ULTRASOUND VELOCITY IN CORTICAL BONE TISSUE WITH EXPERIMENTALLY INDUCED OSTEOPOROSIS

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Abstract

A pulse transmission ultrasonic method was applied to evaluate the quality of cortical bone with experimentally induced osteoporosis. Velocity of ultrasound (1MHz) was measured along femoral shafts in rats with disuse osteopenia. Ultrasonic data were compared with results of mechanical testing (three-point bending). Density and mineralization of bone tissue was also analyzed.

Ultrasound velocity in immobilized femora was reduced with respect to controls, both in immature bone in growing rats and in fully mineralized bones of adult animals. Immobilization induced unloading resulted in a decrease of bone strength in all groups, though in adult individuals any changes in mechanical parameters were preceded by velocity changes. On the basis of quantitative analysis it was stated that subtle changes in cortical bone tissue influenced significantly velocity of ultrasonic pulses along bone shaft. This result justifies application of ultrasound measurements for detection of bone tissue deterioration in early stages of bone diseases.

Introduction

One of the factors essential for normal functioning of bone tissue is cyclic mechanical loading with the intensity depending on the bone function. Reduced loading results in imbalance of bone metabolism followed by rapid bone loss and impairment of bone mechanical function [1].

Mechanical testing of long bones is usually applied in order to quantify the quality of cortical bone in different animal models of bone diseases [2-4]. Mechanical properties in three-point bending, a standard test of long bones, depend on mechanical properties of bone tissue, but also on the size and shape of the bone. In particular, variations of cross-sectional geometrical properties are mainly responsible for the differences in bending behavior of femur [5]. Therefore, three-point bending is a convenient tool in determining the mechanical properties of whole bone as a structure, but it has low sensitivity on estimating properties of cortical bone tissue, especially in small bones in rodent experiments.

It is well documented that ultrasound provides quantitative information on bone quality and, in particular, the velocity of ultrasound in bone is strongly affected by the elasticity of the bone tissue [6-8]. In particular, ultrasonic measurements in the MHz-range characterize well the stiffness properties of the bulk ultrastructure.

Since ultrasound is reported to be a good predictor of mechanical properties of bone, in the present study an ultrasonic method was applied to evaluate the quality of cortical bone tissue in a rat model of disuse osteoporosis. The main question addressed in this paper was whether measurement of ultrasound velocity in femoral shaft is able to detect early changes in cortical tissue.

Material and Methods

Three age groups of male Wistar rats were used in the study: G – intensively growing, Y – young adult, and A – adult. The rats in each age group were randomly divided into control and experimental. The right hindlimb of the experimental animals was immobilized against the abdomen using bandages and padded tape as previously described [9]. The hip joint was in flexion and the knee and ankle joints in extension. After two weeks of immobilization all the experimental rats were released from tapes and bandages. In each age group (G, Y, and A) rats from one experimental group, together with age-matched controls were killed (2+0). The rats from the other groups were allowed to move freely for the next two or four weeks (2+2 or 2+4).

Femora were removed and outer surfaces of the bones were cleaned mechanically from soft tissues. Femora were tested mechanically in three-point bending using a Lloyd LRX Testing Machine. The span of supports was 15 mm and deformation rate 2 mm/min (Fig.1a). The load-deformation curve was analyzed. The maximum bending force and structural stiffness (the slope of the linear part of the curve) were calculated from the curve.

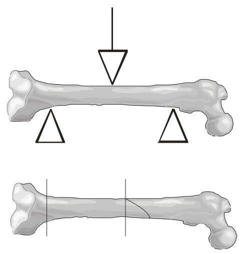

Figure 1. Scheme of bone loading in three-point-bending (a) and location of sample for ultrasonic measurements in a fractured femur (b)

The longer part of the broken femur was cut perpendicular to the long axis of the bone to obtain a tube-like cortical sample convenient for ultrasonic transmission measurements (Fig.1b). Thickness of the tube wall was 0.6 – 1.1 mm and length of the sample was 10 - 25 mm and the depending on the size of bone and location of the fracture site. Wet density of bone samples was determined by the Archimedes principle [10].

Velocity of ultrasound along the femoral shaft was measured using a pulse transmission method. The sample was positioned between two ultrasonic 1 MHz transducers connected to a computer controlled pulse generator and an analogue-digital circuit. The time of flight of ultrasonic pulses along the bone was measured in the circuit by determining the first zero-crossing for the waveform of the pulse analyzed. The measurements were repeated six times in each sample to minimize errors resulting from a large divergence between the cross-sections of the transducer and the sample. During measurements the bones were kept wet in saline solution. Elastic modulus was calculated for each sample using the formula
$E = dV^2$, [6-8].

After mechanical and ultrasonic measurements all pieces of the femora were dried in 105°C for 24 h and weighed (dry weight, m_d). Finally, bones were ashed in a muffled furnace at 630°C for 24 h and weighed again (m_a). Mineralization of the dry bone tissue (%M) was calculated as: $\% M = 100 \cdot (m_a)/ m_d$.

Number of samples was 9 - 10 per group in growing rats (G) and in young (Y) rats and 7 – 10 per group adult (A) rats. Correlations between measured parameters were calculated by using a simple linear regression. The difference in mean values was analyzed using t-test.

Results

Two weeks of unloading of a hindlimb resulted in a decrease of mechanical strength of femur in growing rats (Table 1). During remobilization an increase of measured parameters was noticed though the remobilization period was not sufficient to reach the level of controls. In both adult age groups mechanical parameters were not changed after two weeks of immobilization. However, a significant worsening of bending properties of femora in young adult rats was found during first four weeks of free remobilization. Mineral content in dry bone tissue decreased significantly in growing rats, while in adults was not affected.

Density of cortical samples from immobilized femora as well as the ultrasound velocity and calculated elastic modulus were reduced after immobilization (Table 2) in all age groups.

Remobilization resulted in certain improvement of bone properties in growing rats but not in young adults.

Table 1. Maximum bending force (F_{max}), stiffness and mineral content (by weight) in rat femora after 2 weeks of immobilization of the femur and 0, 2 or 4 weeks of remobilization.

group		F_{max} (N)	Sitffness (N/mm)	Mineral. (%)
G_{2+0}	C	96 (11)	223 (25)	65.7 (0.7)
	E	58 (6)#	136 (20)#	63.6 (1.3)*
G_{2+2}	C	123 (10)	273 (14)	67.8 (0.8)
	E	83 (10)#	214 (25)*	66.3 (1.1)*
G_{2+4}	C	148 (18)	260 (32)	67.5 (0.8)
	E	107 (17)#	241 (21)!	67.4 (0.6)
Y_{2+0}	C	145 (13)	349 (36)	68.4 (0.6)
	E	150 (15)	342 (26)	67.9 (0.7)
Y_{2+4}	C	175 (22)	390 (19)	69.2 (1.1)
	E	155 (9)*	361 (30)!	68.2 (0.8)!
A_{2+0}	C	191 (18)	415 (26)	70.5 (0.8)
	E	184 (14)	408 (18)	70.2 (0.8)

Mean (standard deviation);
!,*,# significantly different from the control, p< 0.05, 0.01 and 0.001, respectively.

Table 2: Density, ultrasound velocity (V) and calculated elastic modulus ($E_{ultr.}$) in femoral diaphyses after 2 weeks of immobilization of the femur and 0, 2 or 4 weeks of remobilization.

group		Density (kg/m^3)	V (m/s)	$E_{ultr.}$ (GPa)
G_{2+0}	C	1920 (18)	3451 (56)	22.8 (0.7)
	E	1873 (15)#	3380 (67)!	21.5 (1.0)*
G_{2+2}	C	1951 (15)	3542 (48)	24.2 (0.7)
	E	1915 (11)#	3491 (55)!	23.3 (0.8)!
G_{2+4}	C	1952 (14)	3598 (38)	25.2 (0.5)
	E	1939 (15)!	3568 (61)	24.8 (1.0)
Y_{2+0}	C	1997 (17)	3641 (46)	26.5 (0.8)
	E	1967 (13)*	3575 (46)*	25.1 (0.7)*
Y_{2+4}	C	2031 (19)	3745 (50)	28.5 (1.0)
	E	2006 (15)*	3688 (41)*	27.2 (0.7)*
A_{2+0}	C	2056 (13)	3780 (49)	29.4 (0.9)
	E	2047 (15)	3721 (49)!	28.4 (1.0)!

Relationship between density of cortical bone and ultrasound velocity in femoral shaft is shown in the Figure 2. Experimental and control samples were analyzed separately, though the difference between regression lines for both groups of samples was not significant.

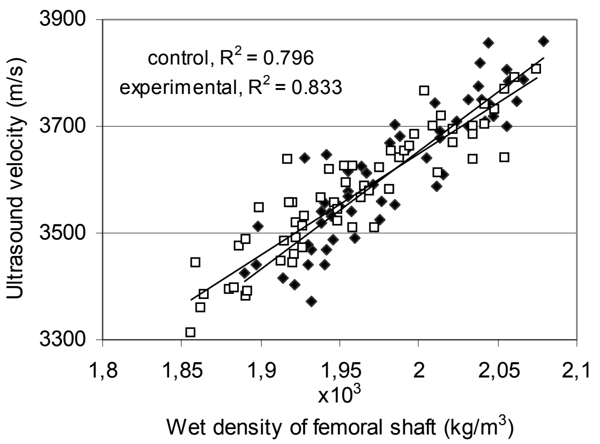

Figure 2. Relationship between density of cortical bone and ultrasound velocity in femoral shaft.

Correlation between mineralization of cortical bone and ultrasound velocity (Figure 3) is much weaker than correlation between wet density and velocity.

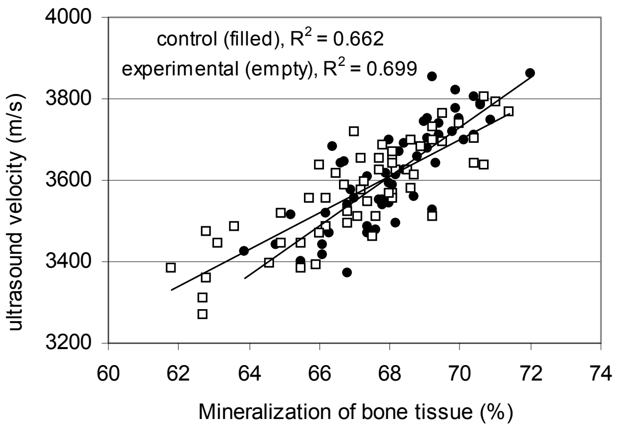

Figure 3. Relationship between mineralization of cortical bone and ultrasound velocity in femoral shaft.

The results of mechanical testing correlated significantly with the results of ultrasonic measurements (Figures 4,5), though the velocity of ultrasonic pulses femoral diaphysis is attributed to properties of cortical tissue while mechanical parameters in three point bending are influenced considerably by the size of the diaphysis cross-section.

Discussion

Ultrasonic method in evaluation of elastic properties of bone tissue was proved to be a sensitive tool for studying properties of cortical bone in different bone disorders [2,10,11].

In this work the ultrasonic method was shown to detect early changes in cortical tissue resulting from unloading of bone. The decrease of ultrasound velocity found in femoral diaphyses indicates a significant decrease of mechanical competence of the cortical tissue after temporary immobilization and after a period of remobilization both in growing and in adult individuals.

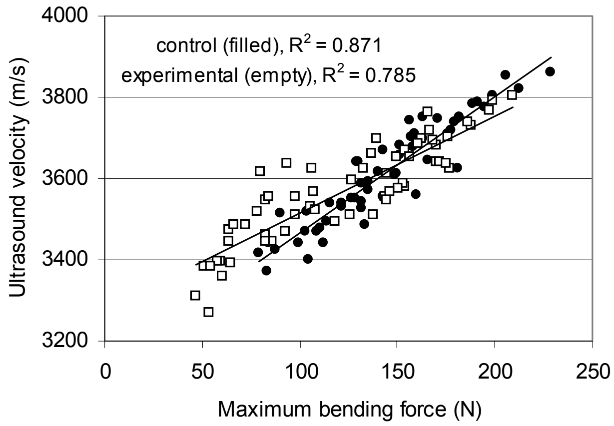

Figure 4. Relationship between maximum bending force of a femur and ultrasound velocity in femoral shaft.

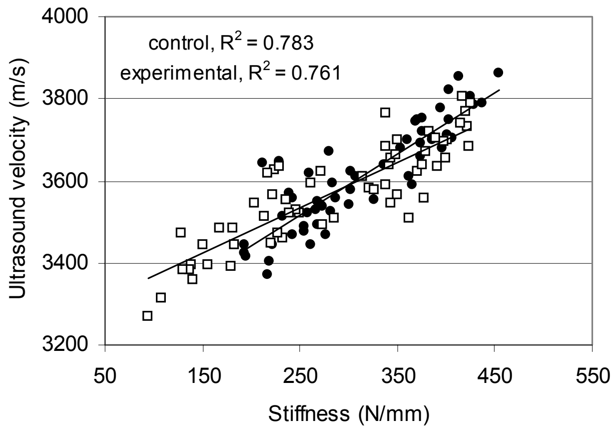

Figure 5. Relationship between stiffness of a femur and ultrasound velocity in femoral shaft.

In adult skeleton deterioration of mechanical properties of cortical tissue appear earlier than decline of the bone strength which decreases during remobilization. However, in growing rats some signs of recovery were seen after remobilization. The differences between age groups in potential to recovery result from differences in rate of bone growth and remodeling [1].

Velocity of ultrasound correlates stronger with wet density of bone tissue than with its mineralization what reflects the fact that mechanical properties of bone tissue are a function of mineralization and porosity [12].

The decrease of ultrasound velocity found in femoral diaphyses indicates a significant decrease in mechanical competence of the cortical tissue after temporary immobilization and after a period of remobilization. This decrease might be attributed to changed density, mineralization, and porosity of cortical bone tissue, as well as to a disturbed collagen fiber orientation [13]. It is possible that even if the bone mass is regained, the nonrecoverable changes in bone structure may cause permanent deterioration of bone strength. However, in the growing group tissue properties seemed to recover after a remobilization period.

On the basis of analysis of the relationships between measured parameters, it was stated that subtle changes in density of bone tissue influenced significantly velocity of ultrasonic pulses along bone shaft. This result encourages to broader application of ultrasound measurements for examination of cortical tissue in early stages of bone diseases.

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