mechanical properties of bone tissue,
diagnosis of osteoporosis, micro hardness

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THE PARAMETERS OF DENSITY AND MECHANICAL STRENGTH OF BONE TISSUE IN DIAGNOSIS OF OSTEOPOROSIS

The paper presents results of a study, in which an incomplete correlation between the mineral bone tissue density and patient susceptibility to bone fractures was confirmed. A simple model describing distribution of internal forces present in the microstructure of trabecular bone is proposed. The purpose of this model is to demonstrate that parameters of mechanical strength depend not only on the quantity of mineral material in the bone, but also on the quality of the trabecular structure. In addition, we present the results of cortical and trabecular bone micro hardness test, which are then used to calculate of the modulus of elasticity. Micro-hardness test was performed using Micro-Combi-Tester equipment. Micro-hardness was measured with Vickers diamond; analysis of displacement prosperities was carried out by Olivier and Pharr method. Young's modulus was calculated directly from the resulting unloading curve. Generally, the results for both cortical and trabecular bone tests correspond to the results known from literature. However, depending on localization of the test, slight deviations in modulus of elasticity occur. In conclusion of this research, it is proposed that the dependence between bone mineral density and Young's modulus can be calculated using samples of a normal bone and a bone with changed biomechanical properties. The aim of this research would be to estimate a quantitative coefficient, which would describe differences between mineral bone density and the bone's real density, which is responsible for the immunity to fractures.

1. INTRODUCTION

A physician diagnoses osteoporosis through a standard diagnostic procedure includes: a patient survey, subject, densitometrical, radiological, and analytical examination of the patient [2]. Well-known international journals are giving increasing attention to the clinical risk factors of fractures. Occurrence of these factors in an examined patient correlates with an increase of a relative fracture risk. Clinical factors and densitometry can be used to calculate an absolute fracture risk [3,4].

A densitometry test forms the basis for the diagnosis of susceptibility to fractures. Such a test can be made using a number of techniques, however World Health Organization (WHO) and International Osteoporosis Foundation guidelines currently recommend a Dual Energy X-ray

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Absorptiometry (DEXA), as an acceptable technique for making densitometry test of proximal femur [1,4].

In principle, DEXA is a measurement of transmission of two different effective X-ray energies through the patient body. This technique enables quantitative estimation of both types of tissue (in this case, bone and soft tissue). Additionally, using fan beam reduces the time of test to 15-30 seconds [12].

A digital X-ray detector eliminates the necessity of using a photographic plate. After diagnosis, the test results are written to a file in a computer memory. Afterwards, the software formulates a densitometry diagnosis with the use of an average algorithm. The obtained result contains information about the sum of superficial bone mineral density both cortical and trabecular. The result is presented in the unit of mass per unit of surface (g/cm^2).

Quantitative Computed Tomography is the most precise diagnosis method of measuring bone density, but in view of higher equipment cost (in comparison to DEXA equipment) it is less cost-effective and therefore not practical in screening tests [1].

Some researchers assert that relation between the bone's mineral density and a risk of fracture is known [2,12,13,16], however, it is worth noting that, from a mechanical point of view, decreasing values in parameters of bone tissue stiffness determine the risk of a fracture. These parameters are not always directly related to the bone's density, as confirmed by known clinical cases. According to Dutch research, the absolute number of fractures is not dependent on BMD [5]. In 63% of cases, fractures occurred despite a correct BMD value, or in osteophenia [6]. The result of Dutch research was also confirmed in Polish study [7].

We conclude that there are factors, other than mineral density, that increase bone's susceptibility to fractures. Among these factors are the parameters of mechanical strength of bone, which depend less on density, and more on the micro-architecture of bone tissue [15].

Bone tissue proximal femur is built with a cortical bone on the outside and a trabecular bone on the inside [1]. It is widely known, mechanical parameters and functions of each type of bone are completely different. Tab.1 contains a collection of values of selected mechanical parameters.

Tab.1.   Selected mechanical parameters of cortical and trabecular bone. (source data: [10])

Quantity	Bone	
	cortical	trabecular
Coefficient of direct elasticity E [Mpa]	$(1,5 - 2,0) \, 10^3$	$(0,3 - 1,5) \, 10^3$
Poisson's ratio V	0,29	0,46

Measurement of bone mineral density is done by averaging X-ray photography of both cortical and trabecular bone. This averaging can have significant influence on scattering of the obtained results.

Osteoporosis can first be observed in the trabecular bone [16]. The simple scheme of trabecular bone fragment is used to illustrate those processes. The two-dimensional schema presents dependence of bone strength on quality of beam structure. Participation of beams in microstructure is represented by B factor, which characterizes quality of structure.

The average Young's modulus for a cortical bone has values that range, depending on a source, from 20 GPa to 30 GPa [10], however for a trabecular bone Young's modulus is much smaller from 0,3 GPa to 1,5 GPa [10].

A participation of the fractured beam in further force transition is negligible, even though its mineral material contributes to the result of the densitometry test.

Expanding the above analysis to the whole bone, we can analyse all the beams in the trabecular bone. Distribution of major loading forces for the minor loading forces impacting each beam, can be described using the formula below:

$$\sum_{i=1}^{k} F_i = \sum_{j=1}^{l} f_j \qquad (1)$$

where:

    F_i value of force loaded of bone,
    f_i value of elementary force loaded of beam,
    k   number of forces loaded of bone
    l   number of elementary forces loaded of beam

Loading forces F_i that impact a bone are distributed for minor forces f_i, which load the beams. Value of a minor force depends on the beam's cross-section and the material building the beam. Presume that those values for all the beams are equal, then the calculation of a minor force can be described with the formula:

$$f_j = \frac{\sum_{i=1}^{k} F_i}{B} \qquad (2)$$

where:

    f_j value of elementary force loaded beam;
    F_i value of force loaded bone;
    k   number of forces loaded bone;
    B   factor characterized activity of participation of beams in microstructure (no dimensional)

B factor in a microstructure can change depending on changes in transversal intersection of every beams and depending on the number of beams. It is obvious that intersections will change and that in turn will influence participation of each beam in distribution of forces.

For the least diameter of every beam, the difference between the expected diameter and the actual diameter can be defined as:

$$\Delta d = d_{expected} - d_{actual} \qquad (3)$$

where:

    Δd value of difference of diameters;

$d_{expected}$ expected diameter, the value of which can be understood as weighted average calculated based on diameter of all beams;

d_{actual} actual diameter, which can be described as value of diameter of a given beam, at a given time.

For correct value of diameter d_{actual}, value of the difference will equal zero. For $d_{actual} < d_{expected}$ value of the difference will be positive. For $d_{actual}=0$, value of difference will be equal $d_{expected}$. For $d_{actual} > d_{expected}$ value of the difference will be negative.

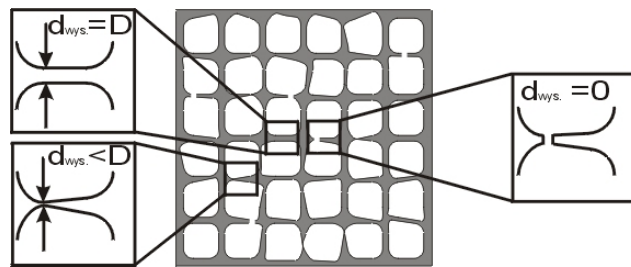Fig.1 below presents the scheme of the trabecular microstructure with each of type of beam marked .

Fig.1. Scheme of trabecular microstructure. There are beams with difference diameter. $d_{wys.}$ - actual diameter
[source data: own]

Rules for calculating B factor are presented below:

$$B = \sum_{i=1}^{b} \left(1 - \frac{\Delta d}{d}\right)_i \qquad (4)$$

where:

b   total number of beams in the structure

Δd value of difference,

Equation (4) presents dependence of the factor B on value of difference Δd for each beam. It takes proportional values to active participation of beams as difference of diameter.

For $\Delta d=0$ value 1 will be added to the sum, as such a beam takes an active (100%) part in force transition.

For $0 < \Delta d < d$ value from range (0,1) will be added to the sum, as such a beam is not fully active in the force transition.

For $\Delta d = d$ value 0 will be added to the sum, as such a beam takes an inactive (0%) role in the force transition.

For $\Delta d < 0$ the value larger than 1 will be added to the sum, as such beam takes a hyperactive (over then 100%) part in the force transition.

Taking under consideration equations (2) and (4), one can show that the force load on beam depends, among other factors, on a coefficient characterizing the microstructure of bone tissue.

This allows us to formulate the following assertions:

$\Rightarrow$ Participation of each beam in the transfer of mechanical loading in the bone depends on:
- material of which they are built (Young's modulus E of this material)
- minimum transversal intersection (diameter d)
- value stress in active beams (normal stresses σ)

$\Rightarrow$ Value force loaded single beam depends on:
- number and diameter of beams (describing by B factor)
- quality of trabecular microstructure
- and, to some extent, on quantity of material contained in trabecular bone

$\Rightarrow$ Reduction of the number of active beams caused by reduction of intersection and micro cracks or fractures increases values of minor forces f_j, which, loaded onto fewer beams, increase presence of stresses and deformations. This in turn, results in formation of subsequent cracks and fractures, which then leads to an escalation in microstructure destruction. The end result is a fracture of a bone.

The above, leads us to asking the question: "How can a densitometry test of bone mineral density be used to estimate bone's susceptibility to fracture?"

As we know, the definition of density does not give information about the material's structure, but only the quantity of material per volume unit [8, 9].

In the DEXA method, the characteristic unit is bone's mineral density measured in g/cm^2 [1].

In view of the two-dimensional character of the data medium – photography of bone – measure mass quantity per volume unit is impossible.

Additionally, only the sum of cortical and trabecular bone measurements is presented.

Quantitative Computer Tomography (QTC) provides better results including mass unit per volume unit (g/cm^3) and separate estimates for cortical and trabeclular bones' measurements. In this respect, QTC is the most precise measurement method [2].

The scale of the image in both methods has low resolution, which does not enable to take into consideration the quality of structure. Only information about quantity of material per surface (or volume) unit is given. The information about structure remains inaccessible [14, 15].

Only BMD of healthy bone will be equal to its real density. For a bone with a similar quantity of material, but a weakener structure, BMD is only seemingly correct. Actual density of the structure, which actively participates in transmitting loading, will be smaller.

In order to examine, whether this problem actually causes mistakes in the diagnosis of strength properties that make bone susceptible to fractures, we propose the study of basic samples of bone tissue, with both correct and modified mechanical properties.

2. MATERIALS AND METHODS

A laboratory test of mechanical properties of bone tissue was carried out using Mikro-Combi-Tester equipment from Swiss CSEM. This tool is capable to gathering and archiving the measurement results. All of the tool's functions are managed using dedicated software, which also permits visualization of results as charts and photographs, and measurement localization [18].

The equipment can calculate hardness and Young's modulus for soft, hard, brittle and plastic materials.

A standard hardness test is based on measurement of diagonal indentation, which is observed through a microscope. This technique relies on a subjective coefficient supplied by a somebody who performs the test. Additionally, reaction of material during such a test has a huge influence on the result. If a sample is soft, some of the material can flow out during penetration, which can cause the measurement of diagonal indentation value to be larger than expected and very diagonal. If a sample is hard during penetration, material will tend to flexion instead of plastic strain. This will cause the measurement of the diagonal indention value to be less than a real diagonal [18].

An indentation test with MCT eliminates these mistakes. The diagonal is calculated based on the depth of penetration thanks to automatic and dynamic estimation of the geometrical quantity of indetication. This main advantage of MCT test was the reason why the method was chosen to carry out our research.

Application of the-above-described method to calculate mechanical parameters of bone tissue seems reasonable due to precise localization of test.

The question was whether calculation of the modulus of elasticity based on the local parameter of the beam's material would deliver new information about the microstructure of the bone.

Pilot research of two samples of animal bone was conducted and results from this test are presented below. The first sample was a basic sample, the second was treated by methanecarboxylic acid solution. As a result of chemical reaction with hydroxiapatite structure, calcium has been removed and biomechanical properties of sample have been changed.

3. RESULTS

- Research included samples of cortical and trabecular animal bone. Samples had dimensions of about 5mmx8mmx8mm and each had one surface polished on abrasive paper of gradation of 1500 in preparation for the test. In the case a trabecular bone, measurement was taken in several distances from a cortical bone. Based on these measurements Young's modulus for each, basic and decalcified, sample was calculated. The basic sample was tested in wet state. The results are presented in
. Next, after 80 hours, both samples were tested again. The results are presented in Tab. 3

Tab.2   Results of tests for basic sample in wet state [source data: own]

distance from cortical one [mm]	Young's modulus E [GPa]			
	test 1	test 2	test 3	Average
0*	23,115	20,214	-	**21,66**
2,2	2,1204	4,3191	-	**3,22**
4,8	1,0177	0,8897	-	**0,95**

- - distance 0 means that test was done for cortical bone

Tab. 3. Results of tests for basic sample in dry state [source data: own]

distance from cortical bone [mm]	Young's modulus E [GPa]			
	test 1	test 2	test 3	Average
0*	21,6	21,8	21,7	**21,70**
1	8,24	7,9	7,38	**7,84**
3	4,73	4,54	5,63	**4,97**
5	3,69	3,98	-	**3,84**

* - distance 0 means that test was done for cortical bone

Tab. 4. Results of tests for basic decalcified sample in dry state [source data: own]

distance from cortical one [mm]	Young's modulus E [GPa]			
	test 1	test 2	test 3	average
0*	16,56	20,02	14,76	**17,11**
1	2,78	2,08	3,94	**2,93**
3	2,68	2,66	2,87	**2,74**
5	2,69	1,39	-	**2,04**

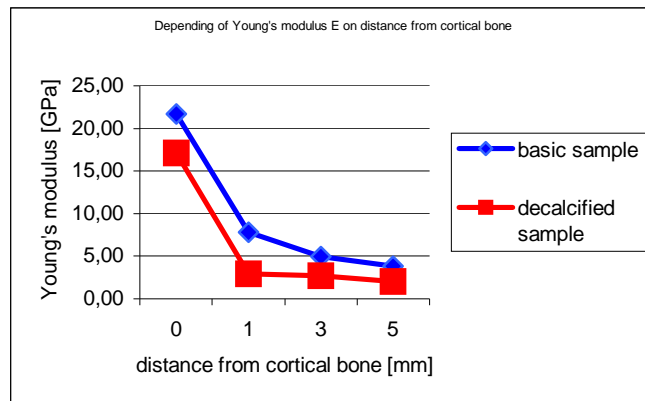* - distance 0 means that test was done for cortical bone

Fig.2. Chart is presents dependence of Young's modulus for trabecular bone on distance from cortical bone. Result for distance 0 means result for cortical bone.

According to our expectations, values of Young's modulus for the decalcified sample are lower than the results for the basic sample. Moreover, properties of a trabecular bone change depending on the distance from the cortical bone.

4. CONCLUSION

This constitutes preliminary research of properties of cortical and trabecular bones. The type of indenter was the most important factor when choosing the method of analysis. Finally, a measurement method, which uses a Vickers diamond, was selected. Similarly study was described

in other publications, where rudiments of method are presented [18]. As could be anticipated, preparation of the tested surface was very important.

The process of making a slide from the bone can cause the beams of the bone to crack and micro fracture, which can then advertently influence the measurement. Additionally, it can impact roughness of the cortical bone's surface, which, in a measurement this precise, can affect the validity of the results. Selecting an adequate loading force, especially for trabecular bone testing, is also very important. Too much loading force can cause beams to fracture.

Comparison of obtained Young's modulus results for the basic sample in wet state to values known from the literature (see: Tab.1) shows that our cortical bone results are in the 3.16% range. Results obtained for trabecular bone depend on the distance from cortical bone. Obtained values are twice as large for distance 2.2mm and they are in the middle range for 4.8mm distance.

The above deviations may be justified due to the construction of the bone's microstructure and higher value of Young's modulus for trabecular bone near cortical bone.

The aim of measuring Young's modulus for basic and decalcified samples was to present differences in biomechanical properties of chemically modified bone tissue.

Results indicate noticeable decrease in the value of Young's modulus for a decalcified sample of a bone. A 26% decrease, in the case of cortical bone, and an 87% to several times the original value decrease in the case trabecular bone. Qualitatively, this process is known from biomechanics theory, but the aim of these tests was to confirm that quantitative classification by indenter test was possible.

Indenter test and calculation of Young's modulus by Olivier and Pharr method can be performed for samples of cortical and trabecular bone. Precision of sample positioning enables accurate localization (to 1μm) of point of prick.

This method enables an indentation test and counting mechanical parameter of a single beam of bone, which has width of about 100μm.

Based on the above results of measurements using single samples, we propose to investigate much more samples to obtain statistical confirmation of the results reported in the paper.

We are especially interested in comparing of the results of the mechanical properties of bone represented by Young's modulus to the bone mineral density, represented by BMD, which is obtained through a densitometry test.

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