World Academy of Science, Engineering and Technology
International Journal of Aerospace and Mechanical Engineering
Vol:8, No:12, 2014

How the Decrease of Collagen or Mineral Affect the Fracture in the Turkey Long Bones

P. Vosynek, T. Návrat, M. Peč, J. Pořízka, P. Diviš

Open Science Index, Aerospace and Mechanical Engineering Vol:8, No:12, 2014 publications.waset.org/9999811/pdf

***Abstract*—**Bone properties and response behavior after static or dynamic activation (loading) are still interesting topics in many fields of the science especially in the biomechanical problems such as bone loss of astronauts in space, osteoporosis, bone remodeling after fracture or remodeling after surgery (endoprosthesis and implants) and in osteointegration. This contribution deals with the relation between physiological, demineralized and deproteinized state of the turkey long bone – tibia. Three methods for comparison were used: 1) densitometry, 2) three point bending and 3) frequency analysis. The main goal of this work was to describe the decrease of the protein (collagen) or mineral of the bone with relation to the fracture in three point bending. The comparison is linked to the problem of different bone mechanical behavior in physiological and osteoporotic state.

***Keywords*—**Bone properties, long bone, osteoporosis, response behavior.

I. Introduction

THE bone is very complicated and hierarchical compound of two phases, mineral (hydroxyapatite) and protein (type I collagen) [1]. Our study is aimed on the experimental investigation of the whole bone properties and behavior compared to the previous work where input material characteristics were deterministic [2]-[5], or stochastic with just estimated normal distribution [6]. It was motivated by the known phenomena related to densitometry and the disease called osteoporosis [7]. The phenomena could be described as opposite inclination to the fracture based on evaluation bone mineral density (BMD). Subject may have low BMD compared to the same gender of population (evaluated by z-score) and did not ever suffer from bone fracture, or it may have high BMD (z-score) and suffer from bone fracture many times. One hypothesis describes this phenomenon as dependence on the other phase which is collagen. With the same amount of mineral (Fig. 1), totally different behavior is obtained when we decrease or increase the amount of collagen (brittle fracture or ductile fracture) [8]. Therefore there is need to find the method which could evaluate also the amount of collagen or the complex behavior (e.g. elasticity of bones). The question is if such property exists and if it could be evaluated also in vivo. This paper present the relations between the usual method for estimating the risk of fracture by BMD (densitometry), by laboratory method for measuring natural frequency [9] and finally by the three point bending [1].

P. Vosynek is with the Institute of Solid Mechanics, Mechatronics and Biomechanics, Faculty of Mechanical Engineering, Brno University of Technology, Technicka 2896/2, 616 69 Brno, Czech Republic (phone: +420 54114 4930; e-mail: vosynek@fme.vutbr.cz).

T. Návrat and M. Peč is with the Institute of Solid Mechanics, Mechatronics and Biomechanics, Faculty of Mechanical Engineering, Brno University of Technology, Technicka 2896/2, 616 69 Brno, Czech Republic (e-mail: navrat@fme.vutbr.cz, y133757@stud.fme.vutbr.cz).

J. Pořízka and P. Divis is with the Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkyňova 464/118, 612 00 Brno, Czech Republic (e-mail: xcporizkaj@fch.vutbr.cz, divis@fch.vutbr.cz).

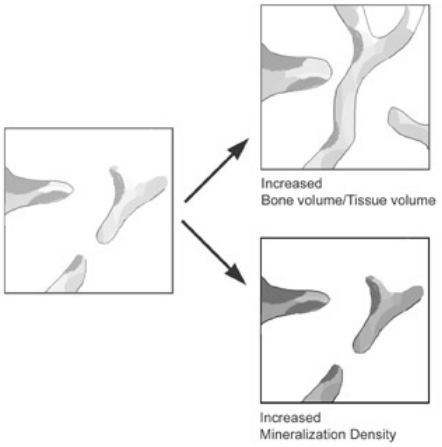


Fig. 1 Different mechanism of increasing BMD [8]

II. Materials and Method

A. Experimental Equipment and Settings

Densitometry: Hologic QDR 4500 C densitometer were used.

Three point bending: Universal testing machine ZWICK with force measuring cell (up to 2.5 kN). Cylindrical supports and loading part has rounded contact area (radius 5 mm). Distance between supports is 120 mm. For the alignment of the sample see Fig. 2. Experimental conditions were set according to the experience of solving similar problems [10], [11].

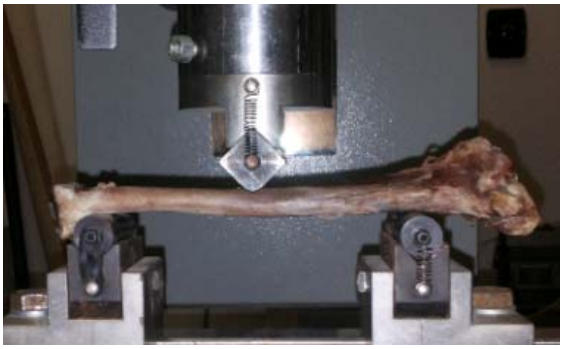


Fig. 2 Alignment of the specimen in three point bending measurement

Open Science Index, Aerospace and Mechanical Engineering Vol:8, No:12, 2014 publications.waset.org/9999811/pdf

Natural frequency analysis: Accelerometer 4370 B&K with sensitivity 10.09 pC/ms^{-2} placed in the middle of bone diaphysis, preamp 2647 A B&K with sensitivity 1 mV/pC, analyzer 3560-B-140 B&K, impact hammer type 8206. For analyzing the results we used PULSE Labshop software. Samples were hanged during excitation on the flexible wire.

B. Samples

All 10 whole bones samples were firstly get rid of the soft tissue. It was done carefully by the knife and the rest of tiny parts by scalpel (no chemicals). This group is called physiological.

Chemical treatment was done in the hydrochlorid acid (5.95 M of HCl) for demineralization and sodium hypochlorite (0.835 M of NaClO) for deproteinization [12].

C. Methods

The experiment has following line up: 1) measure properties and response values in physiological state except three point bending. They are BMD, first natural frequency with bending shape, diameters and length of bones. 2) Divide the physiological group (PL) into demineralization group (DM) with 5 samples and deproteinization group (DP) with 4 samples (one sample left for final fracture with no chemical treatment). 3) Repeat the measurement from the first step. 4) Immerse samples in chemicals for 1 hour (only diaphysis of the bones were exposed to the chemicals, both epiphysis has no chemical treatment). 5) Measure force/displacement dependence up to the bone fracture.

All measurements were done under standard laboratory environment.

From the three point bending measurement the maximum (limit) stress were also obtained next to the maximum force and displacement. Because the cross section is variable along the length of bone we reduce the geometry of bone to the elliptical tube. The geometry of the tube was calculated as a mean value from the two dimension (in the middle of diaphysis). Major axis and minor axis of ellipse were measured on unfractured bones. Thickness was measured after fracture in the middle of diaphysis.

III. Results and Analysis

A. Osteoporosis

Demineralization caused the loss of the mineral. First point was to evaluate the amount of the mineral decrease by the z-score from the BMD values (determine if the specimens undergo osteoporosis or only osteopenia). For the reference group physiological specimens were used. They were described by the normal distribution of BMD (the test of normality at the significance level 5 % was rejected the hypothesis that the data follow a normal distribution, p-value 0.349) with parameters: mean 0.375 g/cm^2, standard deviation 0.019 g/cm^2 (Fig. 3). The mean value of the z-score for DM group is 4.5 with the standard deviation 1.4. The mean value of the z-score for DP group is 0.0 with the standard deviation 1.1. Osteoporosis starts in z-score of 2. The DM group has therefore high level of osteoporosis.

In addition the hypothesis of significantly different mean values between PL samples and DM samples were done and statistically significant difference was found between groups (see bold p-values in Tables I and II). Boxplot in Figs. 4 and 5 presents the graphical relations between mean values.

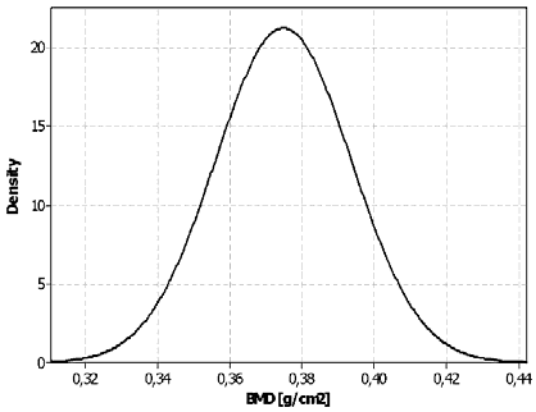


Fig. 3 Normal distribution plot of PL group

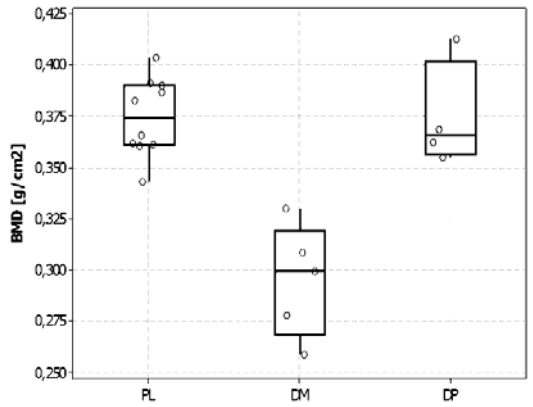


Fig. 4 Boxplot of BMD – PL, DM and DP group

TABLE I
Bone Mineral Density P-Values

p-values	DM	DP
PL	**0.003** paired t-test	0.570 paired t-test
DM		**0.004** t-test

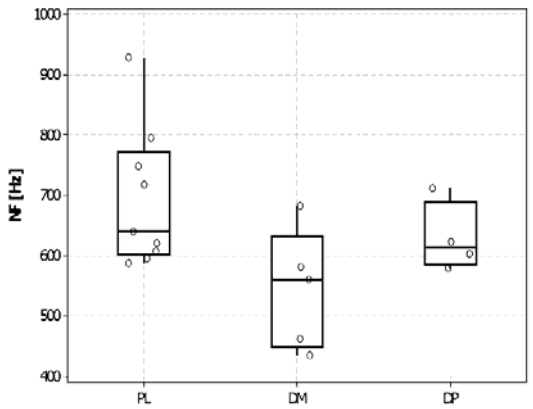


Fig. 5 Boxplot of NF – PL, DM and DP group

TABLE II
NATURAL FREQUENCY P-VALUES

p-values	DM	DP
PL	**0.001** paired t-test	**0.012** paired t-test
DM		0.160 t-test

B. Bone Fracture

From the three point bending measurements we mainly look for the maximum force and displacement at this point. Because in one DP sample the test machine was wrong, the value cannot be compared to the other (it leads to only three samples in DP group for fracture testing). Thanks to these consequences we decided to use only percentage mean value compare, instead of statistical methods.

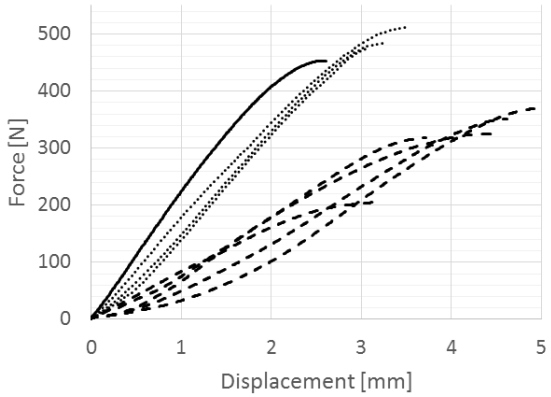


Fig. 6 Force-displacement curves. Groups - line: DM - dashed, DP - dotted, PL – full

Maximal forces for each group of specimens were recalculated to the maximal bending stress. In Fig. 6 the full lines represent PL group, the dashed lines represent the DM group and the dotted line represent the DM specimen.

Mean maximal stress of DM group is 81 MPa with interval of values 47 MPa to 105 MPa. Mean maximal stress of DP group is 113 MPa with interval of values 106 MPa to 119 MPa. Physiological state is represented only by one value of 111 MPa. Difference between mean values of these groups is 28%.

Similarly to the stresses the displacements were evaluated. Mean displacement in the point of maximal force (stress) is in DM group 4.2 mm with interval of values 3.2 mm to 5.0 mm. Mean displacement in DP group is 3.1 mm with interval of values 2.6 mm to 3.5 mm. Difference between mean values of these groups is 26%.

The last evaluated parameter was Young modulus. It was estimated from the stresses and strains with the same tubular elliptical approximation of the cross section. The mean value of DM group is 3144 MPa with interval of values 1576 MPa to 4260 MPa. Mean modulus of DP group is 8222 MPa with interval of values 6997 MPa to 9820 MPa. PL group has only one value 7044 MPa. Difference between DM and DP mean values is 38 %.

The dependence of stress to strain is presented in Fig. 7 (two modulus are the same in DM group therefore only 4 lines could be seen; also there are the same modulus in the groups DP and PL). Notice the switched slope of the curves between groups DM, PL in Figs. 6 and 7.

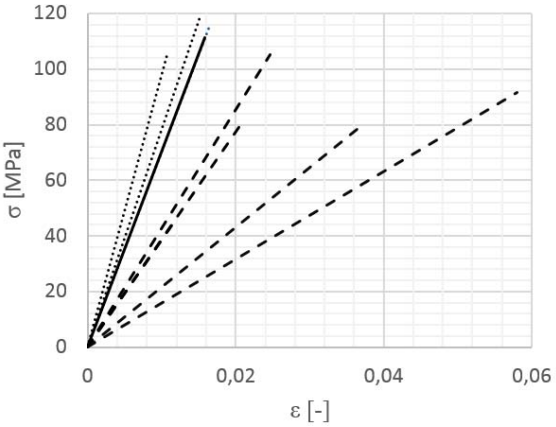


Fig. 7 Stress-strain curves. Groups - line: DM - dashed, DP – doted, PL – full

IV. DISCUSSION

Chemical treatment of samples by hydrochlorid acid is causing higher degree of demineralization. It is seen from the evaluation of the mean and standard deviation of "normal population" (PL group). The setup for this experiment, in meaning of duration of chemical treatment and concentration of hydrochlorid acid, is suitable for simulation the osteoporotic state of bone.

In vitro there is a similar decrement of the bone mineral density and of the natural frequency in demineralized samples. The same behavior is observed in deproteinized samples. Natural frequency has higher variance (Fig. 5) compared to bone mineral density (Fig. 4).

The elasticity behavior cannot be evaluated from the force to displacement dependence (Fig. 6). Young modulus, which is the main characteristic for elasticity behavior, has to be estimated by geometry simplification (elliptical tube). The main interest is not in the precise measurement of the Young modulus (and also maximal stresses and strains) but in proportion of the values to each other in groups DM, DP and PL.

From the mechanical behavior of composites it can be clearly seen that the bone has similar global behavior as composite (Fig. 8) [13]. Matrix is represented by collagen and connective links, fibers are represented by minerals settled along the collagen.

Open Science Index, Aerospace and Mechanical Engineering Vol:8, No:12, 2014 publications.waset.org/9999811/pdf

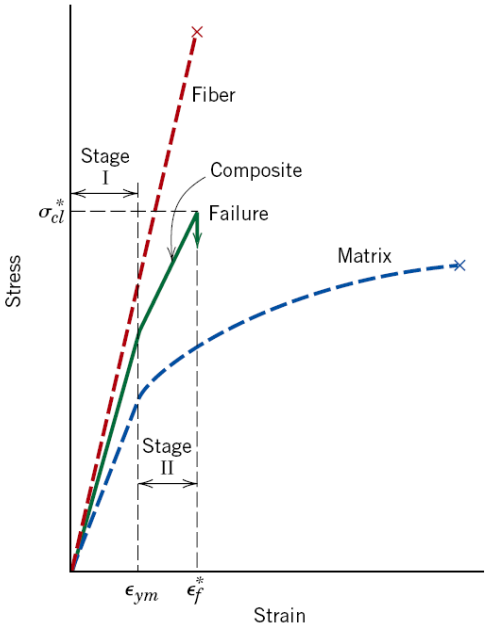


Fig. 8 Schematic stress-strain curves for an aligned fiber-reinforced composite; loaded in direction of fibers, [13]

In future we would like to increase the number of specimens for appropriate statistical evaluation of the measurement output. Next aim should be realization of the vibration measurements in vivo to link the current results with the in vitro measurements.

ACKNOWLEDGMENT

This work is an output of research and scientific activities of NETME Centre, regional R&D centre built with the financial support from the Operational Programme Research and Development for Innovations within the project NETME Centre (New Technologies for Mechanical Engineering), Reg. No.CZ.1.05/2.1.00/01.0002 and, in the follow-up sustainability stage, supported through NETME CENTRE PLUS (LO1202) by financial means from the Ministry of Education, Youth and Sports under the „National Sustainability Programme I".

We would like to thank to MUDr. MVDr. Jan Šlesinger and Dagmar Hrubá of Osteologic Center Brno for kind support in the field of bone mineral density measurements.

REFERENCES

[1] S. Cowin. *Bone mechanics handbook.* 2nd edition. Boca Raton; London; New York; Washington, D.C.: CRC Press, 2001. (800). ISBN 0-8493-9117-2.

[2] P. Vosynek, T. Návrat, P. Krejčí, L. Houfek. *Design of experimental device for loading the femur when standing on one leg* (2011) EAN 2011: 49th International Scientific Conference on Experimental Stress Analysis, pp. 429-434.

[3] Houfek, L., Florian, Z., Březina, T., Houfek, M., Návrat, T., Fuis, V., Houška, P. *Development of Experimental Devices for Testing of the Biomechanical Systems* (2009) IFMBE Proceedings, 23, pp. 2005-2008.

[4] Fuis, V., Janicek, P., *Stress and reliability analyses of damaged ceramic femoral heads*, in Conf. on Damage and Fracture Mechanics, Maui Hawaii, Structures and Materials vol. 12, pp. 475-485, 2002.

[5] Fuis, V., *Stress and reliability analyses of ceramic femoral heads with 3D manufacturing inaccuracies*, in Proc. 11th World Congress in Mechanism and Machine Science, Tianjin, China pp. 2197-2201, 2004

[6] P. Vosynek, T. Návrat, V. Fuis. *Response analysis of bone mass of proximal femur to surface replacement of hip joint* (2010) IFMBE Proceedings, 31 IFMBE, pp. 807-810.

[7] Janů, I., Kočiš, J., Návrat, T., Florian, Z., Wendsche, P. *A comparative analysis of socon CS and socon pedicle screws in view of their use for treatment of osteoporotic fractures of the thoracolumbal Spine. a biomechanical study*, (2011) Acta Chirurgiae Orthopaedicae et Traumatologiae Cechoslovaca, 78 (4), pp. 334-338.

[8] Ott. S., *Osteoporosis and bone physiology* (online). 1998, 9.5.2011 (cit. 2011-08-15). Awailable from WWW: http://courses.washington.edu/bonephys/.

[9] Anastassopoulos, G., S. Panteliou, G. Christopoulou, A. Stavropoulou, E. Panagiotopoulos, G. Lyritis, L. Khaldi, J. Varakis, and N. Karamanos. *Vibrational Bone Characteristics Versus Bone Density for the Assessment of Osteoporosis in Ovariectomized Rats*. Journal of Medical Engineering and Technology 34, no. 1 (2010): 35–42. doi:10.3109/03091900903324056.

[10] Cizmar, I., Florian, Z., Navrat, T., Palousek, D. *A biomechanical study of a suture between the deltoid muscle and a free tendon graft for reconstruction of the elbow extension* (2011) Biomedical Papers, 155 (1), pp. 79-84.

[11] Urbanová, L., Srnec, R., Proks, P., Stehlík, L., Florian, Z., Návrat, T., Nečas, A. *Comparison of the resistance to bending forces of the 4.5 LCP plate-rod construct and of 4.5 LCP alone applied to segmental femoral defects in miniature pigs* (2010) Acta Veterinaria Brno, 79 (4), pp. 613-620.

[12] Chen, Po-Yu, and Joanna McKittrick. *Compressive Mechanical Properties of Demineralized and Deproteinized Cancellous Bone*. Journal of the Mechanical Behavior of Biomedical Materials 4, no. 7 (jen 2011): 961–73. doi:10.1016/j.jmbbm.2011.02.006.

[13] Callister, W. D, Rethwisch D. G. *Materials science and engineering: an introduction*. 8th ed. Hoboken: Wiley, c2010, xxii, 885 s., A40, G15, S4, I22. ISBN 978-0-470-41997-7.

Open Science Index, Aerospace and Mechanical Engineering Vol:8, No:12, 2014 publications.waset.org/9999811/pdf