small housing filled with water (Fig. 3). The wall of the housing opposite to the transducer face was made of a thin latex membrane. The foot under examination was placed between the latex membranes. By positioning the transducers (1 mm step) the area of measurement could be selected. Then, by increasing the pressure in the transducers set up, the heel was surrounded by a "water balloon", which fitted the foot shape. Good transmission at the skin/rubber interface was assured by applying ultrasonic coupling gel.

The transmitting/receiving system was driven by a computer. The transmitter was developed in our laboratory. It generated the burst-like signal of one period duration at the center frequency of 0.5 MHz and peak to peak amplitude of 100 V. The pulse repetition rate was equal to 1kHz. Signals from the receiving transducer were amplified by a wide band receiver (0.1 MHz – 1 MHz) and were next captured by an A/D converter (12 bit, 20 MHz). Up to 32 successive echoes were averaged and stored in the computer for further processing. In order to assure the shortest possible ultrasonic pulse (the wide band transmission) a set

of transmitting-receiving transducers was carefully designed. Both transducers were fabricated out of composite material (diced PZT filled with epoxy resin) resulting in their relatively low acoustic impedance (12.2 MRayl), and high coupling coefficient ($k_t = 0.6$). Acoustic backing together with the front quarter wave matching to water assured the overall 6dB bandwidth better than 400 kHz around 0.58 MHz. A great effort was put into eliminating any spurious reflections coming from the backing of a transducer, which could disturb scattered waves. Both transducers could operate in transmission and receiving modes.

The following set of data was collected for each patient: 1° The reference signal, which is a signal transmitted through water only (Fig. 4A), 2° the signal transmitted through the heel (Fig. 4B), 3° the signal reflected from the heel (Fig. 5), and 4° the signal scattered from the selected heel area, corresponding to the position of trabecular bone (Fig. 6).

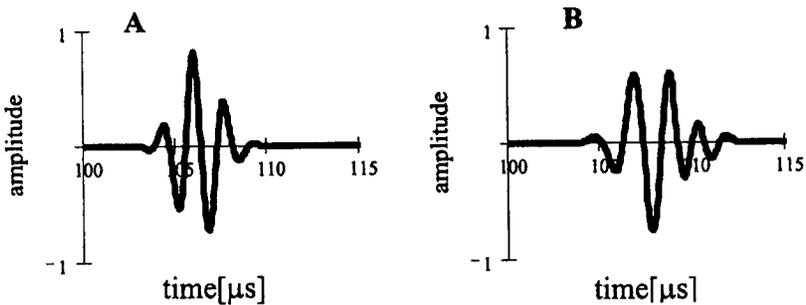


FIG. 4. A - acoustic pulse transmitted through the water and B - the signal transmitted through the heel (multiplied by a factor of 63).

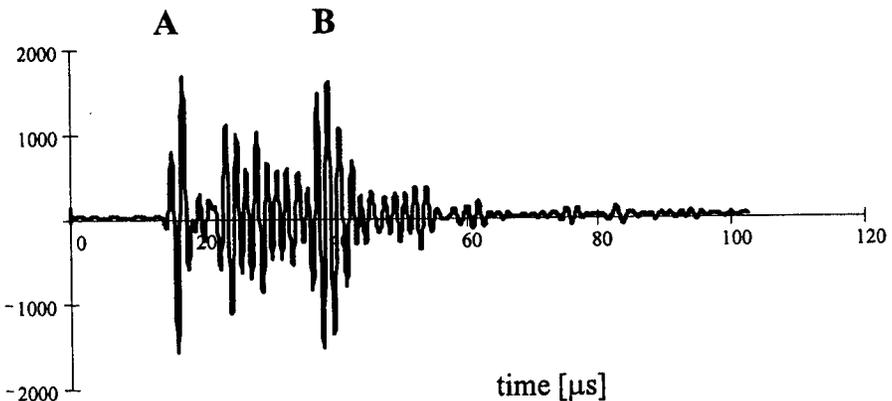


FIG. 5. The signal reflected from the heel, A and B denote reflections at the water-tissue and tissue-bone interfaces respectively.

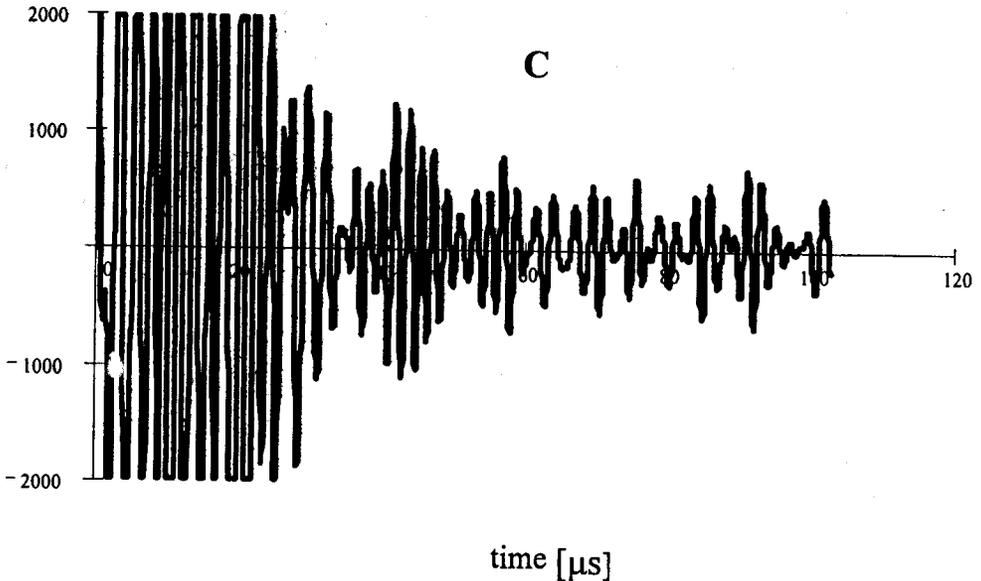


FIG. 6. The signal reflected from the heel received at high amplification. C-area corresponds to the backscattering at the trabecular bone structure.

The transmitted mode was used for BUA, shift of the spectrum and velocity evaluation. We also tried to experiment with velocity dispersion assessment but we could not find any rational interpretation of the results and they were not included in this paper. The reflected signal allowed us to select the trabecular bone area. The scattered signal was used for trabecular structure cross-section function determination.

5. MEASUREMENTS

The ultrasonic measurements were performed on a group of patients (86 women, age 35–88) of the Warsaw Osteoporosis Center (Warsaw, Poland). The approval of Local Ethical Review Board was obtained prior to the measurements. Always, the left heel was examined. For the whole group the transmitted ultrasonic signals were recorded. In the subgroup of 54 women the backscattered echo was also recorded and next processed according to the described procedures allowing BUA coefficient, shift of the signal spectrum and TSC function to be calculated.

For all patients, the left hip BMD was measured using a Hologic QDR-4500A apparatus. The BMD values were compared with the ultrasonic results.

Pearson's correlation coefficient and probability p -value, which describe the significance of the correlation, were used to assess the dependencies between ultrasonic bone status indicators and BMD.

6. TRANSMISSION MODE – ATTENUATION STUDY

Signals transmitted through water and through the heel (Fig. 4) were used to calculate the frequency-dependent attenuation and a shift of signal spectrum. The ratio of amplitude spectrum of a signal transmitted through bone and through water determines the attenuation (Fig. 7). The slope of the attenuation curve for a given frequency range defines the BUA. Attenuation is given in dB instead of dB/m because it was measured for a whole heel thickness and not for a unit length of tissue. BUA values were calculated for all transmission data and these coefficients were correlated with bone mineral density.

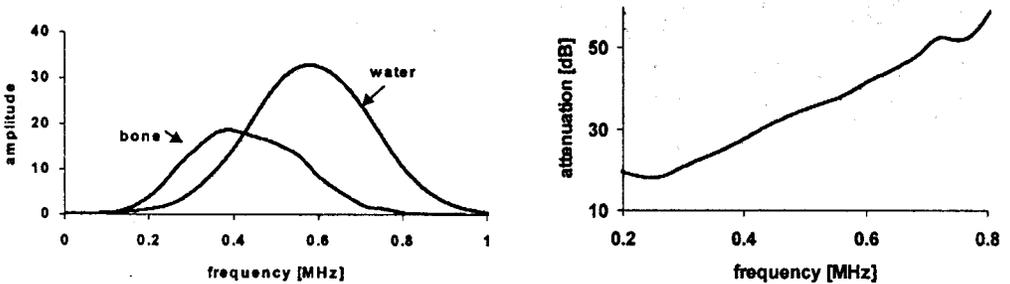


FIG. 7. Amplitude spectra of transmitted pulses (bone spectrum magnified 30 dB) and a ratio of these spectra describing the attenuation/frequency dependence for a heel bone. A slope of a linear regression of this curve determines BUA.

7. TRANSMISSION MODE – FREQUENCY "SHIFT" STUDY

The frequency-dependent attenuation influences the spectrum of transmitted pulses. Since the higher frequencies in the spectrum are more attenuated than the lower ones, the spectrum moves to the lower frequencies. We found it interesting to determine the shift of the frequency of the spectrum (Δf) for the signal transmitted through the trabecular bone and through the water only (Fig. 8).

Values of the frequency shift were correlated with spine and hip BMD.

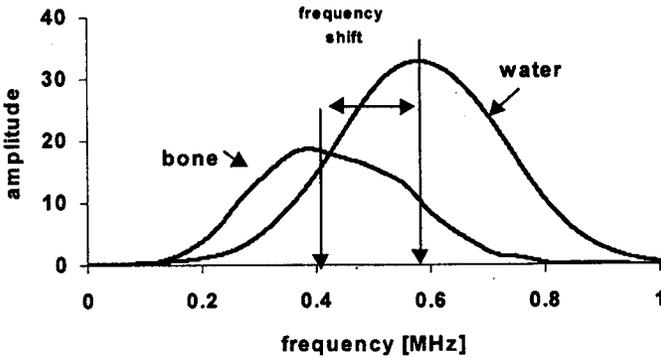


FIG. 8. Amplitude spectra of the pulses transmitted through the water and through the bone. The mean frequency of the pulse transmitted through the bone is shifted towards the lower frequencies.

8. REFLECTION MODE STUDY

In transmission mode the received wave amplitude – $U_0(f)$ depends on reflections at water-tissue and tissue-cortical bone interfaces and attenuation in the bone. In backscattering mode the received waves are additionally reflected by trabecular structure and the amplitude of the reflection depends on a local backscattering coefficient – $R(f)$. Assuming the same overall transmission coefficient at the water-tissue-bone interface for both sides of a heel ($T_1, T_2 = T$), the transmitted – $U_T(f)$ and backscattered – $U_R(f)$ signals amplitudes can be described in the following way:

$$U_T(f) = U_0(f) * T^2 * \exp(-\alpha(f) * d_0),$$

$$U_R(f) = U_0(f) * T^2 * \exp(-\alpha(f) * d * 2) * R(f)$$

where d_0 is the trabecular bone thickness, f denotes frequency, $\alpha(f)$ is the frequency-dependent attenuation coefficient and d is a distance.

For U_R collected from the middle of the bone ($d = \frac{d_0}{2}$), the ratio $\frac{U_R}{U_T}$ yields R . Comparing the amplitude spectra of transmitted and scattered signals, the frequency dependence of $R(f)$ is obtained (TSC-Trabecular Structure Cross-section function).

The spectrum of a back-scattered signal contains many random peaks and valleys (Fig. 10) and it can not be used directly for TSC function calculation. Instead we have used the following averaging method to smooth out the spec-

trum. First the part of the signal corresponding to trabecular bone was selected. Then it was divided (multiplied by semi-Gaussian window) into 16 partly overlapping sections, each $6 \mu\text{s}$ long. The length of the section was equal to the length of a transmitted pulse while the number of sections was limited by the thickness of a trabecular part of a heel bone. For each section the amplitude spectrum was calculated. Using BUA coefficient, the spectra were compensated for attenuation changes resulting from the shift of the section from the middle of the bone. Finally, the averaged spectrum was calculated and compared with the transmission wave spectrum yielding the TSC function (Fig. 10B). The TSC function was divided into several frequency ranges. Next the integrals of TSC over several frequency ranges were calculated giving numerical values (ITSC). ITSC coefficients were correlated with BMD data and BUA coefficients.

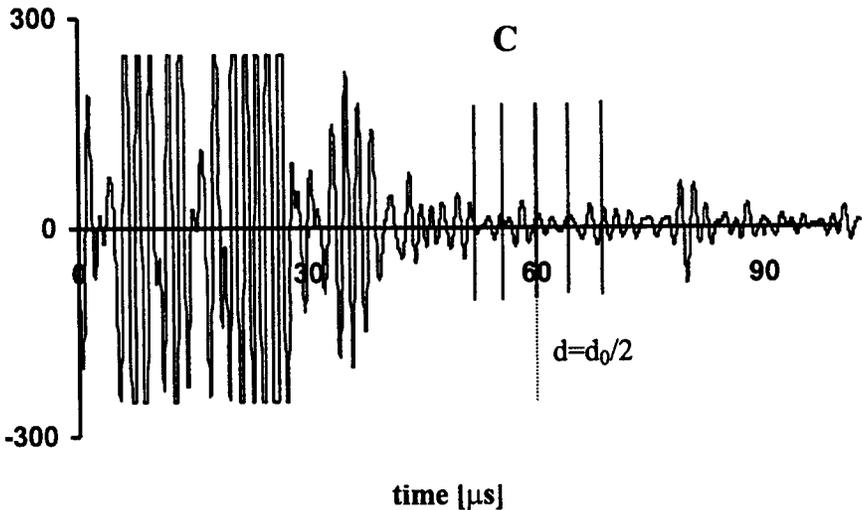


FIG. 9. The reflected signal; C – trabecular bone scattering area divided into $6 \mu\text{s}$ long sections.

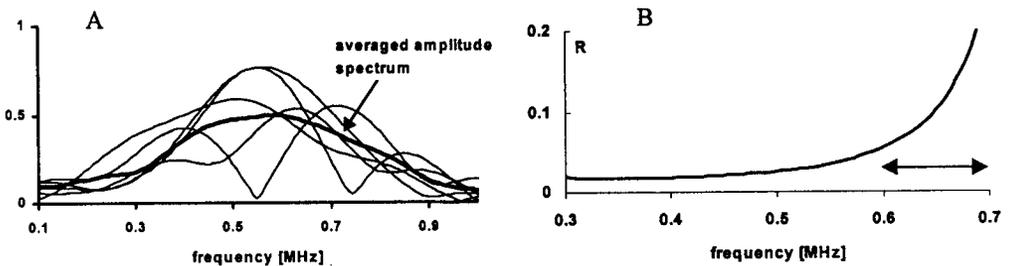


FIG. 10. A – The magnitude spectra calculated for selected signal sections received at different depth (see Fig. 9), thicker line denotes the averaged spectrum, B – TSC function—the backscattered coefficient R as a function of frequency. The marked frequency range (0.6–0.7 MHz) was used for integration.

9. CONCLUSIONS

The results of ultrasonic measurements correlated with hip BMD values are summarized in Table 2. We must stress at the beginning that a limited number of cases allows us to formulate only a weak hypothesis and conclusions.

Table 2. Pearson correlation coefficients (corr) and probability value (p) for ultrasonic and BMD measurements results.

	BUA	Δf	TSC 0.6 - 0.7 MHz
BMD-hip	corr = 0.6 $p = 10^{-3}$	corr = -0.7 $p = 10^{-4}$	corr = 0.6 $p = 10^{-5}$
BUA		corr = 0.9 $p = 10^{-6}$	corr = 0.7 $p = 10^{-6}$

BUA was calculated over the 0.3 MHz - 0.7 MHz frequency range. A moderate correlation with BMD was obtained (corr = 0.6, $p = 10^{-3}$). For some attenuation-frequency curves we have found rather serious deviations from linear dependence on frequency. In these cases, BUA (a slope of a linear regression curve) does not describe properly the attenuation. In our opinion such behavior of the attenuation-frequency curve is caused by positioning error.

The mean frequency shift Δf was found to be better correlated with BMD than BUA (corr = -0.7, $p = 10^{-4}$). These two indicators BUA and shift Δf , are strongly correlated (corr = 0.9) because they describe the same phenomenon. We believe that BUA and Δf can be used with the same efficiency but our results show that the mean frequency estimation was more robust to positioning errors when the flat transducers were used.

The TSC function found from the backscattered signal was increasing rapidly at higher frequency (0.6 MHz - 0.7 MHz) and the best correlation of integrated TSC was found for integration over this frequency range (corr = 0.6, $p = 10^{-5}$). The correlation of BMD with the TSC function integrated over the whole frequency range (0.3 MHz - 0.7 MHz) was slightly inferior. We believe that the backscattered energy depends on bone microarchitecture and describes the trabecular bone connectivity. The total bone mass increases with a number of trabeculae and/or with the single trabecula thickness increase. That explains the correlation of scattering with BMD. On the other hand, the microarchitecture of the bone can also change to some extent without mass variation. The moderate correlation of integrated TSC with BUA (corr = 0.7, $p = 10^{-6}$) suggests that the bone-transmitted wave interaction and the bone-backscattered wave interaction are due to slightly different properties of the trabecular bone. Simultaneous

application of both methods for bone examination can increase the diagnostic sensitivity of ultrasonic detection of osteoporosis and other metabolic bone disease.

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