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Impact of some external factors on the values of mechanical parameters determined in tests on bone tissue

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The knowledge of the mechanical characteristics of bone structures is a prerequisite for theoretical, numerical, and experimental analyses describing the functioning of bone, which is a living organ. The description of the mechanical properties of bone tissue, such as conventional Young's modulus or strength, will enable the assessment of the degree of tissue degeneration through a comparison of the material properties of the examined bones to the properties determined for physiologically normal bones. However, the mechanical parameters published by different research centres often differ from each other by up to several hundred percent. These discrepancies arise primarily from the differences in the research methodology applied, and thus from many additional factors having a direct impact on the values of the mechanical parameters obtained in experimental tests. Therefore, in order to standardize and improve the interpretation of the results of measurements, we should develop universal criteria for the measuring conditions and quantify the impact of the factors being related to sample and measurements on the values obtained. In this paper, the authors present the dependence of some factors, i.e. the site and direction of sample excision as well as the rate and type of loading, on the values of the mechanical parameters. Those values were determined in experimental tests and the additional correlation coefficients proposed enabled an easier comparison of the results obtained with the values presented in the literature.

Key words: *mechanical properties of bone, in vivo and in vitro factors*

1. Introduction

From the viewpoint of mechanician, bone is the most amazing 'material', due to its ability to "sense" changing conditions and adapt to them so as to perform its essential functions. Due to the structure and properties of bone tissue, a number of authors compare bone to engineering materials, looking for similarities in their properties. Consequently, the literature contains claims that bone can be considered as a two-phase composite material. That material consists of a mineralised matrix made of collagen fibres characterised by a low elastic modulus and hydroxyapatite (HA) crystals with a high elastic modulus [24], [28]. The modulus of elasticity of two-phase materials usually ranges within the values of the elastic moduli of the phases, whereas composite strength is higher than the strength of the components tested individually. Another approach is to compare bones to glass laminate, where fibreglass is a high-modulus material (similar to that of HA crystals), whereas epoxy resin is a low-modulus material (similar in this respect to collagen) [9]. According to JACKSON [13] bone belongs to the group of biological ceramics which, like all ceramic materials, are brittle and rigid. Such materials cause measurement problems (samples are difficult to grasp and loading causes small displacements, which require sensitive measuring instruments). However, these materials have several unquestionable advantages, including the possibility of

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applying standard theories that assume linear flexibility and the possibility of using classic methods of measurement of the mechanical properties, whose results show high repeatability.

The values of the mechanical parameters, describing bone tissue response to load, are most often determined by means of mechanical tests. Some of the first and still most popular tests are the compression test and the tensile test [1]. The mechanical parameters of bones were first examined in the compression test conducted by RAUBER [22] as early as in 1876. He showed that the differences in the values of the parameter tested depend on the type of tissue. His results also demonstrate that the compressive strength of the bones parallel to the long bone axis reaches its maximum value in cubic samples. Moreover, this value is higher for tibia than for humerus, therefore the values of the mechanical parameters also depend on the sampling site and the type of the tissue examined [22].

From the time of first measurements, the problem of description of bone tissue, taking account of its mechanics, has been tackled and analysed by numerous domestic and foreign researchers [3], [4], [6], [10], [20]–[22], [27] and [29]. However, on the basis of the data obtained we can observe that the values of the mechanical parameters, determined by means of various tests, very often differ from each other. The discrepancies arise most often because of different methods of measurements and some factors having a great impact on the results obtained. Those significant differences, visible even within the same species, are due to the influence of factors directly related to the sample and the measurement. The above factors can be divided into two groups: in vivo and in vitro [30]. The in vivo group of factors, intravitally related to the research material, can include, among others, age, sex, the level of chemical and hormonal components in the organism, and the activity level and health state of the patient whose material was sampled for tests. In the second in vitro group, the factors are directly related to the sample and the measurement, i.e., the method of storage and preparation of the samples, the type, shape, dimensions, direction, and region of sample excision, the type of mechanical test, and the strain rate.

Despite a high number of the papers dealing with the determination of the mechanical properties of bone tissue, there are no guidelines on how to prepare the research material and to conduct the measurements of bone tissue. Consequently, the results presented in the literature become difficult to compare to each other. Therefore, in order to select properly the measuring conditions, it is of a paramount importance to determine the influence of factors that relate to the sample and to the measurement based on the values of the mechanical parameters determined. In papers determining the impact of factors on the mechanical parameters, we come across the values of the mechanical parameters which are most often determined for compact bone, and much less frequently for cancellous bone tissue. The main reason for this is a complicated and heterogeneous structure of cancellous tissue, which additionally complicates measurements.

The analysis of the literature shows that because of large differences in the results obtained, caused mainly due to the influence of factors connected with the measuring conditions, the main purpose of the present paper was to quantify the impact of changes on the values of mechanical parameters of such factors as: the site and direction of sampling, the loading rate, and the type of mechanical test. An appropriate choice of measuring parameters and the knowledge of the impact of changes of these factors will undoubtedly enable the performance of repeatable, standardized tests and comparison of the results obtained with the literature data.

2. Material and methods

The tests, whose main objective was to determine the impact of factors directly connected with measurement on the mechanical properties of bones, were carried out on a group of preparations consisting of: 30 veal calf femurs, 2 human femurs, and 2 brown bear femurs. The research material was obtained post mortem thanks to cooperation with the Department of Animal Anatomy and Histology of the Faculty of Veterinary Medicine at the Wrocław University of Environmental and Life Sciences and the Forensic Medicine Unit of the Wrocław Medical University. Each of the preparations, until the moment of sample preparation, was stored at a temperature of –20 °C in double plastic packaging.

In order to determine the mechanical properties of bone tissue, strength tests (compression test and three- and four-point bending tests) were conducted on bone samples (figure 1). The dimensions of the samples prepared for testing depended on the type of preparation and the type of a mechanical test applied and were as follows: 4 mm × 4 mm × 20 mm (aspect ratio of 1:1:5) for compression test, due to buckling, and 4 mm × 4 mm × 40 mm (aspect ratio of 1:1:10) for bending test. In order to determine a physical density of each sample, its mass and geometric dimensions were measured. Before mechanical tests the prepared

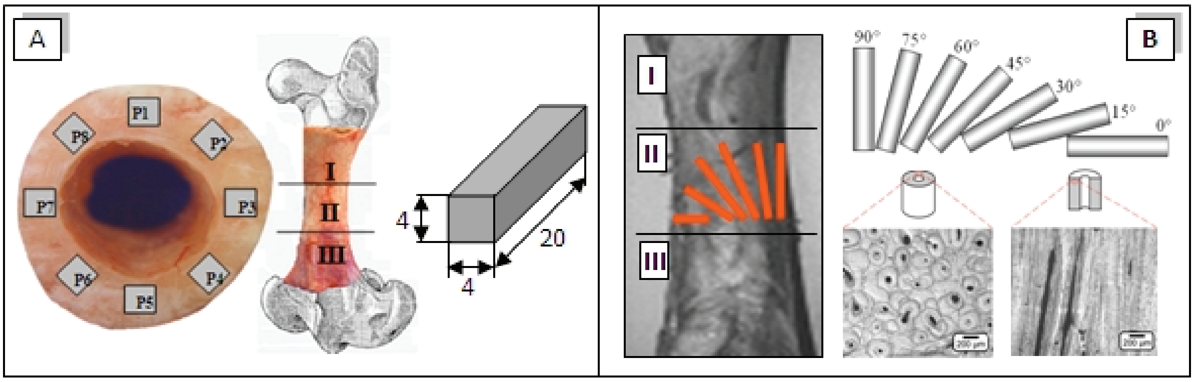


Fig. 1. Region and site of excising compact bone samples: A) used in tests of the impact of the sampling site, the loading rate, and the type of mechanical test on bone properties, B) used in tests of the impact of direction of sample excision on bone properties

samples were stored at a temperature of –20 °C. In order to determine the mechanical properties of bones, we performed uniaxial compression tests on strength testers MTS 858 MiniBionix and MTS Synergie 100. Additionally, bone tissue surface (tissue fibre architecture) was imaged using scanning microscope LEO-Zeiss 435. The samples prepared in the above way were used in tests to determine the sampling site, the excision direction, and the loading rate. In accordance with the objective of assessing the impact of individual factors in each of the test, the measurements were carried out under the same conditions of sample preparation and storage; the only changes pertained to the scope of the parameter examined.

3. Results

The tests were divided into 4 stages according to their purpose and scope. In each stage, we determined the impact of one of the four selected factors (the region and site of sample excision, sample excision direction, sample strain rate, and the type of mechanical test).

3.1. Region and site of sample excision

In the first stage, the region and the site of sample excision were determined. The research material consisted of 10 veal calf femoral diaphyses, divided into 3 regions (I – proximal metaphysis, II – diaphysis proper, III – distal metaphysis), out of which 8 cuboidal samples, 4 mm × 4 mm × 20 mm marked with P1–P8 were excised (figure 1).

The research at this stage involved the compression test at a constant rate of 0.01/s, which corresponded to strains under physiological conditions. The measurements obtained for the I stage show that both the region (I, II, and III) and the site of sample excision at the diaphyses (P1–P8) influence the values of mechanical

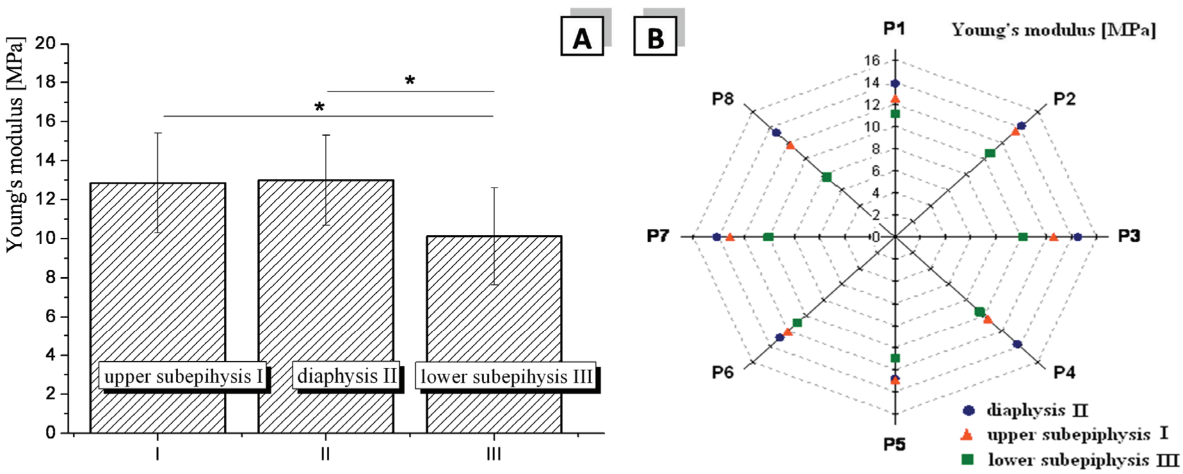


Fig. 2. Comparison of the values of Young's modulus for the compact bone tissue samples from veal calf: A) within the same bone (regions I–III), B) within the same region II (samples P1–P8)

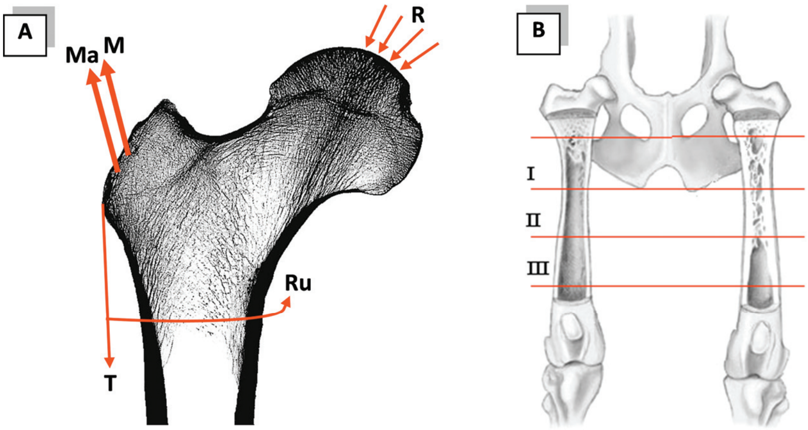


Fig. 3. Active loading model of the hip joint, where: *R* – impact of trunk mass on femoral head, which additionally has impact on the following muscles: M – abductors, Ru – rotators, and T – iliotibial tract [2] (A). Frontal plane cross-section through the pelvis and veal calf femurs with visualisation of bone structure differentiation (B)

parameters. The compact tissue samples excised in the regions I and II differ slightly with respect to the stress–strain characteristics as well as with respect to the values of the mechanical parameters and the strain energy (I~198.61; II~202.05; III~199.63 (mJ/mm^2)). Additionally, in the regions I and III, we can see the greatest differences in the mechanical parameters (I~9% versus III~14%) of the respective samples (P1–P8). Those differences are without a doubt due to differences in the structure and the volume of compact and cancellous tissues in those regions, resulting from the strain model typical of the lower limb (figure 3).

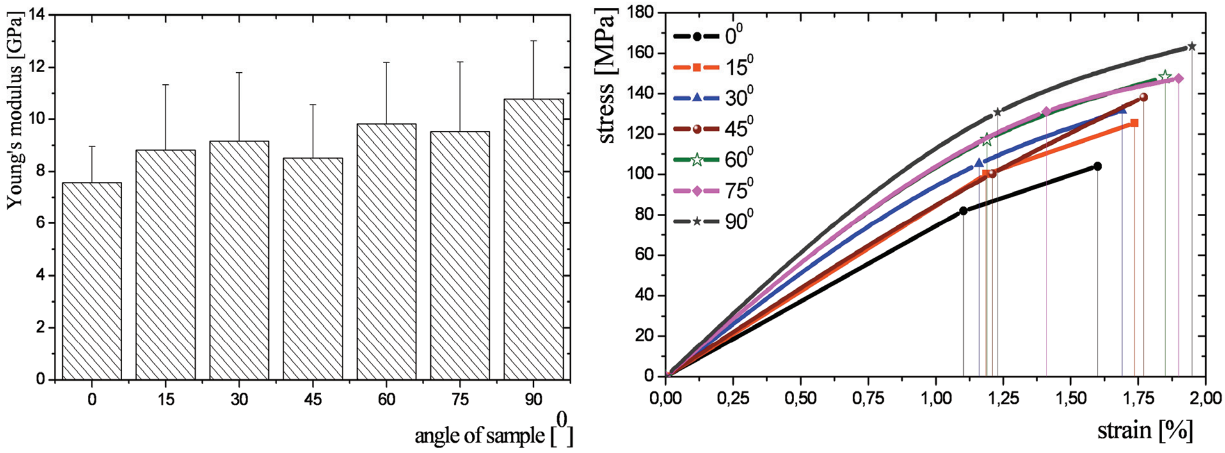
Differentiation in the structure and content of compact and cancellous bone tissues throughout the femoral diaphysis means that even in the same region there are visible differences in the values of the mechanical parameters (figure 2). The smallest differences in the samples analysed (up to 5%) were obtained in the central part of the diaphysis (the region of diaphysis proper), that was why the samples from that area were used in further research.

3.2. Sample excision direction

In the second stage of the research, the influence of the direction of excising the bone sample on the values of the mechanical parameters was established. In that case, the research material consisted of 10 diaphyses proper of the veal calf femurs, which were used to prepare cuboidal samples (4 mm × 4 mm × 20 mm) of compact tissue (n = 103) excised every 15° (from 0° to 90°) in the transverse direction of the long bone (figure 1B). Those samples were subjected to a compression test at a constant rate of 0.01/s.

Figure 4 shows stress–strain characteristics obtained in the compression test for compact bone tissue samples of veal calf, excised every 15° with respect to the transverse axis of the long bone (figure 1B). The analysis of the results obtained shows that a change in the angle of sample excision is responsible not only for mechanical values, but also for the character of the ε–σ curves. With an increase in the angle (from 0° to 90°) of sample excision, the character of bone tissue changes from brittle to more "malleable". The term "malleable" means here the value of strain difference between the strain read out for the material strength and the limit of elasticity. A change in the character of the sample is also visible in the values of strain energies, which range correspondingly from 91.46 (mJ/mm^2) for the samples excised at an angle of 0° and 127.56 (mJ/mm^2) for the samples excised at an angle of 45° to 230.26 (mJ/mm^2) for the samples excised at an angle of 90°. The results obtained are consistent with the observations made by FRANKEL and NORDIN for bone tissue samples excised from the human femoral diaphysis [11].

Additionally, in order to specify the fibre architecture of bone tissue, determining the arrangement of collagen fibres in the sample, measurements were carried out with the use of scanning microscope. The tests were conducted on the samples excised at the angles of 0°, 45° and 90°. Cubical shape and exact dimensions of the samples were obtained by mechanical working using end mill; therefore, in order to remove an external tissue layer, each of the sample surfaces examined under the microscope was treated with concentrated nitric acid for 3 minutes. The surface prepared in the above manner was covered with a layer of gold, and then examined under a scanning microscope (LEO–Zeiss 435). The resulting images (figure 5) provide information on the method and the



A B

Fig. 4. Average values of Young's modulus (GPa) and for veal calf femoral samples excised at different angles (from 0 to 90°) (A). Stress–strain characteristics of compact tissue samples excised every 15° from the veal calf femoral diaphysis (B)

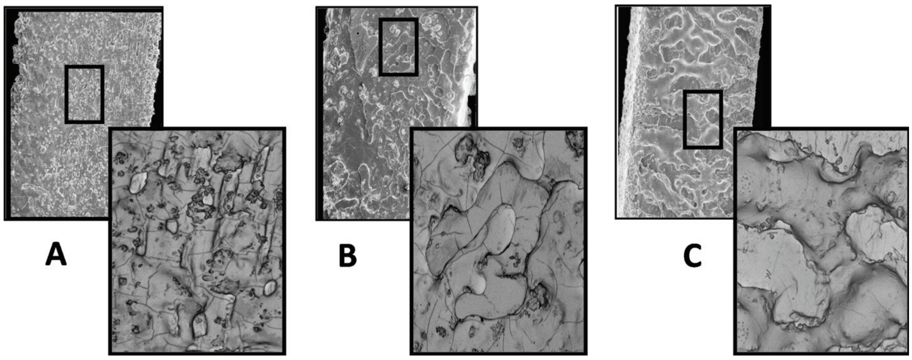


Fig. 5. Examples of fibre architecture of bone tissue samples coming from veal calf femur and excised: A) vertically, 90°; B) obliquely, 45°; C) horizontally, 0° (scanning microscope LEO-Zeiss 435)

direction of the arrangement of collagen fibres in the samples, depending on the direction of excision. As demonstrated by the results of mechanical tests (figure 4), those parameters have a strong impact on the values of the mechanical parameters.

3.3. Sample strain rate

The third stage of the research allowed us to establish the influence of the strain rate of bone tissue samples on the values of the mechanical parameters. As previously, the research material consisted of 10 diaphyses proper of veal calf femurs, which were used to prepare cuboidal test samples (4 mm × 4 mm × 20 mm) of compact tissue (n = 103), parallel to the long axis of the femur. Those samples, as those in the first and second stages of research, were subjected to a compression test, but at variable rates: 0.5; 1; and 2 mm/min.

Figure 6 presents comparative diagrams of the mechanical properties of the tissue, depending on the strain rate and the direction of sample excision. The results obtained prove that the highest values of the conventional coefficient of direct elasticity (Young's modulus) E_1 in the compression test are recorded in the samples excised parallel to the long bone axis (90°) and loaded at a rate of 2 mm/min (0.002/s strain rate); on the other hand, the lowest values are observed in the samples excised at right angles to the long bone axis (0°), loaded at a rate of 0.05 mm/min (0.0005/s).

Viscoelastic materials, which are also the components of bone tissue, are known to be sensitive to the level of strain. At higher loading rates, the tested material becomes stiffer and a greater force is required to destroy it. Additionally, the σ–ε curves

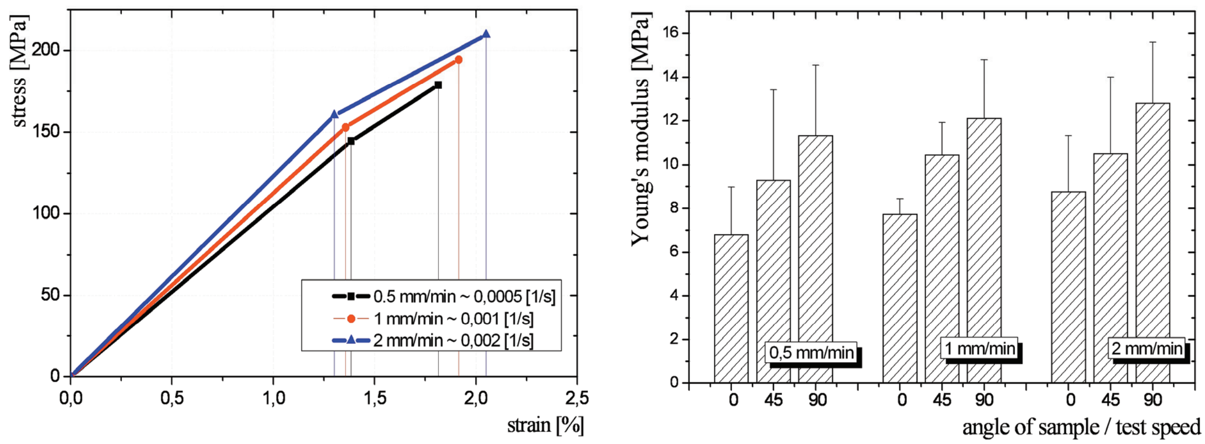


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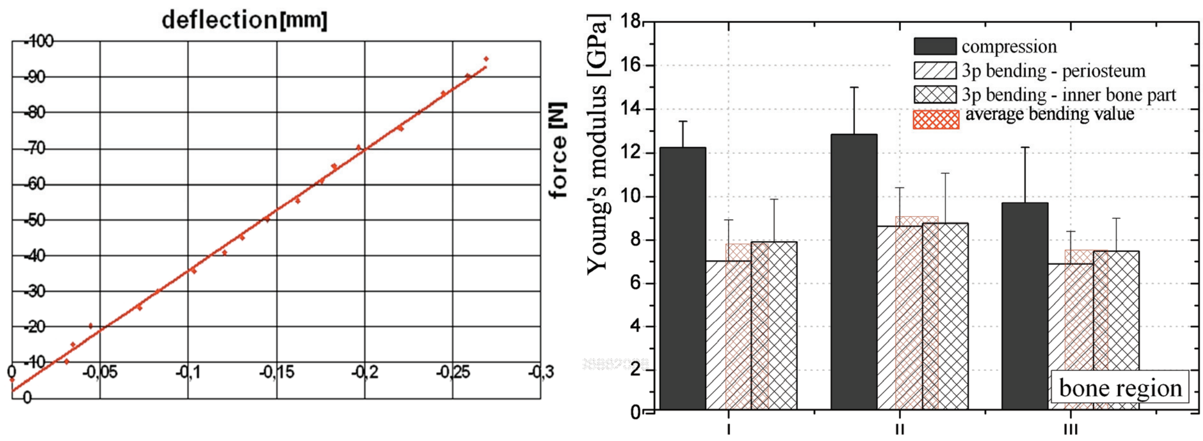
Fig. 6. Characteristics ε–σ of vertical samples (90°) of compact bone tissue from the region of diaphysis of veal calf femur, measured in the compression test at the rates of 0.5, 1, and 2 mm/min (A), average values of Young's modulus (GPa) for samples excised in three directions: 0, 45, 90° and the loading rate values of: 0.5, 1, and 2 mm/min (B)

obtained also show that at higher strain rates the material is more "malleable", and thus its strain energy is also higher (0.5 mm/min~169.5; 1 mm/min~200.86; and 2 mm/min~240.71 (mJ/mm^2)). Therefore, it may be concluded that there is some kind of bone protection mechanism which allows bone to reach a higher strength value during a brisk walk than during a slow stroll.

3.4. Type of mechanical test

The fourth stage of measurements was intended for determining the impact of the type of mechanical test on mechanical parameters of bone tissue. This was done by means of compression tests and three-point bending tests carried out on cuboidal samples of compact bone tissue excised from three regions I–III of the femoral diaphysis of veal calf. The Young's modulus of longitudinal elasticity E_1 was determined in a three-point bending test on 4 mm × 4 mm × 40 mm samples, whereas in the compression tests 4 mm × 4 mm × 20 mm samples were used. Both mechanical tests were conducted at a rate of 1 mm/min on the MTS 858 MiniBionix strength tester. During the three-point bending test each sample was bent in two planes: in the radial direction (from periosteum) and in the peripheral direction (from the inner side of bones). In the compression test, the samples were loaded only parallel to the main axis of the femoral bone.

Figure 7 presents the values of the Young's modulus E_1 obtained in compression tests and three-



A B

Fig. 7. A sample force–displacement (bending) characteristic obtained during measurements (A), values of Young's modulus determined by compression test and three-point bending test on compact bone tissue samples of the human tibial bone (B)

point bending tests of compact bone tissue of the human tibia. The values E_1 from the compression test range from 5.8 (in the region III, on the lateral side) to 16.3 (in the region II, on the medial side). The values E_1 determined by means of the three-point bending test are smaller than those determined during compression. Based on the results obtained we can also observe that the samples bent radially (from the periosteum side) show higher bending strength than the samples bent peripherally.

4. Discussion

In this paper, we present the results of experimental studies aimed at quantifying the impact of factors being related to the samples and the measurement of the values of the mechanical parameters determined during tests. The results obtained confirm previous reports [1], [17], [19], [23], [16], [18] as well as demonstrate unequivocally that each of the factors described has a direct impact on the tissue properties measured in mechanical tests. There are numerous works stressing the importance of age, species, and type of bone tissue [1], [8]. We also know that the mechanical properties of bone tissue, because of its strong viscoelasticity properties, depend on the test speed and set-up. However, comparison of our results to those in literature poses many problems because of huge discrepancies (sometimes up to 400%). Due to those differences, there is still a need to classify individual measurements and observations in order to determine research standards for various types of tissue, so that the results obtained can easily be interpreted and compared. We know from the literature the values of the individual parameters characterising the impact of the factors examined, but so far none of the studies has attempted to quantify that impact. Therefore, it seems reasonable and justified to introduce correlation coefficients which give quantified information on the scope of changes of the parameter examined:

$$A_1 = \frac{E_A}{E_H}, \tag{1}$$

where:

E_A – Young's modulus for animals bone,

E_H – Young's modulus for human compact bone.

On the basis of the results obtained such coefficients can be determined to establish the influence of the species, the direction of sample excision, and the loading rate.

The availability of animal bones and their close metabolic similarity to human bone mean that bovine bones are suitable for investigating phenomena taking place in our bone tissue. However, despite metabolic similarity, the values of the mechanical parameters in those groups differ significantly from each other, so, in order to compare the results for various groups, we can introduce the proportionality coefficient A_1 (figure 8). If the value of this coefficient is known, we can easily compare the results obtained.

Fig. 8. Values of the proportionality coefficient A_1 determined to specify the impact of the species on the values of bone mechanical properties. Value of coefficient A_1 for each group was determined with equation (1), where A_1 equals the ratio of Young's modulus of animal bone to that of human bone

Because a bone tissue is a porous and anisotropic material, its properties depend on the excision and loading directions. The studies conducted show that the highest values of the mechanical parameters determined for femoral bone tissue of veal calf are typical of cuboidal samples excised at 90° from a medial part region of the diaphysis proper (figure 4). The region of the diaphysis proper is entirely composed of a compact tissue whose density approaches 1.98 g/cm^3. Consequently, based on the results obtained, we can determine the function (equation (2)) quantifying change in the value of the conventional Young's modulus from the changing angle of excision of bone samples. As in the case of species and type, we can determine the proportionality coefficients, thus taking into account the influence of that parameter on the mechanical parameters.

$$E_1 = a_1 \cdot \alpha + b_1, \tag{2}$$

where:

E_A – Young's modulus,

α – angle of sample against to the transverse direction of the long bone.

$$E_1 = a_2 \cdot V + b_2 , \quad (3)$$

where:

E_1 – Young's modulus,

V – test speed.

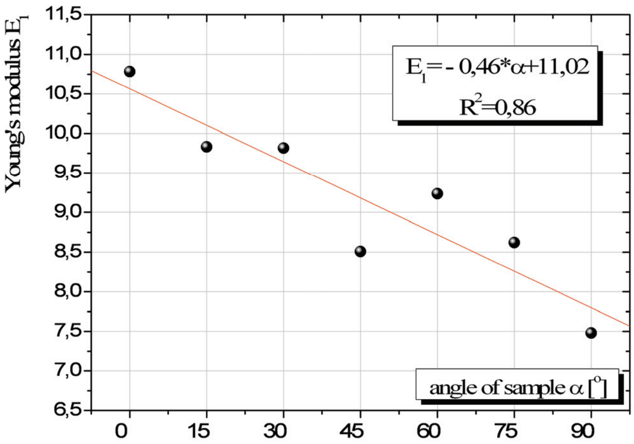


Fig. 9. Determination of parameters a_1, b_1 of the straight line in the form of (2), determining the value of conventional Young's modulus at a variable excision angle of bone samples

The values of the mechanical parameters also depend on the type of mechanical test applied to the samples. The Young's modulus obtained in the compression test carried out on the compact tissue of tibia is approx. 30% greater than that determined in the three-point bending test. The values of the parameter E_1 obtained in the tests on the veal calf femur range from 9.8 to 16.4 GPa, whereas in literature these values range between 14 and 30 GPa [1], [6]. Those discrepancies may be explained by the fact that bone is a viscoelastic material, so its mechanical parameters depend on the strain rate applied. In our studies, we most often used the rate of 1 mm/min (i.e., the strain rate of approx. 0.001/s), while literature studies conducted, among others, by RUBIN [25], [26] suggest that the strain rate under in vivo physiological conditions falls in the range of 0.01/s–0.08/s. According to CARTER and HAYES [3], when the strain rate increases by an order of magnitude, the value of strength increases by approx. 15%. Based on the results obtained, the dependence of the changing rate on the conventional Young's modulus can be represented by equation (3), for which, in accordance with the results obtained, we can calculate the coefficients a_2 and b_2.

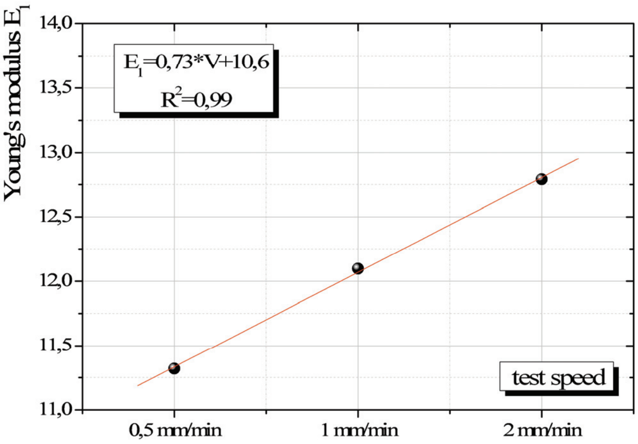


Fig. 10. Determination of parameters a_2, b_2 of the straight line in the form of (3), determining the value of conventional Young's modulus at changing sample strain rate

Lack of a uniform, standard protocol for bone tissue studies inclines us to describe the effect of selected factors on the basis of the mechanical parameters measured. The results of experimental measurements clearly indicate that the selection of each of the factors tested has a significant impact on the measured values of parameters in mechanical tests. The knowledge of the extent of these changes and of the proportionality coefficients enables a detailed and correct comparative analysis of the results obtained.

Obviously, the authors are aware of the limitations and errors that undoubtedly affect the results obtained. Also, it must be remembered that general criteria for impact assessment can only be established through the analysis of both the broad spectrum of changes of individual factors and the large number of biological preparations, which by their nature demonstrate a large spread of the parameters tested. Nevertheless, this paper proposes an approach to the analysis of the results that may facilitate comparison and verification of the results published by other centres.

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