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Relationship between the mineral content of human trabecular bone and selected parameters determined from fatigue test at stepwise-increasing amplitude

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Purpose: The study aimed to investigate a relationship between the mineral content of human trabecular bone and parameters determined from compression fatigue tests at stepwise-increasing amplitude. *Methods*: Mineral content of trabecular bone was estimated comparing density and bone mineral density values. The relationship between the ash density, bone mineral density and factors obtained from fatigue test: fatigue life, cumulative elastic energy and cumulative energy of dissipation was determined. *Results*: The results from the measurements of ash density and bone mineral density show good correlation with the fatigue test results. The relationship was estimated based on the correlation coefficient R within 0.74–0.79 for the particular pairs of factors. *Conclusions*: The study shows that the ash density and the bone mineral density are good predictors to estimate the fatigue life of trabecular bone. The study also validates the applicability of the tests at stepwise-increasing amplitude in determining the mechanical properties of trabecular bone.

Key words: *bone mineral density, trabecular bone, fatigue test, stepwise load, ash density*

1. Introduction

A human skeleton is subjected to loads due to the various forms of human activities. Bones are mostly subjected to dynamic loads variable in time, which may result in bone injuries and fractures as well as fractures without dislocation, observed both in people practicing different sports [1]–[5] and animals, e.g., racing horses [6]–[9]. Thus it is crucial to determine bone resistance to cyclic load.

The decisive factor for the bone resistance is the quality of its inner structure known as trabecular bone. It is a porous structure consisting of spatial network of bone trabeculae. Any disturbances in its structure lead to reduced resistance and may result in bone fractures.

The resistance of the trabecular bone depends on two main factors – the structure of the trabeculae and its tissue quality. A structure of the trabeculae can be measured with high accuracy using computer-aided microtomography. Structural parameters, e.g., the average trabeculae thickness and number, pore size and volume to bone tissue volume ratio etc. can be determined based on the measurement results. A quality of the trabecular tissue depends on the mineral content of its building material. The mineral density of the bones is evaluated *in vivo* based on the bone mineral density (BMD) index using different methods, e.g., dual-energy x-ray absorptiometry (DEXA). The results of *in vitro* tests may not be accurate for small bone samples. Thus, the bone mineral content was determined based on ash density (Ash.D) as a part of this study. This parameter defines the mineral phase content in the bone volume.

Due to prevailing nature of loads variable in time during human activity it is important to use indices allowing to evaluate bone resistance to loads of this kind, i.e., their fatigue strength. For the needs of this paper the authors have decided to use mineral phase content in the trabecular bone as one of indices determining the strength of the whole bones. It must be noted, however,

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that the strength of trabecular bone is also dependent on the architecture of bone trabeculae. Considering that quality of bones is mostly assessed in *in vivo* examinations based on the value of BMD index, the authors have also decided to assess the usability of this index for assessing the fatigue strength of bones. In the scientific literature there is no sufficient amount of papers which discuss the relations between the mineral phase content and strength indices of bones. Those available are mostly related to indices determined through statistical tests [10]–[12]. Additionally, the descriptions often pertain to tests performed on a small number of samples, or a higher number of samples, but collected from a small number of givers [11], or on animal samples [12]. Mosekilde et al. [10] have carried out the compression tests on 42 cylindrical trabecular bone samples from the central section of the first lumbar vertebra from healthy patients (27 women and 15 men, aged 15–87) cut in a vertical and horizontal direction. Maximum stress, maximum rigidity and energy absorption capability were determined based on the S-N curves. Ash density was measured after the samples were incinerated. In the case of examples cut vertically, a 75–80% reduction in stress, rigidity and energy absorption capability at ash density reduced by 48–50% was observed in the age group of 20–80. Similar changes were observed in samples cut in horizontal direction, however the absolute values were lower. A positive correlation between ash density and tested trabecular bone properties were observed in both directions.

Keyak et al. [11] have carried out compression tests on trabecular bone samples until failure to obtain information on the trabecular bone behaviour after failure. The tests were carried out in three anatomical directions. Elastic modulus, compressive strength before, during and after failure were determined based on the S-N curves, and the parameters were correlated with ash density in each anatomical direction. Each value was significantly correlated with ash density in all directions being tested, however, other measured parameters did not show any significant correlation.

Kang et al. [12] carried out tests on dog's trabecular bone. The authors performed compression and indentation tests for samples taken from the head of femoral bone, condyle of femoral bone, tibia and head of humeral bone to determine apparent density and ash density. The results showed that the ultimate load, ultimate strength and rigidity are well correlated at higher values obtained from the indentation test. They have also determined a high correlation between apparent density and ash density and mechanical parameters determined from both tests ($R = 0.737–0.966$).

Therefore the authors have decided to perform the tests on samples collected from human femoral head collected from patients suffering from osteoporosis – OP (patients after fractures at femur neck area) and osteoarthrosis – OA, who have been subjected to alloplastic hip joint procedure. This allowed to carry out tests on a relatively large sample group – collected from 57 givers, with a wide range of apparent density – both measured with the use of medical diagnosis, which also effects in obtaining ash density, as well as trabeculae architecture and strength. In this work, the authors have focused on the relations between fatigue strength of bones and indices indirectly describing their strength, such as Ash.D and BMD. The authors did not seek relations between the age of givers and the values of indices determined in the tests. The authors are aware that such approach results in certain limitations consisting in generalizing the obtained relations down to human population free from bone structure abnormalities.

With the assumed principles in mind, the purpose of the study was to evaluate the effect of the relationship between the mineral phase content in the trabecular tissue and its fatigue strength. The fatigue test was performed at stepwise-increasing amplitude. The load was applied in blocks at a fixed number of cycles at constant amplitude. The minimum load was constant for each block, whereas the maximum load was increased by fixed value at each subsequent block.

2. Materials and methods

2.1. Samples

57 cylindrical trabecular bone samples 10 mm in diameter and 8.5 mm high were used in the tests. The samples were taken from 57 osteoporotic and osteo-

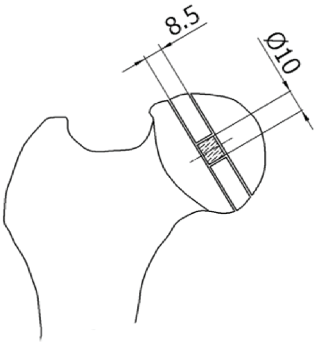

Fig. 1. Manner of a collection of sample.
The sample axis coincided with the axis of femoral neck [17]
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arthritic human femoral heads after hip arthoplasty (Fig. 1). The patients age ranged from 46 to 88 years (average 73, standard deviation SD ± 6,2). The samples were not divided into subgroups neither by gender nor pathology. The samples were stored in 10% formalin solution at room temperature [13], [14].

2.2. BMD measurement

The test was carried out using Lunar Expert (General Electric, USA) densitometer in accordance with the instructions for low density samples. The instructions were provided in the tutorial supplied by the manufacturer.

2.3. Fatigue test

Fatigue compression test of samples was conducted at stepwise-increasing amplitude using INSTRON 8874 (Instron, High Wycombe, England) testing machine. The schematics of sample loading is shown in Fig. 2. The minimum load for each level was 5N. The maximum loading started with 10 N with a gain of 10 N at every subsequent step. A frequency of a load used was 1 Hz. Each loading step included 500 sinusoidal cycles at constant amplitude loading. The temperature during test was 37 ± 2 °C and samples were immersed in 0.9% NaCl solution.

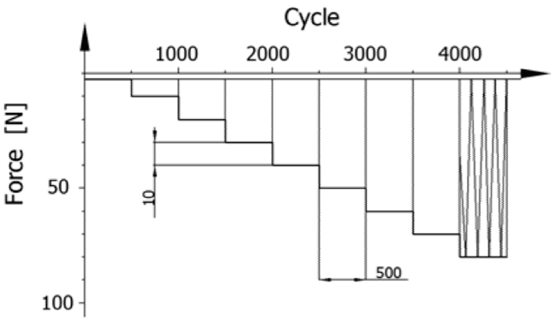

Fig. 2. Sample loading scheme:
minimum loading for every step – 5 N, maximum loading
started from 10 N with a gain of 10 N at every subsequent step,
each loading level included 500 cycles
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The load values were based on the previous tests of the relationship between static compression strength of trabecular bone and BMD carried out by Mazurkiewicz and Topoliński [15]. The data were used to determine the number of cycles for fatigue tests of each load level. The number of cycles in each step should enable to carry out the test of minimum of 6 load levels.

The fatigue life (N_s) was calculated using a modified method by Bowman [16]. Median values of deformation increment were determined and the number of the first loop for which the deformation gain exceeded the value of the median by 10% of its fatigue life was assumed [17].

A cumulative dissipation (SED) and cumulative strain (SES) energy were also calculated for all samples. Cumulative dissipation energy was calculated as a total for all the loop areas determined for all tested samples. Cumulative elastic energy was defined as a total energy minus dissipation energy applied to the sample.

2.4. Ash.D measurement

The mineral content of the samples was measured after the fatigue tests. The samples were incinerated at 500 °C for 18 hours to remove the organic phase and formalin, according to the procedure described in [18]. Ash density was defined as the quotient of the weight of the sample after burning to its volume before measurement, which corresponds to the density of its mineral components.

3. Results

The results of the distributions for bone mineral density, ash density and fatigue life are normal ($p < 0.05$), for cumulative elastic energy and cumulative energy of dissipation are log-normal ($p < 0.05$), as verified by Shapiro–Wilk tests. For distributions for bone mineral

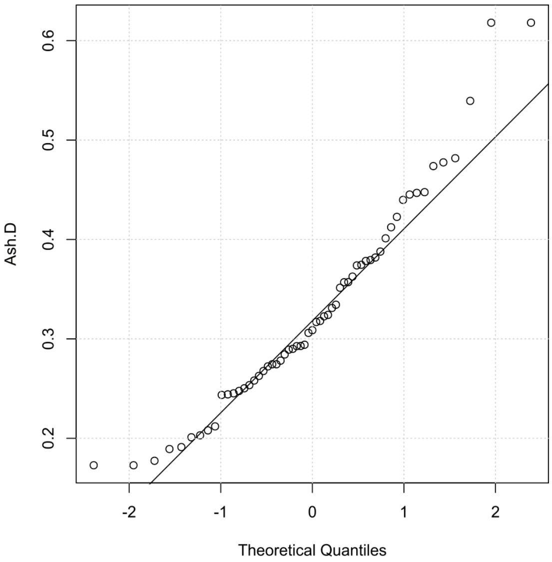

Fig. 3. Distribution of ash density

density and ash density (Fig. 3) the most significant deviations from trend was observed for samples with the highest values of this parameters. For the same samples obtained also the highest values of fatigue life. For distributions for cumulative elastic energy and cumulative energy of dissipation (Fig. 4) the effect is less visible.

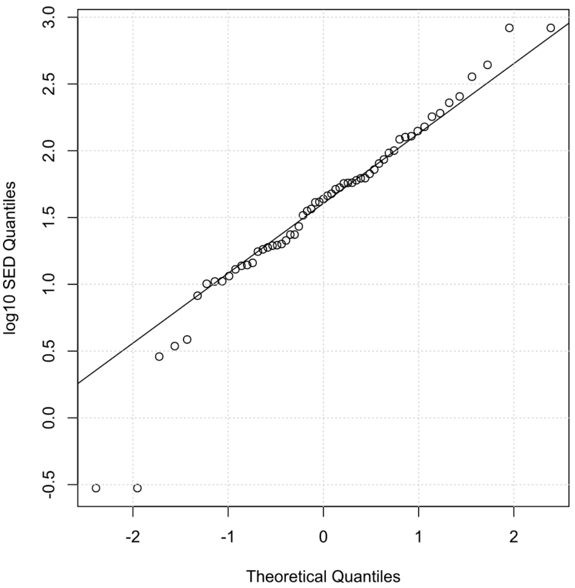

Fig. 4. Distribution for cumulative dissipation energy

The fatigue life values obtained during the tests at stepwise-increasing amplitude were between $3.75 \cdot 10^3$ and $5.02 \cdot 10^4$ cycles (average value Av = $20.99 \cdot 10^3$, standard deviation SD = $11.74 \cdot 10^3$, relative standard deviation RSD = 55%). The values correspond to the test times between 1.04 and 13.95 hours. Cumulative elastic energy values were between 12.16 and 1900.79 N·mm/mm^3 (Av = 412.69; SD = 462.52; RSD = 112%), and dissipation elastic energy between 2.88 and 832.09 N·mm/mm^3 (Av = 85.12; SD = 133.02; RSD = 156%).

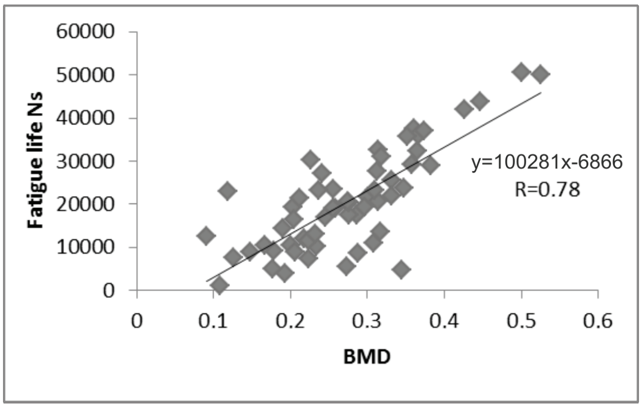

Fig. 5. Relation between bone mineral density and fatigue life

Bone mineral density values are between 0.091 and 0.525 g/cm^2 (Av = 0.277; SD = 0.089; RSD = 32%), ash density between 0.173 and 0.618 g/cm^3 (Av = 0.326; SD = 0.09; RSD = 29%). Values bone mineral density obtained and ash density are comparable with values obtained by others authors [18], [19].

Figures 5–10 show the relationship between bone mineral density/ash density and fatigue life, accumulated elastic and dissipation energy. The results shown

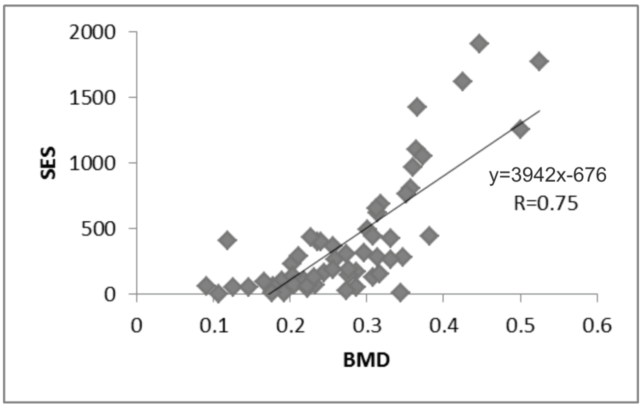

Fig. 6. Relation between bone mineral density and cumulative strain energy

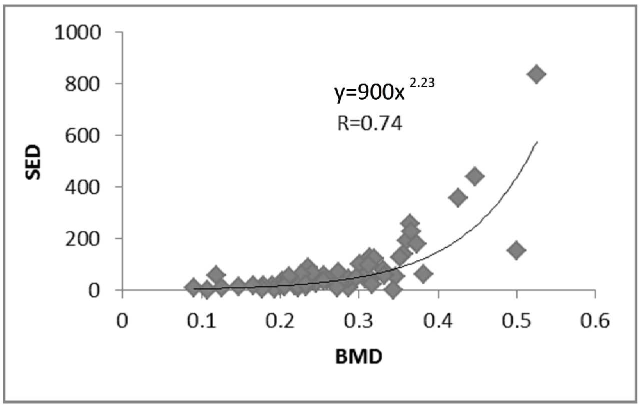

Fig. 7. Relation between bone mineral density and cumulative dissipation energy

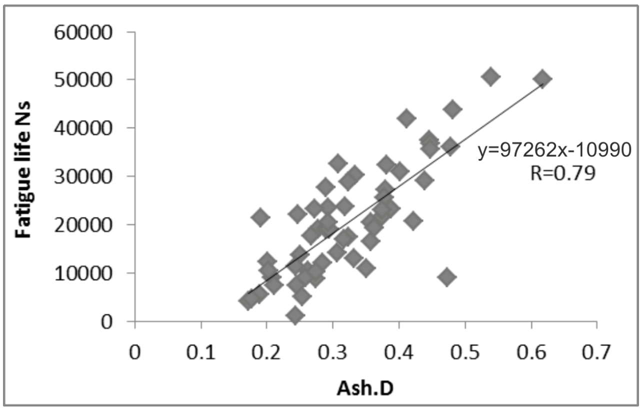

Fig. 8. Relation between ash density and fatigue life

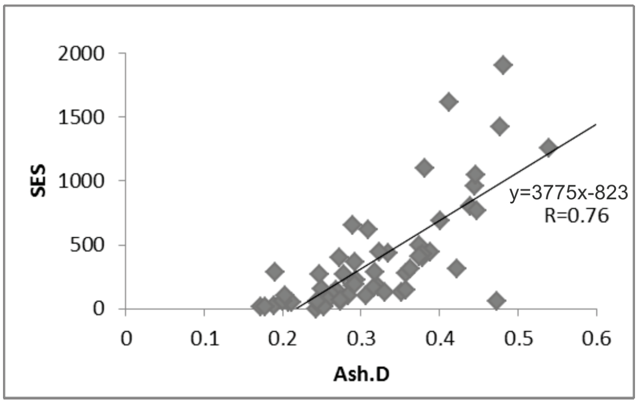

Fig. 9. Relation between ash density and cumulative strain energy

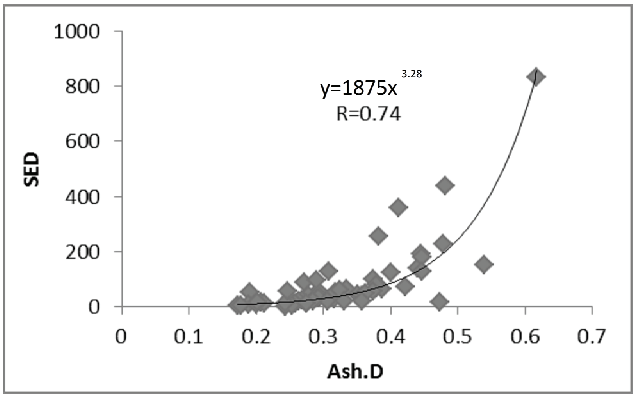

Fig. 10. Relation between ash density and cumulative dissipation energy

in Figs. 5 and 8 confirmed relation between bone mineral density, ash density and fatigue life. Values of cumulative elastic energy (Figs. 6, 9) and cumulative energy of dissipation (Figs. 7, 10) have more significant deviations from trend for samples with highest values of bone mineral density and ash density.

Figure 11 shows the relationship between bone mineral density and ash density. The relationship is described by linear trend with coefficient of correlation $R = 0.67$.

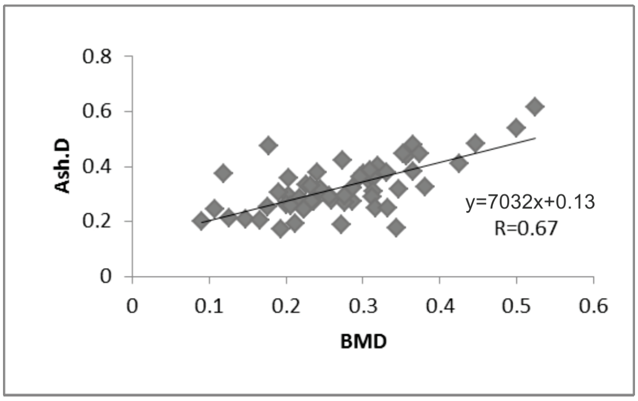

Fig. 11. Relation between bone mineral density and ash density

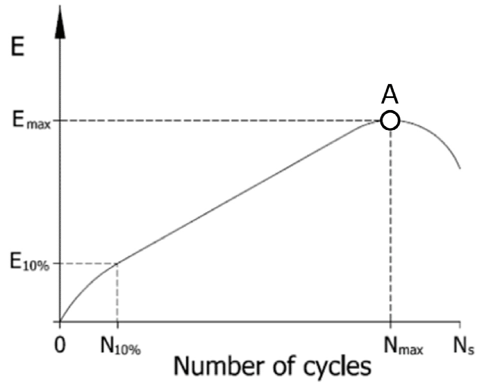

Fig. 12. Diagram of changes in the value of the secant modulus for loading with stepwise increases in amplitude. Point A is proposed criterion of fatigue

Table 1 shows coefficients of correlation R between each pair of factors. Values of the coefficient obtained for relations of both factors describing mineral phase content of the bone and factors from fatigue test were between 0.74 and 0.79.

Table 1. Coefficient of correlation *R* values between each pair of factors

	Fatigue life, Ns	SES	SED
BMD, g/cm^2	0.78	0.75	0.74
Ash.D, g/cm^3	0.79	0.76	0.74

4. Discussion

The purpose of our tests was not to determine changes in mineral density of bones for OP and OA. For OA, in the majority of cases the anomalies relate to bone surface or subchondral bone layer. For our tests, the samples were collected using the method presented in Figure 1. Due to the distance of the sampling point from external surfaces of femoral head, and the visual form of inspection of occurrence of such changes during sampling, the authors have assumed that no anomalies occur in this area. Therefore, neither the influence of age of givers, nor the stage of disease were considered. The authors have focused only on referring the physical value that is mineral density to fatigue strength. This is an undoubtedly different approach than testing relations between strength of bones and their density for givers without bone pathologies, and it appears to be more important from the point of view of predicting the probability of fracture of bones.

The relationships between mineral content of trabecular bone and its mechanical properties were tested by other researches, who found out the relationships between mechanical bone parameters and its ash density. These were, however, compression tests, performed at static loads [10]–[12] – that do not occur most frequently in every-day situations. The values of correlation coefficients obtained by them are at least slightly higher than our results. In the Mosekilde et al. [10] paper, these correlations for the relations between maximum tension, energy absorption capacity and maximum stiffness in ash density function fell within the range of 0.81–0.92 for samples cut out from the first lumbar vertebrae in the horizontal and vertical, direction. However, for maximum deformation they were low, falling within the range of –0.31–0.31. It should be noted that the bone samples under testing were bones collected from corpses of 27 females and 15 males aged 15–87, not exhibiting any bone pathologies.

The authors did not specify any parameters of the architecture of their bones, nor did provide any BMD variables. It can be only assumed that this variability was insignificant. Much higher correlation coefficients for the final elastic modulus and maximum tension in the function of ash density were obtained by Keyak et. al [11] who conducted their strength tests at three anatomic directions. The obtained values fell within the range of 0.894–0.968. However, in this case, the results were obtained for 33 samples collected from only two givers (45 year-old man and 40 year-old woman). This is a definite qualitative difference compared to our results and the results of paper [10]. Another range of variability of the correlation factor (between 0.737 and 0.966) for ash density and ultimate strength and stiffness was obtained by Kang et al. [12] in tests performed on a trabecular bone collected from a dog, for samples obtained from femoral heads, tibia, and humeral head. Those results are difficult to refer directly to the results obtained from human bone tests.

In our examinations the relationships through linear functions or power functions were described. After matching with the test results, the linear functions do not pass through the center of the system of coordinates, which is not in accordance with the physical aspect of sample destruction process. In scientific literature it can be found that both of these functions one in regular use. For instance, in paper [10] both the functions were used for description without making any definite statements on one of them being more useful for description purposes than the other. In paper [11] only the power function was used.

The tests carried out by the authors of the present study also showed correlation between ash density and BMD with indices determined from the fatigue tests. The effects of the mineral content on the fatigue test results were determined using a correlation coefficient R = 0.74–0.79 (Table 1). It may indicate that both indices can be used to predict the fatigue life and assess the value of an accumulated elastic strain energy and dissipation energy in the tests at stepwise-increasing amplitude carried out on the trabecular bone samples.

The tests carried out by the authors of the study showed good correlation between BMD and ash density, however no similar correlations were found in the literature. However, a positive correlation between those values can be assumed based on their definition. In this case, the relation is defined by the correlation coefficient $R = 0.67$ (Fig. 11). BMD density test is the basic test used for assessing the quality of *in vivo* bones. This parameter is evaluated in the majority of cases in clinical practice, and the quality of bones of the patients is determined based on its value. Therefore, in their paper, the authors undertook additional assessment of conformity of this parameter with actual physical content of mineral phase in bones, defined by the ash density parameter. According to authors, this can be useful for evaluating strength resistance of the bones based on *in vivo* tests. However, it should be verified by further examinations taking into account also the spatial architecture of the trabecular [10]–[11]. In our earlier study before the fatigue test a measurement of the structure of trabecular in samples was performed with the use of a microtomograph. Based on these results, in paper [17] we presented the assessment of relations between parameters of bone structure with fatigue life. From among the structure parameters determined in this test, only for BV/TV parameters (bone volume/trabecular volume) a correlation ratio of $R = 0.82$ was achieved. For the remaining structure parameters, the R values were significantly lower. The results obtained suggest that a model taking into account both of these factors should be used to describe strength of trabecular bone.

There are some doubts about the influence of a sample storage method on the mechanical properties of samples in time. The samples were stored in formalin solution. During incineration, organic matter, e.g., collagen, bone marrow and blood is incinerated. Ash.D is defined as the mass to volume ratio of the incinerated sample, i.e., the volume of a cylinder (diameter 10 mm, 8.5 mm high). The sample mass is reduced, whereas the volume remains the same after incineration. During sample storage, some of the pores of the trabecular bone can be filled with formalin. Due to the similar density of bone marrow, blood and formalin, it can be assumed that it has a minor effect on the Ash.D measurement, since the change in sample mass is negligibly small. As for the BMD measurement, the influence of the storage method on the results should not be significant. Another issue which should be considered is the effect of formalin on mechanical properties of the trabecular bone. Some authors have deduced that the storage method improves strength properties of the samples, some that it does not affect it at all or affects it only slightly. Edmondston et al. [13] have investigated fresh and formalin-fixed human and sheep vertebrae, of where fixed samples were stored for 4 weeks in 10% formalin solution. Mechanical testing following storage produced significantly lower average failure for formalin-unfixed (9.3 kN) than fixed (10.0 kN) material. Failure strain did not differ significantly between the groups. Pöpperl et al. [14] have investigated the effects of formalin fixation, storage, and maceration on the reproducibility of the bone's ultrasonic properties. Fourteen fixed calcanei and 12 fresh bones were examined. Fixation with 4% formalin/96% alcohol resulted in a systematic decrease in speed of sound (SOS), a slight increase in broadband ultrasonic attenuation (BUA), and a decrease in stiffness index (SI) with time. However, measurements taken at 6 months of fixation and later were highly correlated with those of fresh specimens ($R = 0.95$ for the SI). Two weeks storage in degassed normal solution had minor effects on the bone's ultrasonic properties. Maceration did not lead to a systematic increase or decrease in ultrasound variables but introduced some unpredictable changes ($R = 0.64–0.94$).

Different results were obtained by Baum et al. [20] who tested 12 thoracic vertebrae from 3 donors. After the samples were taken, multi-detector computed tomography (MDCT) tests were carried out after 3 and 6 months. BMD and structural parameters of the trabecular bone were determined. Two vertebrae from each donor were frozen and stored in formalin between the tests. Changes in BMD, trabecular bone microstructure parameters in formalin-fixed and frozen vertebrae over 6 months ranged between 1.0–5.6% and 1.3–6.1%, respectively. BMD, microstructure parameters of the trabecular bone after the tests correlated significantly with mechanically determined failure load ($R = 0.89–0.99$). The correlation coefficients R were not significantly different for both preservation methods ($p > 0.05$). It is thus difficult to determine the effect of the storage conditions on the results of the fatigue test.

The test temperature is crucial for the bone tests. According to Carter et al. [21], the difference in durability and Young modulus of samples during the static tests at 20 and 37 °C is approx. 4÷5%. The fatigue tests showed that the samples show twice the fatigue life at room temperature than at 37 °C, which is a natural human body temperature. Thus, the fatigue tests were carried out at 37 ± 2 °C in 0.9% NaCl solution to achieve the conditions similar to those of a human body. It also allowed to reduce the effects of drying or decomposition of samples during the test, which might have affected the results.

The test at stepwise-increasing amplitude can be used in qualitative testing. The determination of bone behaviour at subsequent loads allows to define the failure process and the qualitative changes at specific stress levels. The tests were described by Locati [22] and Langraff [23] and were carried out for polymers and composites. In the Locati method, the fatigue limit was determined using an accelerated and approximate method based on the Palmgren–Miner's hypothesis of the accumulation of fatigue damage. In the Landgraf method, the cyclic properties of the material were evaluated by measuring of the hardening or weakening of the material throughout the subsequent test cycles. Landgraf test is carried out on a single sample, and thus was selected for the purpose of this study.

One of the issues with the compression tests at stepwise-increasing amplitude is the way to determine the sample failure criterion. The criterion for constant-amplitude tests may be defined as a 30% reduction in the modulus of elasticity [24]. For tests at stepwise-increasing amplitude, this criterion cannot be used, since the modulus value changes with the increase in load in subsequent cycle blocks.

Topoliński et al. [17] have suggested a sample failure criterion based on the results of tests at stepwise-increasing amplitude as a maximum point at the secant modulus curve, after which the secant modulus begins to drop – designated as A in Fig. 12.

The results show the applicability of tests at stepwise-increasing load in the qualitative evaluation of bone samples. It is very important, since determining the S-N curves apply to samples with uniform composition, structure and geometry, e.g., metals or samples with similar geometry, e.g., like bone samples. In this case, the stress value must be normalized with an initial tangent modulus E_o, which is not obvious to determine. The test at a stepwise-increasing amplitude eliminates this issue.

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Authors have permission of Local Ethic Committee to conduct described investigation.

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