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AN INVESTIGATION ON THE MECHANICAL COMPETENCE OF THE TRABECULAR BONE

Katia Arcaro[a], **Waldir L. Roque**[a] **and Zbislaw Tabor**[b]

[a]*Programa de Pós-Graduação em Matemática Aplicada, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil, roque@mat.ufrgs.br.*

[b]*Department of Image Analysis, Institute of Applied Computer Science, Cracow University of Technology, Cracow, Poland, ztabor@pk.edu.pl.*

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Abstract. Osteoporosis is a disease that has become quite important nowadays due to the longer life expectancy of the population worldwide. It is mainly characterized by bone mass loss and trabecular structure deterioration, which lead to an increase in bone fragility. The bone mass loss is assessed based on measurements of bone mineral density, performed using appropriate densitometry. However, it has been known that the densitometry alone is not able to estimate the risk of fracture of a subject. Another important aspect is the trabecular bone microarchitecture, which can be investigated by image-based examinations, that normally provide 2D or 3D set of images. Some structural parameters have been investigated to describe the mechanical competence of the trabecular bone. In this paper we investigate the relationship between some of these parameters, namely: the trabecular bone connectivity, using the Euler-Poincaré Characteristic (EPC), the trabecular volume fraction (BV/TV) and the Young's Modulus (YM). For that, three distinct set of vertebrae of different subjects, based on their tomographic images were analyzed. The YM has been obtained by simulation using finite element method (FEM) based on grayscale intensity. The results seem consistent and can be grouped according to two distinct aspects: one group has high YM with EPC predominantly negative, suggesting a good trabecular connectivity, and a BV/TV above 50%; the other group has a low YM, with EPC predominantly positive, suggesting a low trabecular connectivity, and a BV/TV less than 50%. The analysis of multiple correlation among BV/TV, EPC and YM have shown a strong positive correlation between BV/TV and YM, and a negative one between EPC and YM, and between EPC and BV/TV. In addition, the principal component analysis confirmed the results.

1 INTRODUCTION

The worldwide improvement of the welfare and of the quality of life of the population have increased quite a lot the life expectancy, as can be seen in the World Health Organization webpage[1]. On the one hand this is very nice, but on the other hand some diseases like osteoporosis have become prevalent among the elderly. Osteoporosis is a silent disease caused by metabolic bone disorder, leading to bone mass loss and deterioration of the bone tissue, with an increase in bone fragility and its consequent risk of fracture. Currently, osteoporosis can be seen as a public health problem with a high treatment cost. New techniques need to be developed to improve the assessment of osteoporosis and the prediction of fracture risk of a patient, making available possible preventive therapies.

The bone mineral density (BMD) is the gold-standard exam to diagnose osteoporosis. The BMD is measured by *Dual Energy X-ray Absorptiometry* (DXA), giving the bone mineral content at a specific location. Although it has been shown that the risk of fracture increases by a factor of 1.5 to 3.0 for each standard deviation decrease in the BMD (Kanis et al., 2009), the BMD reflects the bone quantity by area and this is only one of the aspects of the bone quality (Griffith and Genant, 2008; Goossens et al., 2008). Studies have pointed out that the BMD responds to around 60%-80% of the bone mechanical resistance and 20%-40% of the variability is due to other contributions (Cortet and Marchandise, 2001), such as trabecular microarchitecture (Gomberg et al., 2000), in addition to other concomitant factors (Kanis et al., 2009).

The trabecular or cancelous bone is a spongy bone, as such the trabeculae can be modeled as grains and the marrow cavities as pores, following the nomenclature of porous media (Roberts et al., 1997). There are some procedures for the assessment of the trabecular microarchitecture. The non-invasive techniques for the assessment are image-based, mainly produced by magnetic resonance imaging (MRI) or by computer tomography (CT). A more recent alternative approach is based on quantitative ultrasound (QUS) (Portero-Muzy et al., 2007).

One of the main properties of the trabecular structure is its degree of connectivity, which seems to be a very important factor in establishing the bone quality and consequently, plays a role in the bone mechanical resistance (Roque et al., 2009; Carbonare et al., 2005; Apostol and Peyrin, 2007). The connectivity is a geometrical property which provides information about the structure of the pore space and the Euler-Poincaré Characteristic (EPC) is a geometric measure that can be used to estimate the connectivity degree of the structure. As EPC is a zero-dimensional quantity, it needs to be estimated using a 3D device (Mouton, 2002), in this case, the *disector*, which consists of a pair of parallel images from which the trabecular behavior in the space between them can be inferred (Sterio, 1984; Kroustrup and Gundersen, 2001).

It is also know that the elasticity is an important property of a material. The action of forces (stress) can cause deformations in bone shape (strain) and this can be reflected in the Young's modulus (YM) of elasticity, which is the slope of the linear component of the stress versus strain curve, providing a measure of the material stiffness. The stress-strain ratio can indicate if an object (or part of it) is stiff or malleable. To determine YM for the trabecular structure, the Finite Element Method (FEM) has been used to compute the reaction force exerted by the object structure (van Rietbergen et al., 1995).

According to Gibson (1985), the YM is related to the trabecular bone mass content in a specified volume of tissue. The trabecular bone volume fraction BV/TV can be estimated analyzing binarized two-dimensional images of the object. For each image, the white pixels area is computed, corresponding to trabecular bone, and it is divided by the total number of pixels

[1] http://www.who.int/en/.

comprising the image. An average area is calculated for all images. Following Russ and Dehoff (1999), this area fraction is equivalent to bone volume fraction (BV/TV).

In this paper we are interested in investigating the correlation between the BV/TV, EPC and YM for a set of 15 samples of human vertebrae *in vitro*, where 6 vertebrae were of a subject named A, other 6 were of a subject named B, and 3 vertebrae were from another subject named C. These samples were scanned by computer tomography. In Section 2, we introduce the material and methods used for this study; in Section 3, a discussion about the multiple regression and the principal component analysis is done and the results are presented and, finally in Section 4 some discussion and conclusions are addressed.

2 MATERIALS AND METHODS

This study was based on three sets of vertebrae that were provided by the Legal Medical Department from the State of Rio Grande do Sul, Brazil. The sets were of three different cadavers, here named as A, B and C. The first set, A11 to A16, was from a teenager (15 to 17 years old); the second set, B11 to B16, of a male (26 to 50 years old); and the other one, C11 to C13, was from another male (25 to 45 years old). These vertebrae were submitted to a rigorous process of cleaning and drying, and after that they were wrapped in a substance which simulates the attenuation of the body due to the soft tissue, to carry on the densitometric measurements. The densitometry were performed with the *Hologic* QDR 4500 A. After that, the CT of the set of vertebrae was obtained with the *Siemens* tomograph, model *Somatom Plus* 4, at the São Lucas Hospital, PUCRS, in Porto Alegre. A total of 180 tomographic images were produced with a sectioning of 1mm. From the set images of each vertebrae, the regions of interest (ROI) were chosen inside of the vertebral body, in the anterior part, close to the cortical wall, because this is the most critical region for bone mass loss in the vertebral bodies (see Figure 1).

2.1 Image Processing

To analyze these images, a computer program (*OsteoImage*) has been developed using GTK+, for Windows. *OsteoImage* receives as input the set of sequential ROI images of a vertebra and automatically binarize them (Glasbey and Horgan, 1995; Gonzalez and Woods, 2000) using the *InterMeans* algorithm (Niemistö, 2004). After the binarization, the segmentation oriented to regions is performed for the determination of connected sets which represent the bone structure (trabeculae), defined as the white pixels, and the complement, made by the other tissues (bone marrow), defined as the black regions. The segmentation is performed by pixels aggregation, considering the 8-neighbor technique. The *OsteoImage* has implemented several features, it computes the trabecular and marrow numbers in each image, the trabecular and marrow areas for each image, the trabecular perimeter (following (da Fontoura Costa and Jr., 2000) idea), the area fraction (or, equivalently, BV/TV), and several other important parameters, as defined in Chappard et al. (1999).

The connectivity of the trabecular bone, seeing as a porous media, is a topological property that provides information about the structure of the pore space. The Euler-Poincaré Characteristic (EPC) is an integral geometrical measure that can provide an estimate of the connectivity of the pore space structure. An important aspect of the EPC is that it does not change under deformation or scaling of an object. In other words, it is a topological invariant. Essentially, the EPC for a 3D structure is defined as the number of isolated parts minus the connectivity. The EPC is a zero dimensional quantity and as such it has to be estimated using a 3D probe. Nevertheless, it has been shown for practical purposes that the 3D probe can be approximated by a set of 2D parallel sections chosen such that no change in the morphological structure appears in between them. These two sections form the so called *disector* (Sterio, 1984; Vogel and Kretzschmar, 1996). An implementation of the EPC algorithm was done in the *OsteoImage* (Roque et al., 2009) to estimate the trabecular bone connectivity.

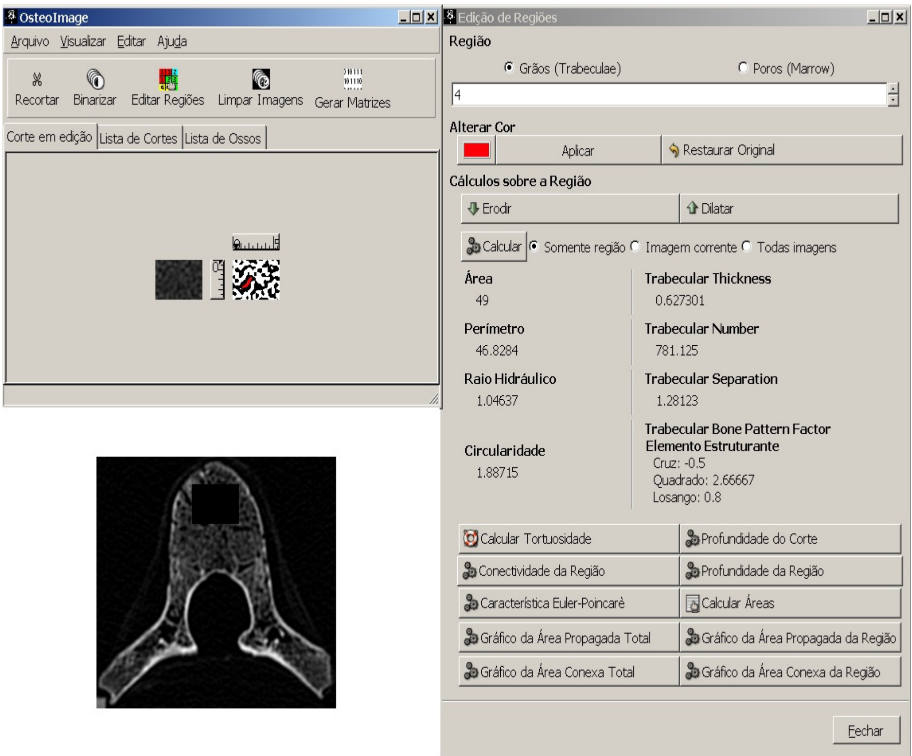

Figure 1: On the top left, the *OsteoImage* initialization window is exhibited, showing the ROI with 59×42 pixels and its binarization, obtained from the tomographic image below. On the right, some of the *OsteoImage* features.

2.2 The YM Simulation

To estimate the Young's modulus for the A, B and C sets of trabecular bones samples, finite element (FE) simulations were performed. The FE software was developed based on the voxel conversion technique (Tabor, 2007; van Rietbergen et al., 1995) modified to account for the range of gray-level intensities present in the CT images (Homminga et al., 2001). In the FE calculations brick finite elements with eight nodes and three internal degrees of freedom were used to account for possible bending modes. Voxels of the CT images were directly converted to finite elements. Typically, CT-based FE models were built with approximately 30000 - 120000 elements and over 100000 degrees of freedom. It was assumed that the bone material is isotropic and linear. Under this assumption, only two material constants have to be specified: Poisson's ratio set equal to 0.3 and Young's modulus E_0 of the bone tissue set equal to 5GPa.

According to the gray-value voxel conversion FE technique (Homminga et al., 2001) implemented in the present study, it has been assumed that the Young's modulus YM of a single element depends on the gray level intensity at the element, in agreement with the following formula:

$$YM = E_0 * (gli)^{exp} \tag{1}$$

where gli is the gray level intensity of a voxel and exp is some exponent. According to the adopted approach, a single stiffness matrix of an element has to be stored, the entries of which have to be appropriately recalculated when assembling the global stiffness matrix, based on the gray-level intensities of the image voxels. The exp has been set equal to 0, 1, or 2. The results for these different cases correlate quite well, in the sense that the proportionality is maintained. In other words, changing the model used for the trabecular bone, the Young's modulus values change proportionally (Gibson, 1985).

In this study a compression test was simulated only for the axial direction of trabecular bone samples. Displacement boundary conditions with infinite friction were imposed. The bottom surface was constrained and unit displacement was prescribed at the top surface of a FE model. The resulting set of linear equations was solved iteratively using the preconditioned conjugate gradient (PCG) method with the diagonal of the global stiffness matrix as preconditioner. The iterations were terminated after the global error measure had dropped below error tolerance equal to 10^{-11}. Moreover, the convergence has been guaranteed testing more than one error tolerance value.

As the result of the simulation, reaction force R at the top surface was calculated. The apparent Young's modulus was computed from the reaction force, just dividing it by the slice area and multiplying by the sample thickness (number of slices minus 1). This is because it has been applied a unit displacement, and the sample deformation can be normalized by $1/$(number of slices minus 1). Fig. 2 shows the trabecular bone built from 20 disectors corresponding to the ROIs for the B12 vertebra. The software ImageJ [2] was used generate the 3D volume.

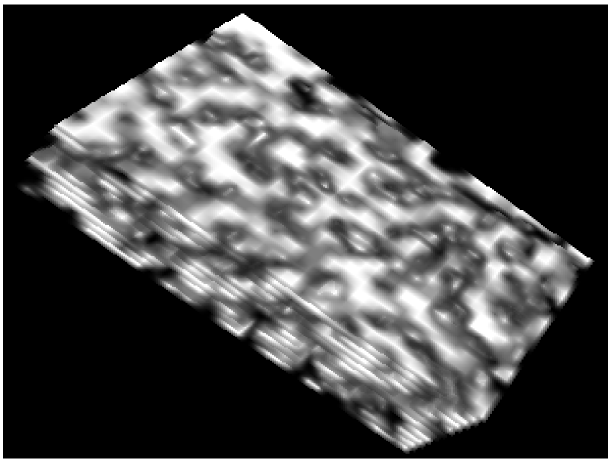

Figure 2: ROI of B12 vertebra. Trabecular bone structure built from 20 disectors by *ImageJ* software.

3 RESULTS

From the estimates of EPC, BV/TV and YM, the following behavior pattern was observed: first, the samples that presented YM values above the average of the 15 vertebra samples, also presented the mean BV/TV above 50% and mean EPC values predominantly negative. This was observed for A and C subjects. Secondly, the samples that presented YM below the average also presented BV/TV below 50% and EPC values predominantly positive, which happened for subject B (see Table 1). The calculations in this section have been done with the software *RGui* (R Development Core Team, 2010).

[2] http://rsbweb.nih.gov/ij/

	YM	BV/TV	EPC
A11	0.813947473	0.522901533	-2.727272727
A12	0.809078708	0.557940379	-7.681818182
A13	0.781908634	0.628191253	-34.31818182
A14	0.811403549	0.633681426	-39.59090909
A15	0.805774694	0.634098765	-44.90909091
A16	0.764700138	0.588976257	-21.22727273
B11	0.732320953	0.417927414	15.27272727
B12	0.760655099	0.449608262	10.31818182
B13	0.759175561	0.474706694	6.5
B14	0.76210217	0.481792717	4.090909091
B15	0.663019285	0.411990837	20.95454545
B16	0.690050758	0.394810745	20.5
C11	0.821903329	0.51742998	-1.363636364
C12	0.80968646	0.559768178	-9.318181818
C13	0.821349942	0.553768201	-7.772727273
mean	0.77380512	-	-

Table 1: The YM values, mean value of BV/TV and mean value of EPC for each sample.

To investigate the correlation between these parameters, first, a pairwise linear regression between YM, EPC and BV/TV was done, and the results are presented in Table 2. In this case, it can be seen that BV/TV and YM have a strong positive correlation, whereas the correlations between EPC and YM and between EPC and BV/TV are strongly negative.

	YM	BV/TV	CEP
YM	1		
BV/TV	0.7475	1	
EPC	-0.6421	-0.9699	1

Table 2: Correlation coefficients for YM, BV/TV and EPC.

To better understand the correlations between these three variables, a multiple regression analysis was performed among the three different parameters, according to: YM as dependent variable and mean BV/TV and mean EPC as independent variables; mean BV/TV as dependent variable and YM and mean EPC as independent variables; and mean EPC as dependent variable and mean BV/TV and YM as independent variables. The correlation coefficients found are shown in Table 3.

Relation	Correlation coefficients
YM in function of BV/TV and EPC	$r^2 = 0.67\ (p < 0.0012)$
BV/TV in function of YM and EPC	$r^2 = 0.96\ (p < 1.2 \times 10^{-9})$
EPC in function of BV/TV and YM	$r^2 = 0.96\ (p < 7 \times 10^{-9})$

Table 3: Results of the multiple variable regression for each one of the cases.

According to multiple variable regression results, the case where YM was considered as the depended variable, the correlation coefficient was the lowest, which was expected as, from

Table 2, we can see that pairwise correlation of the YM with BV/TV or EPC was not so strong. For the other two cases, the correlation determination coefficients were remarkably high (0.96).

Beyond this statistical analysis, it was also performed a principal components analysis. The three principal components (PC) have variances as shown in Table 4. PC1 has 86% of the system variance. It means that the three analyzed variables can be explained by only one principal component: PC1. In other words, YM, BV/TV and EPC are linearly dependent.

	PC1	PC2	PC3
Variance	2.581319	0.4000091	0.01867199
Proportion of Variance	0.8604396	0.1333364	0.006223996

Table 4: Results of the principal components analysis.

4 DISCUSSION AND CONCLUSIONS

The Bone Mineral Density (BMD) is considered the international gold standard for osteoporosis diagnosis. Nevertheless, the measurements of bone density through the use of double energy X-ray absorptiometry equipments have shown to be limited in differentiating between subjects with and without vertebral fractures. During the last couple of years, it has been observed that not just the density, but also the quality of the bone structure plays an important role in defining bone strength.

In this paper we have investigated the correlations between three parameters that seem to play an important role for the mechanical competence of the trabecular bone, namely the bone volume fraction BV/TV, the Euler-Poincaré Characteristic EPC and the Young's modulus of elasticity YM. The study was conducted based on a set of a 180 CT images obtained from 15 vertebral bodies of three distinct subjects.

The analysis has shown that all 9 vertebrae from subjects named A and C presented the same behavior, whereas the 6 vertebrae of subject B presented a different one. That is, the A and C vertebrae have a high YM, a high BV/TV and a negative EPC, while the B vertebrae have shown a low YM, a low BV/TV and a positive EPC, suggesting that the trabecular bone behavior in this case study may be grouped by subject and not necessarily by individual vertebra (see Table 1). In other words, the behavior observed in the set of vertebrae of a subject reflects a global behavior of the subject and not just a local behavior due to one vertebra.

In addition, the statistical analysis has shown that BV/TV and YM have a strong positive correlation, whereas the correlations between EPC and YM, and between EPC and BV/TV are strongly negative. This means that, an increase in the trabecular bone volume fraction BV/TV is accompanied by an increase in YM, while there is an EPC decreases (better connectivity). In contrast, whereas the BV/TV and YM values decrease, EPC values increase (worse connectivity). In other words, the greater the trabecular mass bone is, the greater is the stiffness of the structure (YM values are higher), which agrees with the results in (Gibson, 1985; Cortet and Marchandise, 2001), with better trabeculae connectivity (lower EPC values). On the other hand, for less trabecular area ratio, more malleable and less connected is the structure.

These results can be reinforced by the multiple regression analysis. By the results presented in Table 2, YM works better as independent variable, but all three properties are quite well correlated. According to Gibson (1985), BV/TV and YM are positively correlated, which agrees with our result that YM and EPC are negatively correlated. The relationships between YM and EPC and between BV/TV and EPC are consistent, because when the structure is well connected, its stiffness tends to grow. Through the principal component analysis, we have found a best fit line for the three parameters. This fact corroborates to the results above, where the $PC1$ variance represents 86% of the system total variance (see Table 4).

The correlation observed between the trabecular bone volume fraction and their connectivity plays an important role for the mechanical competence of the trabecular structure in the sense that these two parameters are closely related to its stiffness. Therefore, with the BV/TV and EPC values we can estimate the stiffness of the trabecular bone and, consequently, improve the overall analysis for predicting the fracture risk of this bone structure.

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