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Nondestructive Estimation of Mechanical Orthogonality of Human Trabecular Bone by Computed Tomography and Spherical Indentation Test

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Abstract: The elastic modulus and the apparent density of the trabecular bone were evaluated from spherical indentation tests and Computed Tomography (CT) and their relationship was quantified.

The femurs were prepared for trabecular bone analysis. Embedded with respect to their anatomical orientation, the transverse planes of the trabecular bone specimens were scanned at 1mm intervals using a CT scanner. The metaphyseal regions of femurs were sectioned with a diamond-blade saw, producing 8mm cubes. Using a specially made spherical indentation tester, the cubes were mechanically tested in the anterior-posterior (AP), medial-lateral (ML), and inferior-superior (IS) directions. After determination of modulus from the mechanical testing, the apparent densities of the specimens were measured.

The results showed that the IS modulus was significantly greater than both the AP and ML moduli with the AP modulus greater than the ML modulus. This demonstrated that orthogonality was a structural characteristic of the trabecular bone. The power relationship between the modulus and the apparent density was also found to be statistically significant.

Key words: Trabecular bone, Spherical indentation test, Apparent density, Orthogonality

INTRODUCTION

There is a great variation in strength and elastic modulus between trabecular and cortical bones due to their difference in apparent density and structure [1-2]. Generally, the elastic modulus of the trabecular bone is lower than the cortical bone and this is due to the 15 50% difference in apparent density [3-7]. The strength of the trabecular bone is lower than the cortical bone due to its structural characteristics. However, trabecular bone has been known to absorb a larger amount of energy before damage occurs. The importance of the biomechanical function of the trabecular bone can be noted by its resistance to different impact loads [3,8-13]. Furthermore, measuring an accurate elastic modulus to estimate the other mechanical properties of the trabecular bone is important to evaluate bone growth ability through mathematical analysis, artificial joint modeling, and finite element analysis [14].

Currently, early detection, prognosis, and treatment of osteoporosis have become so important since the population of the elderly has grown rapidly. Accurate assessment of bone quality and its mechanical integrity are essential to evaluate osteoporosis. The density and mechanical properties of the trabecular bone and their relationship to the anisotropy of this bone have to be considered and discovered [15-17]. Previous experimental methods have been generally tensile and compression experiments which are destructive tests [12,18-20]. Recently a non-destructive test, indentation impression, has been introduced to obtain the elastic modulus [21-23].

In this study, taking into account the anisotropic nature of the trabecular bone, the elastic modulus was measured using a spherical indentation test, a novel experimental method, and its apparent density which influences its mechanical properties will be obtained using computed tomography (CT), a noninvasive method. Thus, the relationship between elastic modulus and density was evaluated. Furthermore, the results were compared with previously reported studies to validate this experiment.

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MATERIALS AND METHODS

Experimental Materials

The left and right human distal femurs (31 years old, male) were obtained from the Seoul Municipal Hospital (Boramae) with consent and were preserved at -70°C prior to experiments. The diaphysis of the femurs were fixed with respect to their anatomical orientation in the anterior-posterior (AP), medial-lateral (ML), and inferior-superior (IS) directions, using a Plastic Mix (Vertex, Dentimex Inc., USA) to prevent movement during experiments.

The frontal and sagittal planes of the specimens were sectioned into 8mmX8mm squares using a diamond-blade saw (EXACT Precision Parallel Control 300CP/310CP, EXACT Apparatebau GmbH, USA) with a depth of around 50mm. The specimens were continuously irrigated with water and saline solution to prevent overheating caused by sectioning and to reduce fragmentations. After sectioning, the apparent density was obtained through CT. To determine the mechanical properties the spherical indentation test was conducted. The depth of sectioning along the frontal and sagittal planes was determined in order to assure identical points for the indentation test and CT

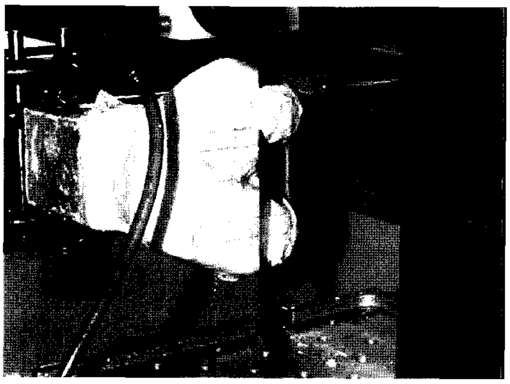

Fig. 1. Sectioning along the transverse plane with a diamond saw

To cut the specimens along the transverse plane, plaster was applied to the surface of the femur to minimize movement caused by the sectioning of the frontal and sagittal planes. To prevent the plaster from entering the gaps between the sections, cotton was placed there. Taking the same precautions when sectioning the frontal and sagittal planes, the specimens were sectioned along the transverse plane producing seventy-two 8mm cubes for each femur (Fig. 1). Immediately after sectioning, the anatomical directions along the three planes for each cube were marked, and the specimens were cleansed in saline solution, wrapped in gauze and stored at -20°C until experiment.

Experimental Equipments

Motor control, measurement, and data acquisition equipments were prepared for experimentation. All the controls were programmed in C-language (Fig. 2). A load cell (Interface Inc. Model SML 25lbs, USA) was selected for use in the experiment. An A/D board (Workmate Series, Strawberry Tree Inc., USA) was utilized to convert the signal generated by the load cell with sampling rate of 20Hz. A Signal Conditioning Amplifier (Measurements Group Inc., Model 2310, USA) was set at a filtering frequency of 10Hz, a power of 3.5V DC, and a gain of 100. To minimize noise all the equipments were grounded. An extremely precise LVDT (LBB series, Excellent linearity of ±0.20% FRO and repeatability of ±0.000004", Schaevitz Inc., USA) was used to measure the indentation depth. Considering that the relationship between the indentation depth and the impression diameter is nonlinear, a spherical indenter was selected which is theoretically capable of detecting all strains below 20%.

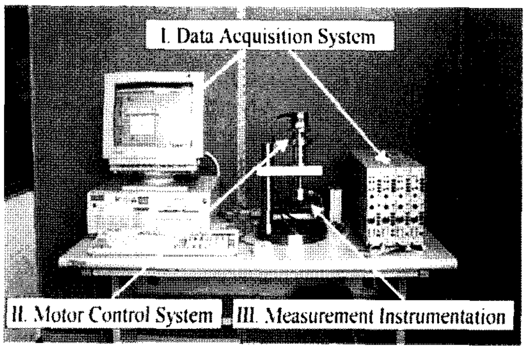

Fig. 2. The configuration of indentation testing system

To verify the validity of the equipments used in this experiment, indentation tests were performed on acryl, aluminum, brass, copper, and steel. The tests showed that modulus values obtained from the testing set-up were within a 5% deviation from actual elastic modulus figures (Fig. 3), which would prove the validity of the system.

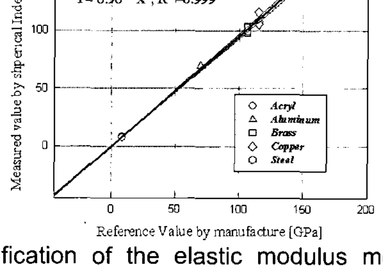

Fig. 3. Verification of the elastic modulus measurements using spherical indentation

Determination of Apparent Density

Using a GE9800 Quick (General Electric Medical System) CT scanner (120kV-100mA, DFOV 11) from the Kyunghee Medical Centre, the transverse plane of the specimens were scanned with a cross-sectional depth of 1.5mm and an interval of 1mm, producing a total of 45 scans for each femur. The same anatomical sites assigned during specimen preparation procedures were assigned here (Fig. 4). Images of the trabecular bone were displayed using an edge-detection programme, Extract (Cornell Univ.), and the Hounsfield Unit (HU or CT number) was determined.

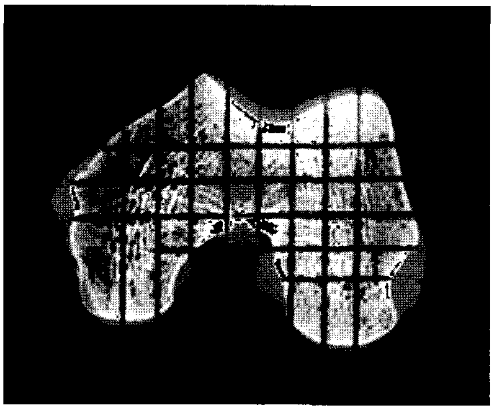

Fig. 4. CT scan of femur

To measure continuous HU values for the femur specimens, standard values need to be set; 0HU for water, -1000HU for air gaps, 1000HU for the cortical bone, and -25HU~714HU for the trabecular bone [15]. For each 8mmX8mm cross section HU was found at 10 different locations and the average value was taken. The average HU of the corresponding sections in 8 consecutive CT scans was designated for each specimen cube (8mmX8mmX8mm). Using the Hvid *et al.* Formula (apparent density=0.0013*HU+0.103, R^2=0.935), measured HU was converted to apparent density.

The apparent density was also obtained through conventional methods. After performing the indentation test, all the bone specimens were collected and put in a 70% ethanol solution for a day. They were then removed from the solution and centrifuged at 12,000rpm for 15min using a high-speed centrifuge (SORVALL Centrifuge). Using an air injector, all the residues inside bone cubes were removed and each bone cube was weighed on an electronic scale. The weight of the bone cubes and the volume of the femurs before experimentation were used to find the real apparent density (Fig. 5).

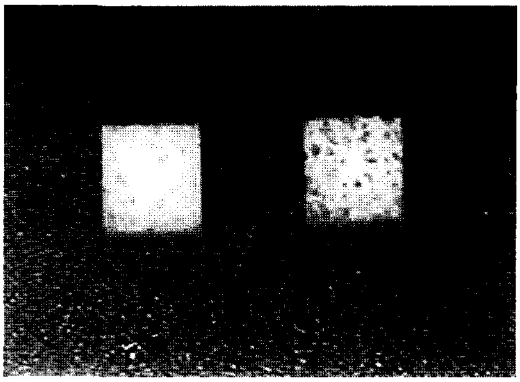

Fig. 5. Specimen cubes of distal femur with marrow (left) and without marrow (right)

Spherical Indentation Test

One of the factors that influence indentation tests is the speed of loading/unloading. This speed was divided and set into four stages; approach, loading, holding, and unloading. All the movements were programmed and controlled (Fig. 6). The approach speed was set at 1 ㎛/sec towards the surface of the specimen and the loading speed was set at 2.5 ㎛/sec which was constant during indentation.

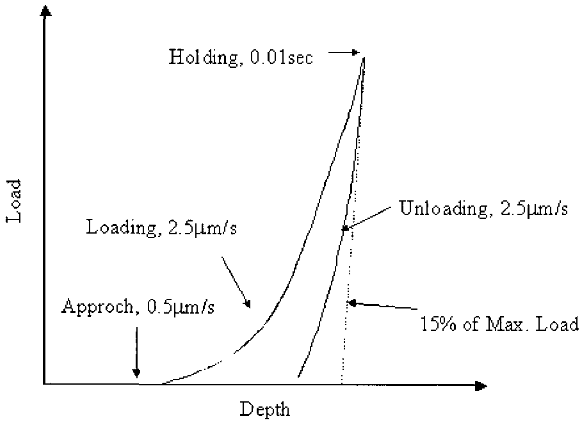

Fig. 6. The different stages of the indentation test : approach, loading, holding, and unloading

After the loading stage, there was a quick holding stage followed by and unloading stage set at the same speed as the loading stage but in the opposite direction (Fig. 7).

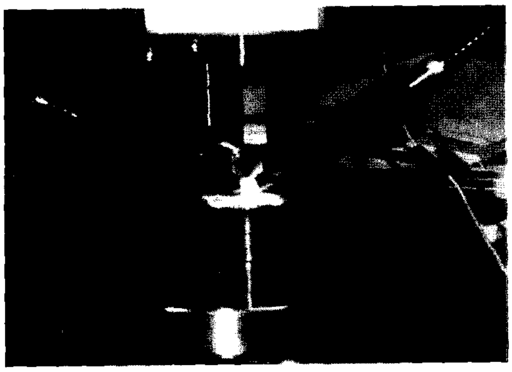

Fig. 7. Spherical indentation test of specimen cube

The prepared bone cubes were removed from -20□ and thawed at room temperature for 30min. In order to maintain their properties, they were washed with saline solution. After washing, the specimens were wrapped in gauze and kept in a box filled with saline solution to maintain their wet-state until experiments. Using a specially made spherical indentation tester, the cubes were mechanically tested in the AP, ML, and IS direction. The force and distance of the spherical tip were measured and recorded. In order to avoid exceeding the yield point, the experiment was conducted within the elastic region by constantly monitoring the data. After testing, the elastic modulus was calculated from the unloading curve with Francis' formula [21].

Statistical Analysis

To quantify the relationship between the elastic modulus obtained from the indentation test and the apparent density determined from CT of the trabecular bone, regression analysis was performed on the linear and power models. To evaluate the anisotropic nature of the trabecular bone, statistical analysis of the elastic modulus in the AP, ML, and IS directions were performed.

RESULTS

The average Hounsfield Unit for each 8mm cube ranged from 76HU to 572HU. The average HU for each of the distal femurs were 324.2HU and 348.5HU. The average HU for all the specimens was 336.45HU. The real apparent density was 0.4g/cm3 after measuring the volume of the distal femurs before experiments and measuring their weight after experiments and centrifugation. It became apparent that there was a relationship between the HU and the real apparent density (Fig. 8).

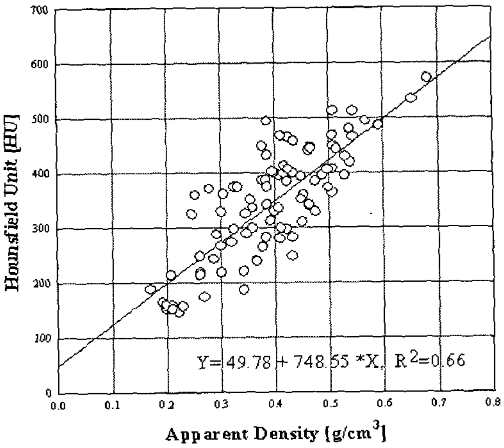

Fig. 8. Relationship between HU and apparent density

The spherical indentation test showed that the average elastic modulus in the IS, AP, and ML directions were 1.48GPa, 1.14GPa, and 0.97GPa, respectively. The majority of the specimens showed that the IS modulus was greater than the AP and ML moduli by an average of 22.5% and 34.5%, respectively. The AP modulus was 15.5% greater than the ML modulus. Statistically, there were significant differences in all three directions (Fig. 9).

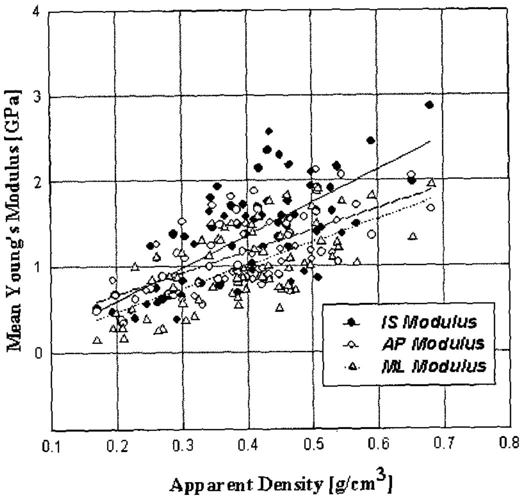

Fig. 9. Relationship between elastic modulus and apparent density

Results showed that there was a relationship between the elastic modulus, HU, and apparent density. The Young's modulus increased as HU became greater.

Table 1. Relationship between HU (Hounsfield Unit), Apparent Density (g/cm3), and Orthogonal Moduli (GPa)

			Linear Model Ei=Slope*(density or HU)+error				Power Model Ei=A(density or HU)^{POWER}+error			
			HU		Apparent Density		HU		Apparent Density	
	Direction	n	Slope	R^2	slope	R^2	Power	R^2	Power	R^2
distal femur	IS	72	0.0036	0.3539	3.8681	0.5111	0.0029	0.4241	4.2773	0.5855
	AP	72	0.0025	0.2844	2.5322	0.4279	0.0110	0.4000	2.6801	0.4966
	ML	72	0.0027	0.3630	2.7020	0.4226	0.0014	0.4120	3.2857	0.5088
	Total	216	0.0030	0.3241	3.0318	0.4165	0.0033	0.3910	3.3479	0.4907

Through regression analysis, the power relationship between the elastic modulus(Ei) and apparent density (Ei=A(density or HU)Power+error, R2=0.4~0.42) was found to be more significant than the linear relationship(Ei=Slope*(density or HU)+error, R2=0.33~0.36) ($p<0.05$). Likewise, the power relationship between the HU and apparent density (R2=0.50~0.59) was more significant than the linear relationship (R2=0.42~0.51). A statistical difference was also noted ($p<0.05$). A proportional increase was found in both cases (table 1).

DISCUSSION

In this study, the apparent density of the trabecular bone was determined both from CT images and conventional methods. There exists a significant linear correlation between the densities from both methods. However, there was a 15% difference in the results of these two methods (Fig. 10). Despite the difference, the results of the two methods were in accordance with the work of other researchers [11,14-15,24]. When measuring bone density through *in vivo* methods, these methods proved to be a source of excellent estimates. All the physical properties of the trabecular bone cannot be explained through its apparent density, however, it is valuable in the early detection of osteoporosis through CT.

Elastic moduli for the trabecular bone were obtained through spherical indentation tests in this study, ranging from 140.3MPa to 2568.7MPa. Previous studies showed that the trabecular modulus ranged from 7.6Mpa to 2942MPa. The IS modulus was greater than the AP and ML moduli, with the AP modulus being greater than the ML modulus. This demonstrated that one of the characteristics of the trabecular bone was its anisotropy and that its structural orientation influenced its elastic modulus [4,15,20,25]. However, our study suggests that trabecular bone tend to be more orthogonal than transversely isotropic which suggested by the studies of Reilly, Burstein, and Goldstein *et al*...

Many researchers have attempted to find a relationship between the structural and mechanical properties of the trabecular bone (table 2). Through the regression analysis on the relationship between the apparent density and the elastic modulus, the power relationship was a more suitable relationship for these two variables than the linear relationship. Many past studies suggested linear relationships between the apparent density and elastic modulus of the trabecular bone [4,15]. However, like the results from this experiment, the majority of recent studies supported the power relationship rather than the linear relationship [8,9]. Thus the spherical indentation test and CT proved to be both effective, non-destructive methods in determining the relationship between the apparent density and the properties of the trabecular bone.

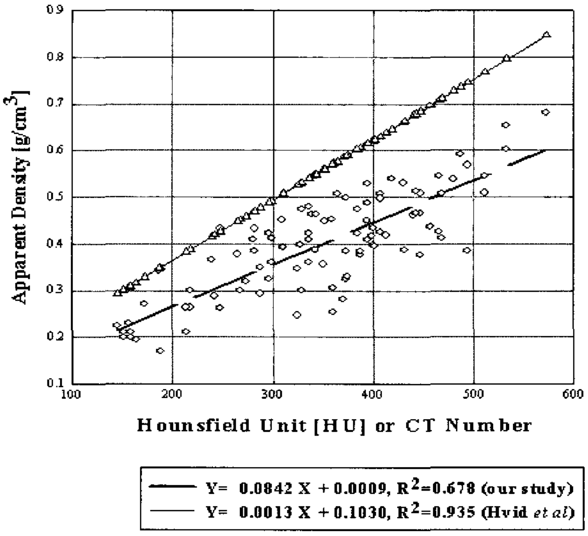

Fig. 10. Data comparison of present study and Hvid *et al.*

CONCLUSION

It has been widely known that determining the mechanical properties of the bone plays a very significant role in diagnosis and treatments of osteoporosis and other bone related orthopedic conditions. In this study, using a spherical indentation tester, the elastic moduli in the IS, AP, and ML directions were determined. The IS modulus was greater than the AP and ML moduli ($p<0.01$) with the AP modulus being greater than the ML modulus ($p<0.01$). This demonstrated that orthogonality was a structural characteristic of the trabecular bone.

Generally DEXA (Dual Energy X-Ray Absorptiometry) is used to examine bone density in clinical situations, however, in this study, CT was used. While the apparent density of the trabecular bone obtained both from CT and conventional method demonstrated a linear relationship, measuring the apparent density from CT is more realistic for clinical cases.

Finally, when comparing the relationship between the apparent density obtained from CT and the elastic modulus derived from the spherical indentation test, a power relationship was observed. Through obtaining the HU and the elastic moduli of real femurs of osteoporosis patients, dangers of bone fracture can be prevented and an early treatment prognosis can be administered, providing great assistance to the patients.

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