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Research Paper

Prediction of Young's modulus of trabeculae in microscale using macro-scale's relationships between bone density and mechanical properties



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ARTICLE INFO

Article history:

Received 9 January 2014

Received in revised form

17 April 2014

Accepted 20 April 2014

Available online 29 April 2014

Keywords:

Trabecular bone

Young's modulus

Micro-computed tomography

Bone mineral density

Digital Image Correlation

Microscale properties

ABSTRACT

According to the literature, there are many mathematical relationships between density of the trabecular bone and mechanical properties obtained in macro-scale testing. In micro-scale, the measurements provide only the ranges of Young's modulus of trabeculae, but there are no experimentally tested relationships allowing the calculation of the distribution of Young's modulus of trabeculae within these experimental ranges. This study examined the applicability of relationships between bone density and mechanical properties obtained in macro-scale testing for the calculation of Young's modulus distribution in micro-scale. Twelve cubic specimens from eleven femoral heads were cut out and micro-computed tomography (micro-CT) scanned. A mechanical compression test and Digital Image Correlation (DIC) measurements were performed to obtain the experimental displacement and strain full-field evaluation for each specimen. Five relationships between bone density and Young's modulus were selected for the test; those were given by Carter and Hayes (1977), Ciarelli et al. (2000), Kaneko et al. (2004), Keller (1994) for the human femur, and Li and Aspden, 1997. Using these relationships, five separate finite element (FE) models were prepared, with different distribution of Young's modulus of trabeculae for each specimen. In total, 60 FE analyses were carried out. The obtained displacement and strain full-field measurements from numerical calculations and

Abbreviations: micro-CT, micro-computed tomography; DIC, Digital Image Correlation; FEM, finite element method; FEA, finite element analysis; BMD, bone mineral density; MTD, main trabecular direction; GD, grey density; HAD, hydroxyapatite density

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<http://dx.doi.org/10.1016/j.jmbbm.2014.04.011>

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experiment were compared. The results indicate that the highest accuracy of the numerical calculation was obtained for the [Ciarelli et al. \(2000\)](#) relationship, where the relative error was 17.87% for displacements and 50.94 % for strains. Therefore, the application of the [Ciarelli et al. \(2000\)](#) relationship in the microscale linear FE analysis is possible, but mainly to determine bone displacement.

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1. Introduction

In clinical practice, the gold standard of non-invasive assessment of bone structure is the evaluation of bone mineral density (BMD) as recommended by the World Health Organization (WHO) ([Kanis et al., 2000](#); [WHO, 1994](#)). As a result only partial estimation of patients' bone condition is obtained, which directly affects the probability of future osteoporotic lesions. Application of densitometry without additional testing in the mechanical context does not allow a full description and evaluation of the trabecular bone micro-architecture. A number of histomorphometric parameters describing bone tissue, determined on the basis of tomographic imaging, should be the set of input data for the three-dimensional model of cancellous bone structure followed by mechanical testing analysis using FEA.

The basic technique for providing high-resolution data translating into high accuracy of calculations performed on their basis is micro-computed tomography. Micro-computed tomography (micro-CT) and computed tomography (CT) are methods commonly used in clinical studies ([Binkowski et al., 2010](#); [Muller et al., 1998](#); [Rüegsegger et al., 1996](#); [Shefelbine et al., 2005](#); [Ulrich et al., 1998](#)), laboratory studies ([Lin et al., 2003](#); [McDonald et al., 2003](#); [Mercer et al., 2003](#); [van Garderen et al., 2012](#)) as well as in industry ([Badde and Illerhaus, 2008](#)). Micro-CT delivers high resolution of the cross-sectional images, which can also be processed to three-dimensional views based on surface or volume rendering. CT and micro-CT scans combined with the finite element method (FEM) are powerful tools for prediction of the mechanical properties of the bone ([Depalle et al., 2013](#); [Hambli, 2013](#); [Kokot et al., 2012](#)). Especially interesting is the use of multiscale FEM simulations, which allow one to achieve a significantly better accuracy than the one-scale simulations ([Makowski et al., 2013](#)). However, the multiscale simulations require a preparation of FE models individually for each of the scale, which is associated with the appropriate selection of Young's modulus and Poisson's ratio in the numerical model. The selection of Young's modulus has a great influence on the accuracy of finite element analysis (FEA) and requires special relationships between Young's modulus and tissue density, which allow one to obtain Young's modulus from the micro-CT scans. According to the literature, there are many mathematical relationships between bone density and mechanical properties established on the basis of research in a macro-scale ([Carter and Hayes, 1977](#); [Ciarelli et al., 2000](#); [Helgason et al., 2008](#); [Kaneko et al., 2004](#); [Keller, 1994](#); [Keyak et al., 1994](#); [Li and Aspden, 1997](#)). Macro-scale refers to mechanical compression tests on cylindrical or cubic specimens ([Carter and Hayes, 1977](#); [Ciarelli et al., 2000](#); [Kaneko et al., 2004](#);

[Keller, 1994](#); [Li and Aspden, 1997](#)). Most of these relationships work well in predicting the mechanical behavior of bone in the range of applicability defined by the authors. Micro-scale refers to Young's modulus of trabeculae measured by nanoindentation, 3-point microbending test, compression test of microscopic cubic specimens or uniaxial testing of single trabeculae ([Choi et al., 1990](#); [Hong et al., 2007](#); [Jiroušek et al., 2011](#); [Rho et al., 1997](#); [Turner et al., 1999](#); [Zysset et al., 1999](#)). In micro-scale, there is no appropriate relationship that would allow for the accurate prediction of the mechanical behavior of trabeculae. There is only one theoretical relationship given by [Wagner et al. \(2011\)](#), which is included in their theory of determining the voxel-specific tissue density by means of micro-CT data.

However, in this article we focused on finding a mathematical relationship that will allow for the accurate prediction of Young's modulus of trabeculae in the micro-scale. One of the possibilities is treating the relationships estimated in the macro-scale only as a mathematical function without taking into account the ranges of applicability defined by the authors. Then the applicability of these mathematical functions in micro-scale should be verified experimentally. For this purpose, an experiment should be performed (e.g., bone compression test), and then a numerical simulation of the same experiment performed, where Young's modulus values will be calculated and assigned to the finite element (FE) models using mathematical functions that are verified. Finite elements should build micro-scale components such as trabeculae. We expect that a good compatibility between experimental and numerical results will be achieved, if Young's modulus distributions calculated from mathematical functions are similar to Young's modulus ranges of trabeculae measured in micro-scale. Such experimental measurements of Young's modulus of trabeculae depend on the measurement technique and measurement conditions (wet/dry) used in research. Nanoindentation measurements in dry conditions were used for example by [Rho et al. \(1997\)](#), [Turner et al. \(1999\)](#) and [Jiroušek et al. \(2011\)](#), who also compared nanoindentation results with the results of uniaxial testing of single trabeculae. Young's modulus ranges given by these authors were 13.4 ± 2.0 GPa, 18.14 ± 1.7 GPa and 16.34 ± 1.76 GPa (nanoindentation)/ 9.21 ± 1.26 GPa (uniaxial testing), respectively. The measurements in wet conditions were performed by [Choi et al. \(1990\)](#) using the 3-point microbending test, [Hong et al. \(2007\)](#) using the compression test of microscopic cubic specimens and [Zysset et al. \(1999\)](#) using nanoindentation. Young's modulus ranges were 3.27–10.58 GPa, 3.47 ± 0.41 GPa and 11.4 ± 5.6 GPa, respectively. The above-mentioned experimental ranges overlap partially, but in general the differences between them are significant. Thus, the question is which experimental range should be used in numerical modeling and

how to calculate the distribution of Young's modulus of trabeculae within this range.

The aim of the present study was to examine the applicability of the relationships between Young's modulus and bone density, which were obtained in macro-scale testing for the calculation of Young's modulus distribution in micro-scale. In addition, the present study is an attempt to find out which of the relationships is the optimal one for predicting bone mechanical behavior.

2. Material and methods

2.1. Specimen preparation

Nine femoral heads from patients undergoing total hip replacement surgery (Provincial Specialist Hospital No. 5 in Sosnowiec, Poland) and two proximal ends of the human femur from cadavers (Department of Forensic Medicine, Jagiellonian University Medical College in Cracow, Poland) were obtained. The donors were aged 45–77. The hip specimens from the hospital were collected with permission from the Bioethics Committee from the Medical Silesian University in Katowice, Poland. Hip degenerative disease was diagnosed in eight of them, dysplasia and necrosis in one, in another there was a femoral neck fracture; for the last two no diagnosis information was available [see Table 1]. Cubic specimens (total number=12) for mechanical testing were cut out from the femoral heads, using a precision saw machine with a diamond-coated cut-off wheel (STRUERS Secotom – 15, Willich, Germany, 2200 rpm, $f=0.1$ mm/s). The cutting planes of the femoral heads were located at the thinnest point of the femoral neck and were perpendicular to the femoral neck axis (Fig. 1a). The main trabecular direction (MTD) was identified and marked on the femoral head using the CT scans. The trabecular bone layers were cut out of the femoral heads according to MTD (Fig. 1b). The target thickness of the layers was 10 mm. MTD was marked on the layers again, and the cubic specimens were cut out of these layers. The orientation of the specimens in the femoral heads was marked. The specimens' planes parallel to the anterior–posterior axis were marked with a single dot, and the planes perpendicular to MTD were marked with two dots. The specimens were divided into two categories: “core” and “side”. The specimens located in the center of the layer, which had a vertical direction of trabeculae, were classified into the “core” category (number of specimens=7) (Table 1, Fig. 1c). The other specimens located in one of the sides of the layer, which had a diagonal direction of trabeculae, were classified into the “side” category (number of specimens=5). The specimens were fixed in 70% ethanol and stored in 4 °C between research to reduce the risk of infection. The procedure of specimen's cutting, micro-CT scanning and mechanical testing lasted about 2 days. The time of specimen's fixation in ethanol was several hours.

Distribution of the mechanical parameters in the femoral head region is widely known (Augat et al., 1998; Łukowski et al., 2000). Therefore, for the purpose of this study samples from different regions were distinguished using the “side” and “core” categorization.

Table 1 – Specimen data.

No.	Specimen no.	Sex	Age	Side	Diagnosis	Specimen position in femur head	Specimen dimensions		
							Length	Width	Height
1	8AII	M	45	Left	Left hip degenerative disease	Side	10.5	11.3	10.7
2	12AI	M	64	Right	Both hips degenerative disease	Side	10.4	10.4	10.7
3	12BII	M	64	Right	Both hips degenerative disease	Core	10.4	10.4	10.7
4	13BII	F	62	Right	Right hip degenerative disease	Core	10.4	10.4	10.4
5	14AII	F	74	Left	Left hip degenerative disease	Core	10.4	10.4	10.7
6	15AII	M	54	Right	Right hip degenerative disease	Core	10.4	10.4	10.4
7	16BII	F	70	Left	Left hip degenerative disease	Core	10.4	10.5	10.4
8	17AII	F	69	Right	Femoral neck fracture	Side	10.4	10.4	10.4
9	18CI	F	59	Left	Dysplasia, necrosis	Core	10.4	10.4	10.9
10	20BII	M	46	Left	Left hip degenerative disease	Core	10.3	10.4	10.4
11	23AI	M	75	Left	No data	Side	10.3	10.4	10.7
12	24AII	M	77	Right	No data	Side	10.3	10.5	10.5

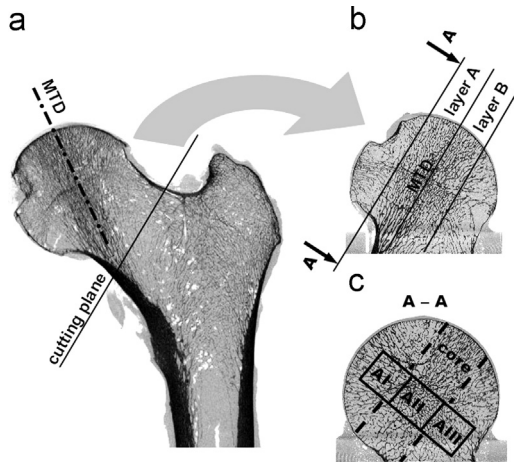


Fig. 1 – Micro-CT cross-sections for femur no. 12. (a) Proximal end of the femur, showing the main trabecular direction (MTD) and the position of cutting plane. (b) Position of layers in the femoral head and the position of A-A cross-section. (c) A-A cross-section showing the surface of layer A with marked position of specimens: 12AI, 12AII and 12AIII. The area between the dashed lines is classified into the “core” category.

2.2. Quantitative micro-computed tomography scanning

All the femoral heads were scanned with a micro-CT scanner (vitomelx s, GE Sensing & Inspection Technologies, Phoenixix-ray, Wunstorf, Germany). The device is a laboratory scanner (also called an industrial scanner) consisting of two open X-ray tubes: direct tube (240 kV) and transmission nano-tube (180 kV). In this study the direct tube was applied. It is equipped with both tungsten cathode (filament) and anode (target), and the polychromatic X-ray cone-beam is used there. The results of X-ray beam attenuation propagated through the sample located on the rotary table in the scanner chamber is recorded by the 16" Flat Panel Detector (2024 × 2024 pixels, pixel size = 200 μm²). The manufacturer's software (Datos 2.0) was applied to obtain projection and image reconstruction.

The femoral heads were scanned to obtain the view of the total specimen in order to identify the main trabecular direction (MTD). Having the proper orientation of MTD, the cubic samples dissected from the specimen were tested using higher scanning resolution. The scanning parameters are presented in Table 2.

A hydroxyapatite phantom was used while scanning all the cubic specimens. The density phantom is a custom-made product designed by authors a few years back and ordered to manufacture by QRM (QRM GmbH, Moehrendorf, Germany). The phantom is delivered with individual protocol described amount of HA in each cylinder. The quality of phantom was a subject of previous studies (Binkowski et al., 2012, 2010). The phantom consists of 5 cylinders of varied HA concentrations (100, 200, 400, 800, and 1000 mg HA/cm³). The cylinders are held parallel to each other in the resin cube. The mean gray density values (GD) for every cylinder were calculated against the known mineral density at each cross-section of the dataset, and individual linear calibration curves for each sample were estimated as follows: based on the images of individual samples obtained from

Table 2 – Scan parameters of femoral heads and the specimens.

Scan parameters		
Parameter	Femur heads	Specimens
Magnification	5.985	19.518
Voxel size [μm]	33.416	10.246
Binning	–	–
FOD [mm]	–	48.08
FDD [mm]	–	938.54
Scan time [min]	34	26
Number of images	1000	1000
Image width	2024	2024
Image height	2024	2024
Fast scan	No	No
Type	Microfocus	Microfocus
Timing [ms]	500	500
Average	3	2
Skip	1	1
Voltage [kV]	140	70
Current [μA]	200	140
Mode	0	0
Filter	Cu 0.5	Cu 0.1
Phantom	2.2	2.2

Table 3 – Coefficients of Eq. (1).

Specimen no.	Coefficients	
	α	β
8AII	0.07	–2.589
12AI	0.022	–2.346
12BII	0.019	–1.92
13BII	0.02	–2.008
14AII	0.019	–1.686
15AII	0.02	–2.075
16BII	0.021	–2.343
17AII	0.02	–1.849
18CI	0.018	–1.772
20BII	0.04	–1.567
23AI	0.019	–1.515
24AII	0.023	–2.485

micro-CT in the ImageJ 1.44p (Wayne Rasband, National Institutes of Health, USA), GD were calculated in correlation with the hydroxyapatite density (HAD, g/cm³). Then the α and β coefficients of linear calibration curves were estimated for each sample (Table 3). The calibration curves were applied to calculate the Quantitative Bone Mineral Density (ρ_{qBMD}) parameter (Eq. 1), which defines the mineral density of bone in each cubic sample as an equivalent of HA:

$$\rho_{qBMD} = \alpha GD + \beta [g/cm^3] \tag{1}$$

2.3. Mechanical testing and Digital Image Correlation (DIC)

The specimens were mechanically tested in a uniaxial compression test using a platen technique on an MTS machine (MTS Insight 2, Eden Prairie, MN, USA). Digital Image Correlation system (model Q-400, Dantec Dynamics GmbH,

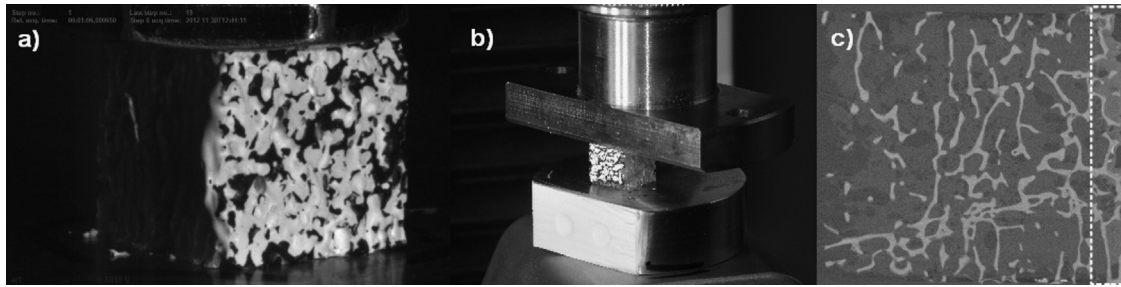


Fig. 2 – Specimen no. 13BII. (a) Front surface prepared for DIC measurement. (b) Specimen during compression test and (c) the micro-CT cross section of the specimen with indicated (white dashed frame) region of acrylic paint penetration.

Ulm, Germany) was used to measure displacement and strain fields in two axes (x , y) during the compression test.

Digital Image Correlation technique uses a special image correlation algorithm, based on a pseudo-affine coordinate transformation from one camera image to another for full-field displacement and strain evaluation. This algorithm compares the so-called facets of digitized images obtained by observing the specimen surface with two digital cameras while loading. The position of each point of an object in three dimensions is calculated based on knowledge of the imaging parameters for each camera and their mutual orientation. As the series of measurements is taken, while the specimen surface is moved due to loading, the displacements and strains on the object surface can be assessed. What is specific for this method is that the specimen surface must have a randomly generated speckle pattern on it, which is used to track the facets. On this ground the correlation algorithm transforms the corresponding facet positions in both cameras into 3D coordinates for each step, resulting in tracking of each facet surface in 3D space. It is the basis for the displacement and strain evaluation by the image correlation algorithm (Becker et al., 2006).

To perform measurements, the cubic specimens were painted with non-toxic white acrylic paint (background paint) by means of a paintbrush. Black acrylic paint was used to create a stochastic speckle pattern (Fig. 2a), which was sprayed from a distance of 20 mm. Only one side surface of each specimen was painted. The mean speckle size was 0.3 mm and there were in average about 200 speckles per specimen surface (10 mm \times 10 mm). Fig. 2c indicates in a white dashed frame a region of penetration of acrylic paint into the trabecular bone. The mean depth of the penetration equaled to less than 1 mm. The orientation of all the specimens in the compression test was the same, and the direction of compression corresponded to the MTD. The specimen's surface used for the DIC measurement was parallel to the anterior–posterior (AP) axis. The compression tests were performed at the displacement rate of 0.5 mm/min at room temperature (≈ 25 °C). The load range was from 0 to 2000 N (or till the specimen's failure). The pre-load of specimens was 1 N. The DIC system was set up to take pictures every 100 N of the force increment.

2.4. Selection of relationships between bone density and Young's modulus

Five relationships between bone density and Young's modulus were selected for the tests; those were given by Carter

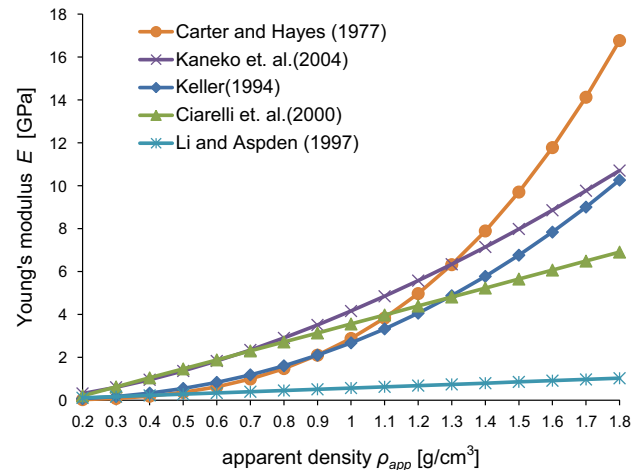


Fig. 3 – Relationships between density and Young's modulus selected for the tests (Helgason et al., 2008).

and Hayes (1977), Ciarelli et al. (2000), Kaneko et al. (2004), Keller (1994) and Li and Aspden (1997) (Fig. 3, Table 4). The decision about the selection of the relationships was made based on the significant differences between the coefficients of the functions describing these relationships. Young's modulus range of the relationships and the fact that most of them were estimated in research on the human femur (except Carter and Hayes, 1977, which was estimated in pooled site research) were also taken into account. In addition, cubic specimens were used in three of them: the ones given by Keller (1994), Ciarelli et al. (2000) and Kaneko et al. (2004). In the other two cylindrical specimens were used. In majority of the selected relationships Young's modulus range at least partially corresponded to the experimental Young's modulus ranges given by Choi et al. (1990), Hong et al. (2007) or Zysset et al. (1999).

Having selected the relationships, the required variables were calculated as follows. The first step was to determine the distribution of quantitative bone mineral density ρ_{qBMD} in each specimen using the GD values from the micro-CT scans and the previously calculated α and β coefficients (Eq. 1). Then, the quantitative bone mineral density ρ_{qBMD} was an input data to calculate the distribution of ash density ρ_{ASH} . In order to calculate the distribution of ash density ρ_{ASH} Eq. (2) provided by Kaneko et al. (2004) for the no cancer (NC) group of specimens was used (Helgason et al., 2008; Kaneko et al., 2004). The ash density ρ_{ASH} was used for the calculation of

Table 4 – Relationships between density and Young's modulus selected for the tests (Helgason et al., 2008).

Study	Site	Specimen's shape	Densitometric measure	ρ -Range [g/cm ³]	Young's modulus function E [GPa]
Carter and Hayes (1977)	Pooled	cylindrical	ρ_{app}	0.07–2.0	$E = 3.79\varepsilon^{0.06}\rho_{app}^3$
Keller (1994)	Femur	Cubic	ρ_{ash}	0.092–1.221	$E = 10.5\rho_{ash}^{2.29}$
Li and Aspden (1997)	Femoral head	Cylindrical	ρ_{app}	0.14–1.4	$E = 0.573\rho_{app} - 0.0094$
Ciarelli et al. (2000)	Proximal femur	Cubic	BV/TV	0.15–0.40 ^{RFG}	$E = 7.541(BV/TV) - 0.637$
Kaneko et al. (2004)	Distal femur	Cubic	ρ_{ash}	0.102–0.331	$E = 10.88\rho_{ash}^{1.61}$

Young's modulus (E) in GPa and density in g/cm³. RFG=read from graph.

Table 5 – Numerical models data.

Specimen no.	Type of element	Number of elements	Number of different $E_i^{(sn)}$ values	Load [N]
8AII	Tet10	1,114,130	11	1200
12AI	Tet10	769,950	11	600
12BII	Tet10	853,516	10	1200
13BII	Hex8	1,119,803	8	1020
14AII	Hex8	1,126,503	8	1000
15AII	Tet10	1,069,141	10	1000
16BII	Hex8	1,242,083	11	1000
17AII	Hex8	751,447	11	700
18CI	Tet10	935,911	10	900
20BII	Tet10	1,087,268	12	1000
23AI	Hex8	870,917	9	627
24AII	Tet10	802,708	11	1200

Young's modulus of two relationships: those given by Keller (1994) and Kaneko et al. (2004). In the other three relationships, which are the functions of apparent density ρ_{app} (Carter and Hayes, 1977; Li and Aspden, 1997) or bone volume fraction BV/TV (Ciarelli et al., 2000), Eqs. (3) and (4)¹ were used (Helgason et al., 2008; Keyak et al., 1994).

$$\rho_{ASH} = 79.8 + 0.792\rho_{qBMD} \text{ (mg/cm}^3\text{)} \quad (2)$$

$$\rho_{app} = \frac{\rho_{ASH}}{0.55} \text{ (g/cm}^3\text{)} \quad (3)$$

$$BV/TV = \frac{\rho_{app}}{1.8} \text{ (g/cm}^3\text{)} \quad (4)$$

Young's modulus relationships were considered only as mathematical functions, without taking into account the ρ – ranges of these relationships given by the authors (Table 4). The reason for this methodology is that the range of applicability of the relationships in microscale was the subject of the research. Therefore, although the ρ_{ASH} , ρ_{app} or BV/TV values exceeded the ρ – ranges given by the authors, we used them in the numerical model.

2.5. Numerical model

The trabecular bone structures were segmented from each of the micro-CT scans, and then the 3D bone surface models

were created. The 3D bone surface models were optimized using the surface smoothing and wrapping operations. Finite element meshes were created with two different methods: directly converting each bone voxel into an eight-noded hexahedral element (Hex8), or by generating the mesh from the 3D bone surface model using a ten-noded tetrahedral element (Tet10). FE meshes and 3D bone surface models were created using Mimics 15.0 (HEX8) and 3-matic (Tet10) software (Materialise, Leuven, Belgium). The number of elements for each specimen's mesh is presented in Table 5. Young's modulus distributions were calculated using the GD values from the micro-CT scans, formulas (1)–(4) and the five selected Young's modulus relationships. Therefore, five meshes for each specimen's model were created, which differed only with the distribution of Young's modulus values ($E_i^{(sn)}$, sn – specimen's number, $i = 1, 2, \dots, 5$) according to one of the selected relationships. In total, 60 numerical models were generated. Each of the five meshes for one specimen had the same mesh structure. Young's modulus distributions ($E_i^{(sn)}$) were calculated using the uniform material assignment method implemented in the Mimics 15.0, in which the calculation procedure was performed using the following manufacturer protocol. The GD range for each micro-CT scan was divided into 20 intervals and the center GD value in each interval was chosen as a representative for that material ($GD_{ij}^{(sn)}$, $i = 1, 2, \dots, 5$, $j = 1, 2, \dots, 20$). Young's modulus value in each interval was calculated using the representative GD value [$E_{ij}^{(sn)}(GD_{ij}^{(sn)})$, $i = 1, 2, \dots, 5$, $j = 1, 2, \dots, 20$]. Therefore, Young's modulus distribution could have had a maximum

¹Based on the assumption that real density is equal to compact bone density, i.e. 1.8 g/cm³ (Carter and Hayes, 1977; Helgason et al., 2008).

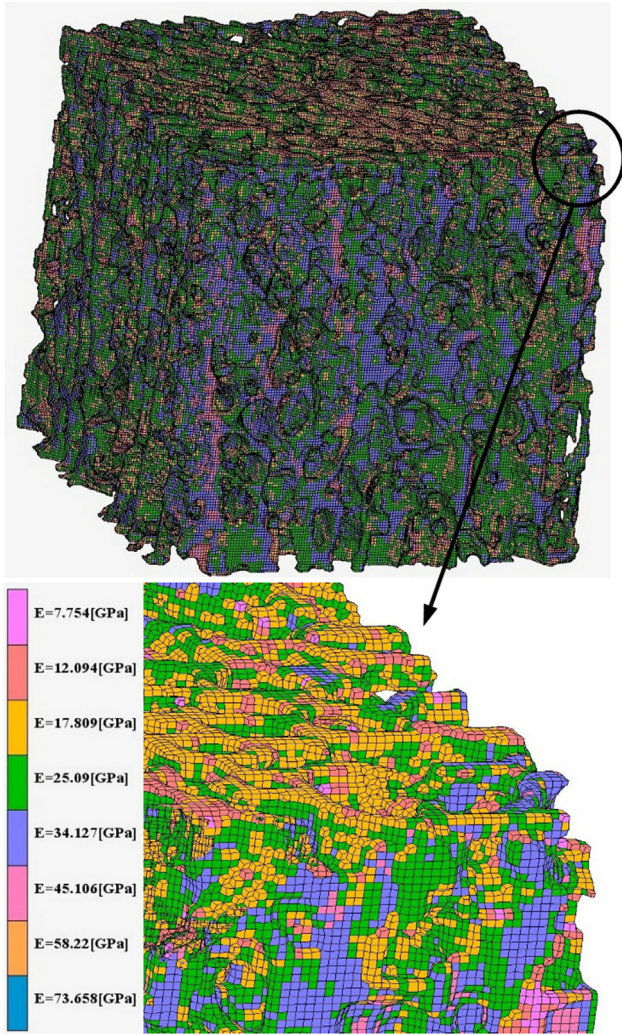


Fig. 4 – Young's modulus distribution obtained for the relationship given by Carter and Hayes (1977) for specimen no. 13BII.

number of 20 different Young's modulus values. However, the real number of different Young's modulus values ($E_{ij}^{(SN)}$) was within the range of 8–12 (Fig. 4, Tables 5 and 6). It was due to the used algorithm of dividing the GD range, which is taking the starting GD=0 instead of the minimal GD in the segmented micro-CT images. Poisson's ratio of 0.3 was used.

The FE models had the same orientation during simulation as the specimens during the experiment, and the boundary conditions were also the same for all models and represented the experimental uniaxial compression tests. The FE mesh was virtually placed between two plates. The glue contact was used between the mesh elements and the plate surfaces with a contact tolerance of 0.01 mm. The bottom plate was fixed, but the top plate was free to move with the applied load. The load values for the tested models are presented in Table 5. The numerical analysis was performed only in the elastic range. Therefore, the load was applied in a single step of calculation, but only for the models that had a constant contact area. If there was a relief on the top mesh surface, the initial contact was applied to the highest point of the mesh and changed with

Table 6 – Example of Young's modulus distributions obtained for specimen no. 13BII.

13BII – Young's modulus distributions

j-index	Number of elements	Ciarelli et al. (2000) BV/TV	$E_j^{(13BII)}$ [GPa]	Keller (1994) ρ_{ash}	$E_j^{(13BII)}$ [GPa]	Kaneko et al. (2004) ρ_{ash}	$E_j^{(13BII)}$ [GPa]	Carter and Hayes (1977) ρ_{app}	$E_j^{(13BII)}$ [GPa]	Li and Aspden (1997) ρ_{app}	$E_j^{(13BII)}$ [GPa]
1	5211	0.773	5.195	0.766	5.696	0.766	7.077	1.392	7.754	1.392	0.788
2	39,286	0.897	6.126	0.888	7.996	0.888	8.984	1.614	12.094	1.614	0.916
3	160,548	1.02	7.057	1.01	10.745	1.01	11.058	1.837	17.81	1.837	1.043
4	406,704	1.144	7.988	1.132	13.958	1.132	13.291	2.059	25.091	2.059	1.17
5	462,358	1.267	8.92	1.255	17.652	1.255	15.676	2.281	34.127	2.281	1.298
6	44,373	1.391	9.851	1.377	21.841	1.377	18.,208	2.503	45.107	2.503	1.425
7	1005	1.514	10.782	1.499	26.538	1.499	20.88	2.726	58.221	2.726	1.552
8	318	1.638	11.713	1.621	31.757	1.621	23.689	2.948	73.658	2.948	1.68

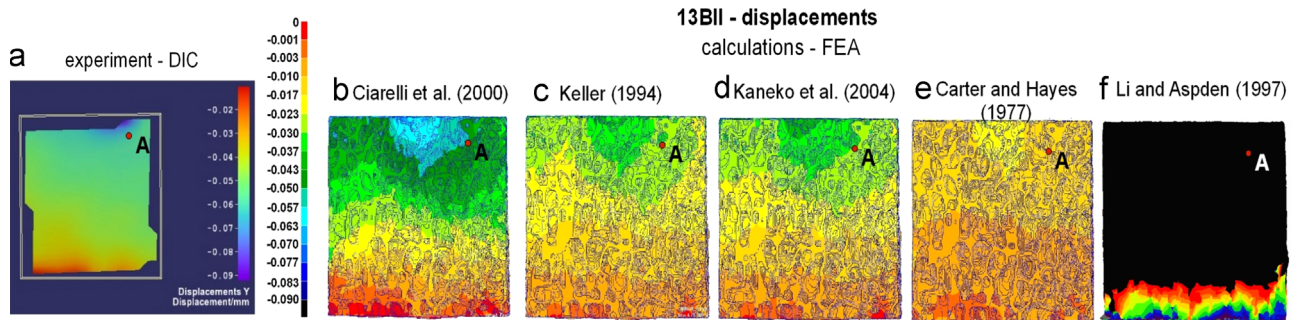


Fig. 5 – Comparison of displacement distributions obtained from (a) experiment (DIC) and (b–f) numerical analyses (FEA) for specimen no. 13BII.

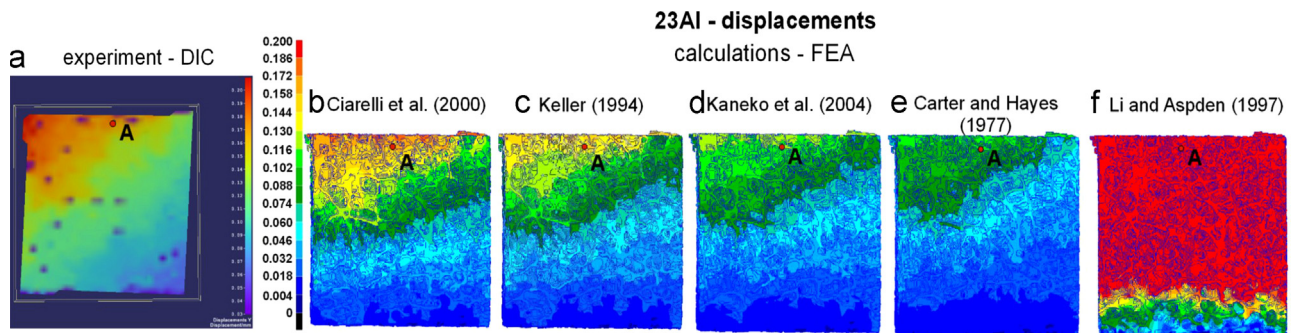


Fig. 6 – Comparison of displacement distributions obtained from (a) experiment (DIC) and (b–f) numerical analyses (FEA) for specimen no. 23AI.

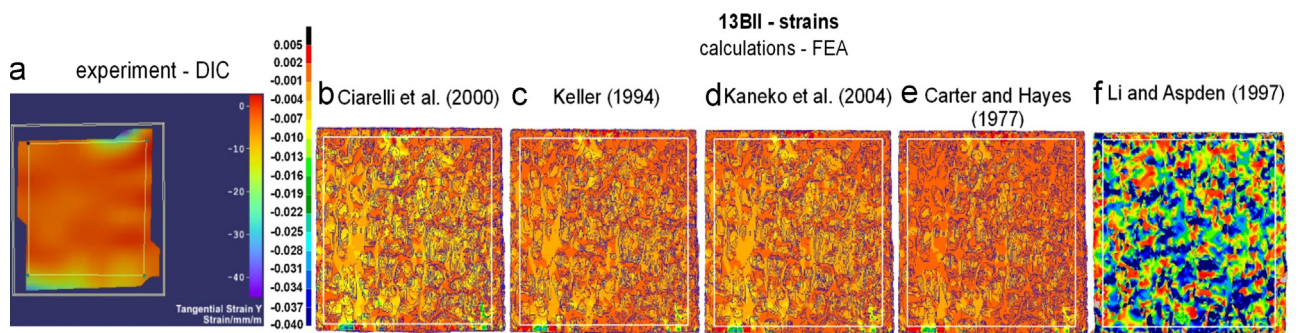


Fig. 7 – Comparison of strain distributions obtained from (a) experiment (DIC) and (b–f) numerical analyses (FEA) for specimen no. 13BII.

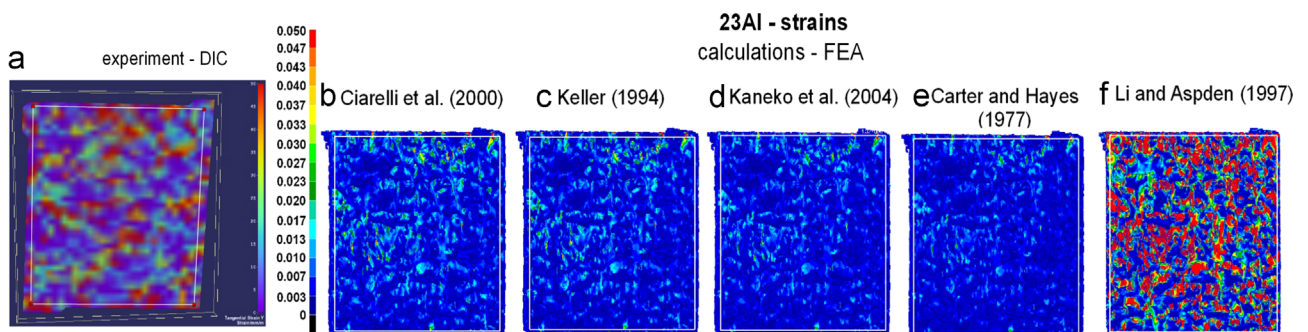


Fig. 8 – Comparison of strain distributions obtained from (a) experiment (DIC) and (b–f) numerical analyses (FEA) for specimen no. 23AI. The distribution obtained from the relationship given by [Li and Aspden \(1997\)](#) seems to be the most similar to the experimental distribution. In fact, the average strain relative error δ_e is 78.75% ([Table 7](#)).

the movement of the top plate. In this case, the load was applied in five steps of calculation with a linear increase in load.

The results of the numerical analyses were compared with the experimental full-field measurements obtained from the DIC system. Three quantities were compared: the maximum displacement, the displacement in control point A, and the average strain value in the selected area. The control points were placed in the top part of specimens, near the top loading surfaces, where the high displacement values minimized the influence of the reading error on the results (Figs. 5 and 6). The areas in which the average strains were calculated are marked with a white frame on Figs. 7 and 8.

The numerical models were calculated using the MSC. Marc&Mentat 2012 software (MSC Software Corporation, Santa Ana, California, USA) and a symmetric multi-frontal sparse solver. The calculations were run on a PC (Dell T7500 Intel[®] Xeon[®] X5650, 2 processors 2.67 GHz, 96 GB RAM).

3. Results

The displacement and strain full-field measurements for each specimen were obtained from the numerical analyses and the experiment. The measured values of the maximum displacement, the displacement in control point A and the average strain values in the selected area are presented in Table 7. The displacement and strain distributions for two exemplary specimens are shown in Figs. 5–8. The experimental full-field measurements from the DIC system are shown in Figs. 5a–8a, whereas the other figures show the results of numerical simulations. In the case of perfect accuracy of the numerical simulation, numerical displacement and strain distributions should be identical with the experimental measurements. Therefore, the best accuracy of numerical analysis should be found in the relationship for which obtained numerical displacements and strains distributions were the most similar to the experimental results.

The comparison of the experimental data with the numerical analyses as for the displacement and strain distributions indicates that the best accuracy of the numerical analysis was obtained for the relationship given by Ciarelli et al. (2000) ($\bar{\delta}_{max} = 14.11\%$, $\bar{\delta}_A = 17.87\%$, $\bar{\delta}_\epsilon = 50.94\%$) as compared to others Keller (1994) ($\bar{\delta}_{max} = 23.20\%$, $\bar{\delta}_A = 25.88\%$, $\bar{\delta}_\epsilon = 60.27\%$), Kaneko et al. (2004) ($\bar{\delta}_{max} = 29.42\%$, $\bar{\delta}_A = 37.26\%$, $\bar{\delta}_\epsilon = 62.29\%$), Carter and Hayes (1977) ($\bar{\delta}_{max} = 35.47\%$, $\bar{\delta}_A = 45.18\%$, $\bar{\delta}_\epsilon = 67.99\%$), Li and Aspden (1997) ($\bar{\delta}_{max} = 328.81\%$, $\bar{\delta}_A = 364.74\%$, $\bar{\delta}_\epsilon = 216.62\%$). However, the good accuracy for the relationship given by Ciarelli et al. (2000), which allows for practical application, was obtained only for displacement values ($\bar{\delta}_{max} = 14.11\%$, $\bar{\delta}_A = 17.87\%$). Despite this, the calculations also indicate that the accuracy for average strain values was much lower. However, the smallest average relative error for the strain ($\bar{\delta}_\epsilon = 50.94\%$) was obtained also for the relationship from Ciarelli et al. (2000). Accuracy for the other relationships was even lower.

As the relationship given by Ciarelli et al. (2000) provides the highest accuracy of the numerical analysis of all the relationships taken into account in this study, the relative errors obtained using this relationship were compared for the specimens divided into four categories: “core”, “side”, “tet10”,

and “hex8”. The average relative errors calculated for the two categories depending on the position of the specimen in the femoral head were as follows: “core” ($\bar{\delta}_{max}^{core} = 16.25\%$, $\bar{\delta}_A^{core} = 15.75\%$, $\bar{\delta}_\epsilon^{core} = 47.89\%$), and “side” ($\bar{\delta}_{max}^{side} = 11.11\%$, $\bar{\delta}_A^{side} = 20.83\%$, $\bar{\delta}_\epsilon^{side} = 55.22\%$). The average relative errors calculated for the categories depending on the type of the finite element used in the numerical model were as follows: “tet10” ($\bar{\delta}_{max}^{tet} = 15.14\%$, $\bar{\delta}_A^{tet} = 19.69\%$, $\bar{\delta}_\epsilon^{tet} = 57.16\%$) and “hex8” ($\bar{\delta}_{max}^{hex} = 12.67\%$, $\bar{\delta}_A^{hex} = 15.32\%$, $\bar{\delta}_\epsilon^{hex} = 42.24\%$). These average relative errors show that the accuracy of numerical results for displacements was similar for all the categories (maximum difference of average relative errors for displacements was 5.14% between $\bar{\delta}_{max}^{core}$ and $\bar{\delta}_{max}^{side}$). The maximum difference for strain was higher (14.92% between $\bar{\delta}_\epsilon^{tet}$ and $\bar{\delta}_\epsilon^{hex}$), but the values of average relative errors for strains were also much higher. The obtained results indicate that there is no basis to conclude that the accuracy of the numerical solution in this study depended on the specimen's position in the femoral head or on the type of the finite element used in the numerical model.

4. Discussion and conclusions

This paper describes the research on the applicability of relationships derived from the macro-scale compression tests for the calculation of Young's modulus distribution of trabeculae in micro-scale. The compression tests of 11 specimens with DIC full-field measurements of displacement and strains were combined with the numerical analyses.

The results of the numerical calculations indicate that most of Young's modulus relationships obtained in macro-scale testing cannot be used in micro-scale. Only the relationship given by Ciarelli et al. (2000) provides a high accuracy of the numerical solution. This is a surprising result, especially that experimenting with new applications of macro-scale's relationships could seem unreasonable. In order to account for these results, the experimental Young's modulus ranges of trabeculae (Choi et al., 1990; Hong et al., 2007; Jiroušek et al., 2011; Rho et al., 1997; Turner et al., 1999; Zysset et al., 1999) need to be compared with Young's modulus distributions calculated in this study using GD values ($GD_{ij}^{(sn)}$) obtained from micro-CT scans and calibration curves for each specimen (Table 6). Young's modulus distributions for each specimen calculated from the relationship given by Li and Aspden (1997) were too narrow and limited to the range of 0.277–1.695 GPa. This range was significantly below the lower limits of the experimental ranges of Young's modulus of trabeculae (Choi et al., 1990; Hong et al., 2007; Jiroušek et al., 2011; Rho et al., 1997; Turner et al., 1999; Zysset et al., 1999). Therefore, applying this relationship to the numerical calculations causes too low stiffness of the numerical model, resulting in large displacements and deformations. Also, the results of numerical calculations are highly different from the experimental data for this relationship (Table 7). On the other hand, Young's modulus distributions for each specimen calculated from the relationship given by Carter and Hayes (1977) were included in the range of 0.532–75.753 GPa, which is too wide compared to the experimental ranges (Choi et al., 1990; Hong et al., 2007; Jiroušek et al., 2011; Rho et al., 1997;

Table 7 – Comparison of experimental (DIC) and numerical (FEA) results.

The comparison of experimental (DIC) and numerical (FEA) results

Specimen number	Relationship	Absolute value of maximum displacements [mm]			Absolute value of displacements in control point A [mm]			Absolute value of average strain y-component		
		Experiment (DIC)	FEA	Relative error δ_{max} [%]	Experiment (DIC)	FEA	Relative error δ_A [%]	Experiment (DIC)	FEA	Relative error δ_ϵ [%]
8AII	Ciarelli et al. (2000)	0.17	0.195	14.71	0.164	0.112	31.71	0.0108	0.0028	74.02
	Keller (1994)		0.189	11.18		0.106	35.37		0.0025	76.57
	Carter and Hayes (1977)		0.169	0.59		0.044	73.17		0.0016	84.92
	Kaneko et al. (2004)		0.172	1.18		0.049	70.12		0.0018	83.67
	Li and Aspden (1997)		0.636	274.12		0.457	178.66		0.0131	21.61
12AI	Ciarelli et al. (2000)	0.08	0.077	3.75	0.08	0.055	31.25	0.003	0.0026	13.87
	Keller (1994)		0.079	1.25		0.057	28.75		0.0027	11.44
	Carter and Hayes (1977)		0.065	18.75		0.045	43.75		0.002	34.98
	Kaneko et al. (2004)		0.063	21.25		0.042	47.50		0.0021	30.59
	Li and Aspden (1997)		0.332	315.00		0.287	258.75		0.0149	397.74
12BII	Ciarelli et al. (2000)	0.24	0.164	31.67	0.103	0.084	18.45	0.0064	0.0031	51.42
	Keller (1994)		0.127	47.08		0.063	38.83		0.0027	58.23
	Carter and Hayes (1977)		0.113	52.92		0.052	49.51		0.002	68.60
	Kaneko et al. (2004)		0.114	52.50		0.056	45.63		0.0021	67.00
	Li and Aspden (1997)		0.384	60.00		0.314	204.85		0.0183	185.22
13BII	Ciarelli et al. (2000)	0.09	0.077	14.44	0.06	0.054	10.00	0.003	0.0025	18.01
	Keller (1994)		0.048	46.67		0.034	43.33		0.0013	56.31
	Carter and Hayes (1977)		0.031	65.56		0.018	70.00		0.0007	77.19
	Kaneko et al. (2004)		0.05	44.44		0.034	43.33		0.0014	52.54
	Li and Aspden (1997)		0.44	388.89		0.375	525.00		0.0112	272.40
14AII	Ciarelli et al. (2000)	0.1	0.104	4.00	0.067	0.083	23.88	0.0045	0.0037	17.51
	Keller (1994)		0.068	32.00		0.054	19.40		0.0026	43.20
	Carter and Hayes (1977)		0.044	56.00		0.035	47.76		0.0014	68.19
	Kaneko et al. (2004)		0.062	38.00		0.05	25.37		0.0021	52.36
	Li and Aspden (1997)		0.512	412.00		0.423	531.34		0.0157	248.22
15AII	Ciarelli et al. (2000)	0.105	0.071	32.38	0.1	0.07	30.00	0.02	0.0026	86.94
	Keller (1994)		0.064	39.05		0.06	40.00		0.0022	89.17

Table 7 (continued)

The comparison of experimental (DIC) and numerical (FEA) results										
Specimen number	Relationship	Absolute value of maximum displacements [mm]			Absolute value of displacements in control point A [mm]			Absolute value of average strain y-component		
		Experiment (DIC)	FEA	Relative error δ_{max} [%]	Experiment (DIC)	FEA	Relative error δ_A [%]	Experiment (DIC)	FEA	Relative error δ_ϵ [%]
	Carter and Hayes (1977)		0.05	52.38		0.046	54.00		0.0016	91.93
	Kaneko et al. (2004)		0.054	48.57		0.053	47.00		0.002	89.79
	Li and Aspden (1997)		0.412	292.38		0.375	275.00		0.0172	13.80
16BII	Ciarelli et al. (2000)	0.2	0.169	15.50	0.19	0.154	18.95	0.013	0.0059	54.53
	Keller (1994)		0.149	25.50		0.135	28.95		0.0031	76.39
	Carter and Hayes (1977)		0.101	49.50		0.091	52.11		0.0034	73.99
	Kaneko et al. (2004)		0.122	39.00		0.11	42.11		0.0042	67.81
	Li and Aspden (1997)		0.606	203.00		0.552	190.53		0.0133	2.47
17AII	Ciarelli et al. (2000)	0.125	0.153	22.40	0.115	0.136	18.26	0.006	0.0028	52.86
	Keller (1994)		0.147	17.60		0.13	13.04		0.0027	54.91
	Carter and Hayes (1977)		0.114	8.80		0.098	14.78		0.002	66.21
	Kaneko et al. (2004)		0.117	6.40		0.102	11.30		0.0021	64.82
	Li and Aspden (1997)		0.869	595.20		0.807	601.74		0.0195	225.38
18CI	Ciarelli et al. (2000)	0.14	0.13	7.14	0.09	0.09	0.00	0.004	0.0057	43.16
	Keller (1994)		0.129	7.86		0.088	2.22		0.0056	39.46
	Carter and Hayes (1977)		0.105	25.00		0.065	27.78		0.0042	5.97
	Kaneko et al. (2004)		0.106	24.29		0.067	25.56		0.0043	8.04
	Li and Aspden (1997)		0.711	407.86		0.626	595.56		0.0368	819.08
20BII	Ciarelli et al. (2000)	0.22	0.201	8.64	0.2	0.182	9.00	0.0068	0.0025	63.63
	Keller (1994)		0.18	18.18		0.161	19.50		0.0018	74.13
	Carter and Hayes (1977)		0.16	27.27		0.141	29.50		0.0011	84.53
	Kaneko et al. (2004)		0.173	21.36		0.153	23.50		0.0015	77.75

23AI	Li and Aspden (1997)	0.953	333.18	0.934	367.00	0.0221	224.88
	Ciarelli et al. (2000)	0.186	7.00	0.172	5.49	0.004	68.30
	Keller (1994)	0.162	19.00	0.149	18.13	0.0034	73.13
	Carter and Hayes (1977)	0.116	42.00	0.106	41.76	0.0024	81.19
	Kaneko et al. (2004)	0.134	33.00	0.123	32.42	0.0028	77.51
24AII	Li and Aspden (1997)	0.905	352.50	0.858	371.43	0.0225	78.75
	Ciarelli et al. (2000)	0.12	7.69	0.104	17.46	0.0036	67.06
	Keller (1994)	0.113	13.08	0.097	23.02	0.0032	70.29
	Carter and Hayes (1977)	0.095	26.92	0.078	38.10	0.0024	78.16
	Kaneko et al. (2004)	0.1	23.08	0.084	33.33	0.0027	75.63
	Li and Aspden (1997)	0.535	311.54	0.475	276.98	0.0229	109.92

Turner et al., 1999; Zysset et al., 1999). As a result, the stiffness of the numerical models was high, which resulted in significantly lower displacements and deformations than in the experiment. Therefore, applying both Li and Aspden (1997) and/or Carter and Hayes (1977) relationships provides extreme Young's modulus distributions, which are too narrow or too wide, respectively. The relationships given by Ciarelli et al. (2000), Keller (1994) and Kaneko et al. (2004) provide Young's modulus distributions more similar to the experimental ranges (Choi et al., 1990; Hong et al., 2007; Jiroušek et al., 2011; Rho et al., 1997; Turner et al., 1999; Zysset et al., 1999), which are 1.461–11.829 GPa, 0.548–32.444 GPa and 1.365–24.048 GPa, respectively. The highest accuracy of numerical calculations is obtained using the Ciarelli et al. (2000) relationship with Young's modulus distributions within the range of 1.461–11.829 GPa, which is the most similar to the experimental ranges given by Choi et al. (1990), Hong et al. (2007) and Zysset et al. (1999). This accounts for the high accuracy of the numerical calculations obtained using this relationship, especially that the compression tests and measurement of these experimental ranges in the studies (Choi et al., 1990; Hong et al., 2007; Zysset et al., 1999) were both performed in wet conditions.

Apart from Young's modulus, Poisson's ratio also had an influence on the solution of the numerical calculation. In this study Poisson's ratio of 0.3 was used, but it was an assumption taken based on other numerical studies of the human femur in the literature (Couteau et al., 1998; Nareliya and Kumar, 2011; Sherekar and Pawar, 2013; Trabelsi et al., 2011). In contradiction to this, the experimentally determined value of Poisson's ratio of microscopic cubic specimens in three different directions was reported by Hong et al. (2007). They reported Poisson's ratio of 0.199 (longitudinal), 0.152 (anterior–posterior) and 0.154 (latero-medial). This shows a big difference between the commonly used value of 0.3 of Poisson's ratio and the values determined experimentally. Therefore, accuracy of the numerical calculations can be increased using the experimentally determined values of Poisson's ratio rather than Poisson's ratio of 0.3 commonly used in the literature.

There are also many other factors that could influence our experiments such as fixing in ethanol, a testing in the air, painting of the specimens and the radiation dose. The negative influence of fixation in ethanol on the specimens was presented by Hammer et al. (2014), where alterations of the mechanical properties of bone specimens were observed during long-term immersion in ethanol. However, we tried to minimize the time of specimen's fixation in ethanol, which was several hours. The other factor was a testing in the air. During the compression tests, the air partially filled the pores between the trabeculae instead of the bone marrow. The bone marrow remaining in the interaction with trabeculae affects the mechanical properties of trabeculae and also carries the external loads. However, our research was focused on a micro-scale and the mechanical properties of trabeculae without taking into account a bone marrow contained in the pores between trabeculae (this is rather a meso-scale). Thus, the ability of carrying external loads by a bone marrow contained in the pores is less important in our research. However, the interaction of bone marrow and trabeculae and

the possible changes of mechanical properties of trabeculae caused by an air presence instead of bone marrow could significantly influence the obtained results. Painting of the specimens could also make some disturbances on the measured values of displacements and strains as the used acrylic paint could also carry the applied loads. Only one side surface of each specimen was painted, so there was no obstacle for marrow escape during compression tests. However, the average depth of the penetration of acrylic paint into the specimens equaled to less than 1 mm (Fig. 2c). In this region the pores were filled with acrylic paint and this could affect the mechanical behavior of the specimens. Young's modulus of acrylic paint is significantly lower than Young's modulus of trabeculae and is approximately equal to $E=1$ GPa (Wright et al., 2014). A 1 mm-thin layer of acrylic paint that Young's modulus is significantly lower than Young's modulus of trabeculae might make its impact negligible. Effect of radiation dose on the mechanical behavior of specimens could also make it negligible. This is proved by the work of Currey et al. (1997), in which the effects of ionizing radiation on, inter alia, mechanical properties of human bone were examined. Young's modulus was unchanged by any level of radiation. Similarly, in the work of Barth et al. (2011), on the basis of the obtained stress–strain curves from the three-point bending test, it can be concluded that Young's moduli are constant for various degrees of X-ray irradiation. In addition, Brouwers et al. (2007) found no effects of micro-CT radiation on structural parameters of bone, which we also analyzed in our research.

The application of the relationship by Ciarelli et al. (2000) to the numerical calculation allows one to obtain the displacement distribution with a relative error of 17.87%. In the strain distribution the relative error is much higher and is about 50.94%. Therefore, the relationship given by Ciarelli et al. (2000) is not dedicated to determine the strain and stress in the bone. Although this study involved the numerical analyses only in the elastic range, it could be concluded that the application of this relationship in nonlinear simulations such as cracking, where the accuracy of determined stress concentrations should be very high, is not to be recommended, either. However, the application of the Ciarelli et al. (2000) relationship in the linear simulations is possible, but only to determine displacement.

The comparison of average relative errors for categories: “core”, “side”, “tet10” and “hex8” indicates that there is no basis to conclude that the accuracy of the numerical solution in this study depended on the specimen's position in the femoral head or the type of the finite element used in the numerical model. What could influence the results of the calculations were the mesh density and the boundary conditions. The results of the numerical calculations were obtained using the number of elements from the range of 750,000–1,250,000 (Table 5). Therefore, increasing the number of elements or using the adaptive finite element techniques can provide a higher accuracy of calculations, but the computing time will be extended. The appropriate selection of boundary conditions has the greatest impact on the accuracy of calculations. In this study the boundary conditions represented a mechanical compression test in which a platen technique was used. As a result, the compression of the

specimens could be influenced by the end artifacts and friction between ends of specimens and platens. However, a DIC system was used that measures the displacement and strain distributions on the specimen's surface and even they are influenced by the end artifact and friction. As a result on the obtained maps of displacement and strain distributions concentrations of displacements/strains caused by these end artifacts are visible (e.g. the concentration of the displacements and strains visible in the top part of Figs. 5a and 7a). Thus, the end artifacts influence our results, but this influence is measured by the DIC system, thereby causing the accuracy of the measurements to be limited to the accuracy of the DIC system. However, the representation of these boundary conditions (end artifacts, friction) in the numerical simulation can be a bigger problem. Although the representation of the end artifacts in the FE models of specimens was quite accurate as we have the voxel size of 10.246 μm in the micro-CT scans, the inaccuracy could appear in modeling of friction. We used in the FE models the glue contact between the plates and mesh of the bone structure. This simplification could influence the obtained numerical results, especially for strains, and as a result the calculated average relative errors for strains. The use of touching contact with an accurately determined value of friction coefficient may slightly increase the estimated accuracy of numerical simulations.

Acknowledgments

The authors would like to thank Jolanta Filipek for her help using the Mimics 15.0 and Anna Wójcicka for help in the analysis of speckle patterns. The numerical simulations were calculated using the license for MSC Software provided by the Academic Computer Centre in Gdańsk, Poland. Łukasz Cyganik and Paulina Popik are beneficiaries of the “Silesian Cooperation: Innovations For Efficient Development (SWIDER)” project realized within Human Capital Operational Programme, Priority VIII: Regional human resources for the economy, 8.2 Transfer of knowledge, Sub-measure 8.2.1 Support for cooperation of scientific environment and enterprises. The project was partially funded from European Union Project based on European Social Funds 8.2.1. Marcin Binkowski was partially supported by postdoctoral fellowship (KOLUMB) and supportive Grant from the Foundation for Polish Science, Warsaw, Poland.

The project was partially supported by Grant no. N N518 425036 from the Ministry of Science and Higher Education, Warsaw, Poland.

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