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Original Article

ON THE ROLE OF HISTOMORPHOMETRIC (STEREOLOGICAL) MICROSTRUCTURE PARAMETERS IN THE PREDICTION OF VERTEBRAE COMPRESSION STRENGTH

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ABSTRACT

The well-documented relation between bone mineral density (BMD) and bone compression strength constitutes the basis for osteoporosis diagnostics and the assessment of fracture risk. Simultaneously, this relation demonstrates a considerable scatter of results as bones of identical mineral density may have significantly different properties. The experimentally confirmed theorem that two materials or tissues of identical microstructure have identical properties leads to the evaluation of various quantitative stereological parameters (also referred to in biomedicine as histomorphology). These parameters, obtained from analysis of 2D or 3D images, have been used in numerous attempts to explain changes in bone strength. Although numerous correlation dependencies, often with high correlation coefficients, were evaluated, we do not know which parameters are worth evaluating, and there is no physical interpretation of these relations. An extended statistical analysis was accomplished on the basis of analysis of 3D images from 23 lumbar (L3) vertebrae scanned with micro-CT and the results of subsequent compression tests. A new parameter called SDF (structure destruction factor) was proposed in order to characterise the quality of 3D trabecular structures, and its significance was demonstrated. The final correlation function, which uses only three stereological parameters, made it possible to predict compression strength with considerable precision. The estimated values correlated very well with the apparent values (correlation coefficient r=0.96). Finally, the stereological parameters most suitable for characterisation of bone compression strength were chosen and a mechanism responsible for the changes in mechanical properties was proposed. The results obtained defined the necessary improvements in diagnostic techniques that would allow for more efficient quantitative microstructure evaluation and guidelines on how to improve treatment of patients with weakened bones.

Keywords: 3D image analysis, bone structure, compression strength, histomorphometry, osteoporosis, spine.

INTRODUCTION

Bone mineral density (BMD) is a good predictor of the mechanical properties of bones. However, numerous fractures are observed in patients with higher than average BMD for their age. Closer analysis showed that bones with the same BMD could significantly differ in geometry, structure, and properties (Kazakia et al, 2011). So, variation in the mechanical properties of bones seems to be a consequence of microstructural differentiation (Ulrich et al, 1999). Consequently, this opens a discussion on the choice of proper (stereological) parameters that quantify bone microstructure and the methodology of appropriate measurements. One of the most important difficulties in bone structure quantification is the lack of a so-called gold standard. In other words, we have no method that gives widely accepted and sufficiently precise results that can be used as a reference for other methods. Let us look at some works devoted to this problem.

In some works (Mullender et al 2005, Khoo et al 2005) various parameters were evaluated and compared within different groups of patients: for example, males and females, osteoporotic and healthy. Unfortunately, the mechanical properties of vertebrae remain unknown and therefore these results cannot be used to predict them. Consequently, they cannot be applied for objective in vivo judgement of the bone quality of a given patient.

Shahtaferi (2007) examined flat two-dimensional sections from biopsies of the iliac crest of young (mean age 30) and elderly (mean age 60) men with osteoporosis. The measurements were performed automatically. However, no precise information concerning the equipment and software used was given. The measurements showed that almost all the parameters (trabecular bone volume fraction, trabecular bone specific surface, mean trabecular thickness) as well as the number of end points and nodes per unit area were statistically more significant in young men's bones. Consequently, mean trabecular separation was significantly higher in elderly men's bones.

Unfortunately, the aforementioned paper gives no information about the relations between the various parameters. We have only the mean values and ranges for two groups of significantly different ages. In addition, there is no critical stereological analysis of the results. For example, according to the results presented, the mean trabecular length is ten times higher in young bones, which is practically impossible. The lack of precise information on the measurement methodology precludes deeper discussion and analysis of the results.

Shipilov et al (2013) reported an extensive study on 95 young volunteers (59 females and 36 males aged between 16 and 30 years) in which they tried to explain how impact loading influences bone microstructure, which is a key determinant of bone strength. The in vivo tests of the distal radius and distal tibia were performed using high-resolution peripheral quantitative computed tomography (QCT) with a resolution of 82 □m. Three groups of athletes who practised alpine skiing (high-impact loading), soccer (medium-impact loading) and swimming (low-impact loading) were analysed and compared with a control group that was not active in sport.

The results clearly demonstrated that sport activity, especially related to high-impact loading, influenced bone microstructure and compression strength when evaluated using FEA (the finite elements method). An increase in bone density, cortical thickness, and failure stress was observed. Moreover, these differences were observed mostly in the distal tibia, which is heavily loaded in sporting activity associated with high-impact loading. However, this work gives no answer on how to predict bone strength on the basis of the histomorphometric parameters of the bone microstructure.

Interesting results can be found in the work by Thomsen et al (2005), in which the problem of vertebral bone strength prediction on the basis of histomorphometric (i.e. stereological) analysis of vertebral bone was examined. The second and third vertebral bodies (L2 and L3) were used for histomorphometry and destructive compression tests, respectively. The test materials covered a wide age range (19–96 years), both women (21) and men (24). Statistically significant correlations were found between trabecular bone volume fraction (BV/TV), trabecular number (Tb.N) or trabecular separation (Tb.S), and the compression strength of vertebral bodies. The correlation coefficients for these relations were evaluated as 0.84, 0.86 and -0.86, respectively. Correlations with other histomorphometric parameters were also statistically significant, but with lower correlation coefficients. Similar correlation coefficients were reported for correlations with bone density, evaluated by means of quantitative computed tomography (QCT), peripheral QCT (pQCT), and dual-energy X-ray absorptiometry (DEXA).

A similar study was reported by Beuf et al (2001). They examined fourteen L3 lumbar vertebrae obtained from cadavers (aged 22–76 years). The results of high-resolution MRI structural measurements and bone mineral density (BMD) were compared with the results of mechanical tests. Good correlations were reported between age, BMD, morphological parameters, and the results of mechanical tests. The absolute correlation coefficients were in the range 0.79–0.91. In addition, the application of a stepwise multiple regression model allowed for even better correlations when BMD or volume fraction was coupled with the mean intercept length. However, the correlation coefficient never exceeded 0.92.

An improvement in this correlation with mechanical properties was reported by Ulrich et al (1999) after combining several structural parameters. They obtained very high regression coefficients (up to 0.96) after taking into account volume fraction, trabecular spacing, and mean intercept length. However, one should take into consideration that they analysed data sets covering only bone structures that were clearly osteoporotic.

Let us summarise this brief review. The conclusions of all the works discussed above are, in fact, in good agreement; they might be of a more qualitative or more quantitative character, but the general trend is always the same. A good correlation between BMD and mechanical properties constitutes the background for the use of densitometry as the main tool for osteoporosis diagnostics. Unfortunately, numerous cases have proved that using BMD as the only parameter for estimation of compression strength can either overestimate or underestimate bone strength

(Kazakia et al, 2011). Therefore, research should be oriented towards looking for structure–property relationships in bones.

It seems to be evident that the volume fraction of the trabecular bone was always taken into account in such analyses because it should correlate well with BMD. The correlation coefficient for this relation was evaluated as 0.91 (Beuf et al, 2001). The other parameters most frequently used were mean intercept length and trabecular spacing. Even if these parameters gave the best correlations, it did not prove any real dependence of the properties upon microstructure.

The lack of deeper analysis of the correlations observed was the main disadvantage of all the works analysed above. We can, for example, select a group of people in such a way that their height monotonically decreases with age. If we next correlate height with BMD, we will probably get a relation indicating that taller people have better bone mineral density, which is obviously a false conclusion.

This necessity to combine microstructure and properties is a key problem in material engineering. It is obvious that the absolutely basic theorem of material engineering can be expressed as follows: two materials with identical microstructures have identical properties. This rule is also valid for biological structures and bones. The main problem lies in the fact that biological structures are much more varied than, for example, the microstructures of metal alloys.

Building an appropriate model of bone microstructure evolution will allow much more than just a reduction of the quantitative microstructural parameters necessary for precise prediction of mechanical bone properties. The main goal is to understand the mechanism of bone strengthening/weakening, especially in the case of comparable BMD. Precise information on how changes in the trabecular structure of a bone affect its mechanical properties would be a first step in the development of more efficient treatment of patients with high risk of fracture.

MATERIAL AND METHODS

The whole experiment was devoted to analysis of the effect of microstructure on the compression strength of bone. Lumbar vertebral bodies, most often L2 and L3, are commonly used for such experiments: for example, Beuf et al (2001), Bevill et al (2009), Thomsen et al (2002), Ulrich et al (1999) and others. In this work, L3 lumber vertebral bodies were used for analysis of both microstructure and mechanical properties.

Vertebral bodies for tests were obtained from 23 cadavers, both women and men, aged 25–89. The age distribution, illustrated in Fig. 1, was roughly uniform; this enables analysis of the effect of age on bone microstructure. Due to the limited number of specimens, the sex of patients was excluded from analysis. Patients who had died due to multi-organ injuries were excluded since there might have been hidden vertebrae damage.

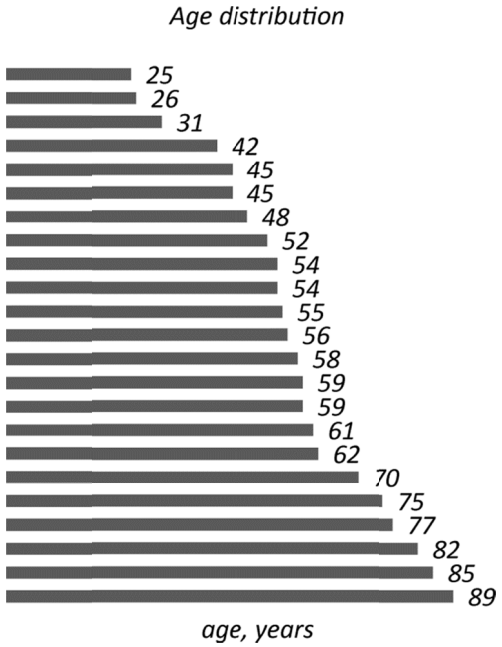

Fig. 1. Age distribution of patients.

Specimens were immersed in 96% ethanol and stored in plastic containers. In the first stage of research, the specimens were scanned by μCT, and then 7 parameters were evaluated by image analysis methods: volume fraction of bone (BV/TV), trabecular bone thickness (Tb.Th), trabecular bone separation (Tb.Sp), trabecular bone pattern factor (Tb.Pf), mean number of branches per unit volume, mean number of junctions per unit volume, and average branch length. The symbols in parentheses are commonly used in medical sciences (see Table 1). Most of the parameters listed above are intuitively understandable; however, the meaning of the trabecular bone pattern factor should be explained. This parameter represents the proportion between the changes in perimeter and the area of the trabecular bone visible in flat section before and after dilation (Hahn et al, 1992). Changes in this parameter, however, gave no significant effect in this study and therefore it will not be analysed in more

detail. The Aphelion 4 package (ADCIS, France) and the ImageJ environment were used for image quantification.

The necessary μCT data were collected from an X-tek Benchtop CT160Xi microtomograph (Nikon Metrology, Tring, UK), and the final images were obtained with a voxel size of 34.6μm x 34.6μm x 34.6μm. The next step of the experimental procedure was mechanical compression. Compression tests were completed using an MTS Mini Bionix 858.02 tensile/compression machine at the Laboratory of Biomechanics, Faculty of Mechanical Engineering, Technical University of Prague (Czech Republic). Compression strength, Rc, was calculated as the ratio between maximum compression force and the minimum cross section area of the vertebrae, calculated from μCT data.

Table 1. Selected parameters characterizing bone microstructure

parameter characterizing bone microstructure (in parentheses: additional stereological description)	unit	symbols used in medical science	symbols used in stereology
trabecular bone volume (fraction)	no unit or [%]	TBV, BV/TV	V_V
trabecular bone (specific) surface	[mm^2/mm^3]	BS	S_V
node number (per unit area or volume)	[$1/mm^2$], [$1/mm^3$]	NND	N_A or N_V
terminus number (per unit area or volume)	[$1/mm^2$], [$1/mm^3$]	NTM	N_A or N_V
trabecular number (per unit area or volume)	[$1/mm^2$], [$1/mm^3$]	TB NO, Tb.N	NA or NV
mean intercept length	[mm]	MIL	$\bar{l}$
(mean) trabecular length	[mm]	TB LE	variable
(mean) trabecular thickness	[mm]	Tb TH, Tb.Th	variable
(mean) trabecular separation	[mm]	TB SP, Tb.Sp	variable

RESULTS

The results of the experiments are shown in Table 2.

DISCUSSION

It is a well-known and documented observation that compression strength decreases after the age of twenty. This is shown in Fig. 2. Linear regression gave the following formula:

$$R_c = 0.1886 \cdot (age) + 21.23 \qquad (1)$$

Equation (1) is illustrated in Fig. 2 by the dashed line segment. This is statistically significant correlation as the correlation coefficient was r=0.80; however, the errors of estimation are considerably high, and their values shown in Table 3.

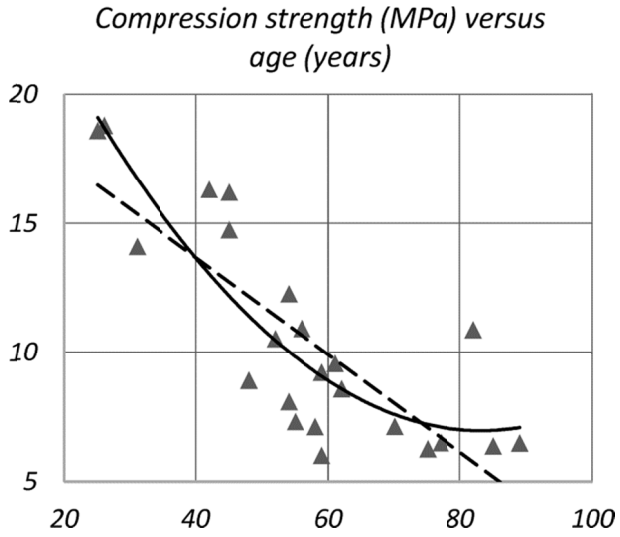

Fig. 2. Correlation between age of patients and compression strength of spine. Details in text.

Table 2. Results of experiments

L.p.	Rc,	Age	BV/TV	Tb.Th	Tb.Sp	Tb.Pf	Bran-ches	Junc-tions	Average branch length
unit	MPa	year	-	mm	mm	-	$1/mm^3$	$1/mm^3$	mm
1	8.08	54	0.1166	0.2029	1.0785	0.3439	5.8627	3.4724	0.4544
2	18.81	26	0.2341	0.2094	0.7913	0.1125	10.2597	5.9767	0.3833
3	7.31	55	0.1603	0.1762	0.8508	0.2806	11.1857	6.5363	0.3717
4	18.61	25	0.2495	0.2247	0.8415	0.1131	9.4670	5.5925	0.3736
5	12.27	54	0.1704	0.1956	0.7951	0.3046	9.3460	5.5847	0.3845
6	16.21	45	0.1959	0.2066	0.8528	0.1756	7.8127	4.5305	0.3985
7	6.50	77	0.0727	0.1875	1.2737	0.5187	3.1667	1.8487	0.4344
8	14.76	45	0.2181	0.2153	0.6879	0.2822	8.0648	4.7760	0.3523
9	10.87	82	0.1156	0.2382	1.1200	0.3909	2.4981	1.4564	0.4439
10	8.91	48	0.1459	0.2022	0.9893	0.2620	6.0010	3.5627	0.4378
11	10.52	52	0.1392	0.1874	0.8702	0.3689	8.3790	4.8488	0.3877
12	7.13	70	0.0956	0.2123	1.3613	0.3446	3.2275	1.9075	0.4935
13	14.10	31	0.2139	0.2161	0.8665	0.1653	9.8098	5.8136	0.3843
14	6.01	59	0.1061	0.2087	1.0807	0.4010	5.2762	3.1694	0.4590
15	6.36	85	0.1022	0.2054	1.3677	0.3038	4.0528	2.4041	0.4520
16	7.11	58	0.1065	0.2002	1.0568	0.4026	7.8878	4.5475	0.4359
17	6.26	75	0.0837	0.1798	1.1577	0.5220	6.7055	3.8857	0.4117
18	10.92	56	0.1546	0.2543	1.0252	0.2788	4.7831	2.8448	0.4509
19	16.32	42	0.1957	0.1982	0.8219	0.1895	9.6873	5.6839	0.3953
20	6.48	89	0.0938	0.1843	1.1621	0.4056	5.8117	3.4954	0.4466
21	9.55	61	0.1410	0.2132	1.0205	0.3022	9.2607	5.4136	0.4130
22	9.21	59	0.1407	0.1949	1.0492	0.2516	6.8481	4.0794	0.4340
23	8.57	62	0.1220	0.1924	1.0340	0.3293	6.0651	3.6250	0.4429

Linear regression is not a good choice in this case as practically all the parameters (including sport achievements) tend to (approximately) monotonically decrease with age but almost never exceed a constant final value. Consequently, approximation using the 2nd degree multinomial would be better but still simple:

$$R_c = 0.0036 \cdot (age)^2 - 0.5875 \cdot (age) + 31.809 \quad (2)$$

Equation (2), illustrated in Fig. 2 by the solid line, is also statistically significant and gives better approximation than equation (1), as is demonstrated by the results of error analysis shown in Table 3.

Application of a nonlinear function slightly improved the correlation, but the errors were still very high. So, equations (1) and (2) are insufficient for application as predictors of compression strength. One should remember that there is no physical relation between time (more precisely, the age of the patient) and the compression strength of the spine. Changes observed in compression strength are induced by changes in the microstructure of vertebrae. This microstructure, in turn, evolves with time, but the rate and range of these changes are significantly different in various patients.

Table 3. Parameters characterising accuracy of compression strength estimation using various parameters. Some of the presented data is explained later in the paper

Approximating function	Equation number	Correlation coefficient	Maximum relative error %	Mean relative error %	Median relative error %
Age, linear	(1)	0.800	68.10	21.25	13.20
Age, 2nd degree multinomial	(2)	0.862	51.22	16.60	15.76
Volume fraction, linear	(3)	0.931	57.34	11.21	8.78
Volume fraction and density of junctions, linear	(6)	0.944	32.4	11.5	9.09
Volume fraction, density of junctions and SDF, linear	(9)	0.959	40.15	9.18	6.71

Consequently, we can evaluate equations describing the correlation between age and properties, but these equations have no physical meaning. The real factors responsible for compression strength are related to microstructure and are discussed below.

Intuitively, having more (in the sense of volume or weight) of any material should allow a stronger construction. This assumption has been both theoretically and experimentally confirmed in material engineering. Fibre-reinforced composites whose strength is proportional to the contents of strong fibres are a good example. Similarly, the calcium content, which is the main component of the trabecular bone, should be a significantly better predictor of compression strength than age. Consequently, many densitometric measurements that estimate the contents of calcium in bones (BMD) constitute the basis for osteoporosis diagnostics because the standard BMD values change with the age of the patient. Unfortunately, in some cases fractures were observed in bones with above average calcium content and sometimes no fractures were found in bones with a clear calcium deficit. This observation proved that more precise characterisation of bone microstructure was necessary.

The volume fraction (V_V or BV/TV – see Table 1) of the trabecular bone can be treated as an equivalent of BMD (bone mineral density), and compression strength should be a linear function of V_V. From experimental data we can calculate the following correlation function:

$$R_c = 75.645 \cdot V_V - 0.6245 \quad (3)$$

This relation is shown as the solid line in Fig. 3. As expected, the estimation errors were smaller than in the case of regression with age (see Table 3). However, the scatter of results is still considerably high and therefore a more thorough analysis was performed.

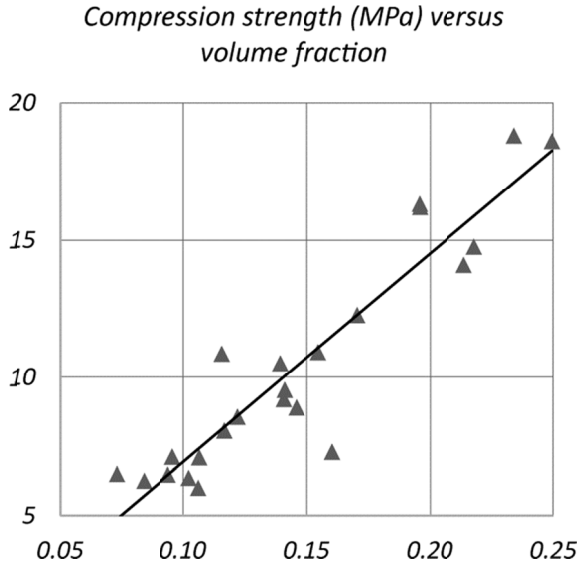

Fig. 3. Correlation of volume fraction of the trabecular bone and compression strength. Triangles denote experimental results; the solid line illustrates the linear correlation function – equation (3).

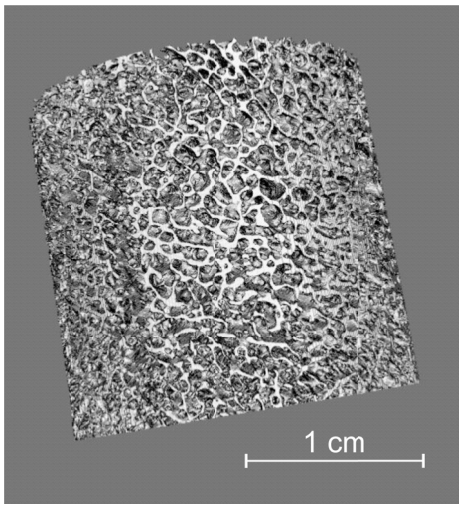

Fig. 4. An example of trabecular bone structure reconstructed on the basis of microtomography.

The trabecular bone has a clearly visible 3-dimensional structure (Fig. 4) that is somewhat similar to some engineering structures (Fig.5). We know from mechanical engineering and countless case histories that application of a space truss leads to relatively light but stiff and strong constructions. However, any breaks or fractures of individual bars lead to collapse of the whole structure. So, it seems logical to analyse the structure of the trabecular bone in more detail.

Fig. 5. Two examples of engineering structures which are built of beams and are therefore similar to the trabecular bone.

The trabecular bone can be simplified to a collection of nodes or junctions and bars or branches connecting these junctions. If we want to build a 3D structure, we need to join 4 branches at a single node (junction). Simultaneously, every branch connects 2 junctions. Consequently, $N_V(branches)$ can be easily calculated as:

$$N_V(branches) = 2\, N_V(junctions) \quad (4)$$

One can check this relation using data from Table 2. As a result, we obtain the following regression function:

$$N_V(branches\ calculated) = 1.1665\ N_V(branches\ measured) + 0.0772 \quad (5)$$

This relation (see Fig.6) gives an almost perfect linear alignment of data points and an extremely high correlation coefficient of r=0.999. However, the calculated number of branches is overestimated by approximately 17% in comparison with the measured values. This problem is discussed later in the paper. Here we analyse the join effect of the volume fraction of the trabecular bone and the density of its junctions. Statistical analysis leads to the following formula (results shown in Fig. 7):

$$R_c = 83.79179 \cdot V_V - 0.66172 \cdot N_V(junctions) + 0.571676 \quad (6)$$

The correlation coefficient and the relative errors for this function are shown in Table 3. A very similar correlation can be evaluated for the volume fraction and density of branches instead of junctions. Nevertheless, this relation gives slightly higher errors, which might suggest that estimation of the number of junctions during image analysis is more accurate than estimation of the number of branches.

It is interesting that the coefficient evaluating the effect of the density of nodes has a negative value in equation (6) and equation (9). This can be explained as follows: There is possibly an optimum bone structure. If there are too many thin branches (increasing N_v of nodes), the whole structure is less stiff and, consequently, less resistant. On the other hand, excessively thick branches (low N_V of nodes) would probably lead to a very stiff and consequently brittle bone. Advanced simulations using FEM (finite elements method) could possibly confirm this hypothesis.

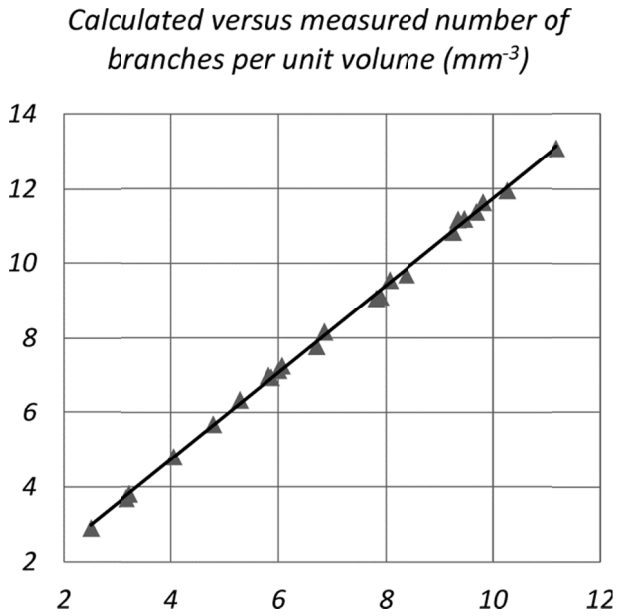

Fig. 6. Correlation between the measured and calculated density of branches in the trabecular bone.

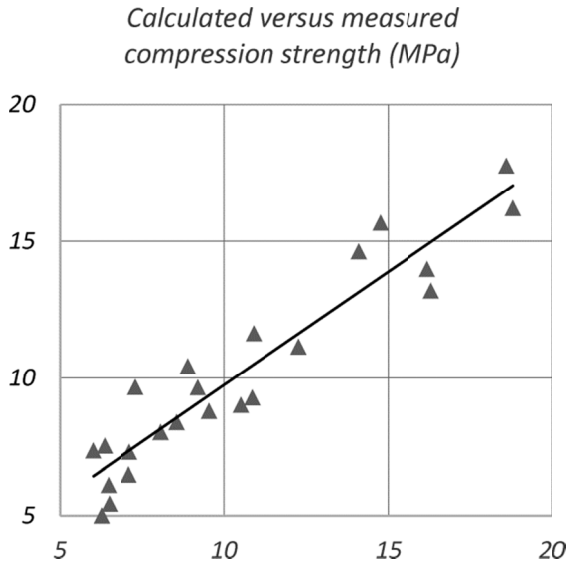

Fig. 7. Measured compression strength versus compression strength calculated from the volume fraction and density of nodes in trabeculae in vertebral bodies.

Now, let us analyse in more detail the overestimation of the number of branches per unit volume, as described by equation (5) and illustrated in Fig. 6. In the case of an ideal 3-dimensional structure, the number of branches per unit volume fulfils equation (4). On the other hand, when we analyse a network which can be unfolded and placed on a flat surface (such a network is generated, for example, by Voronoi tessellation), we have 3 branches at each junction and:

$$N_V(branches) = 1.5\, N_V(junctions) \qquad (7)$$

The real structure of vertebrae lies somewhere between these two extremes, as described by equations (4) and (7), respectively. In order to characterise the quality of a 3-dimensional network, we introduce the structure degradation factor (SDF), defined as:

$$SDF = 4 - 2\frac{N_V(branches)}{N_V(junctions)} \qquad (8)$$

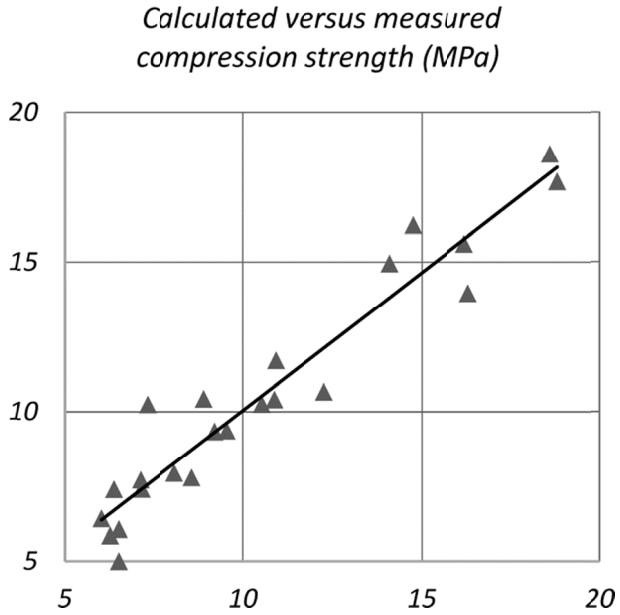

Fig. 8. Measured compression strength versus compression strength calculated from volume fraction, number of nodes, and structure degradation index in vertebrae.

It is clear from equations (4) and (7) that SDF=0 for an ideal network with 4 branches at every node, and SDF=1 for a 2-dimensional (and possibly folded) network with 3 branches at every node. Interpretation of SDF is very simple: its value is equal to the relative number of nodes with 3 branches in all nodes.

Due to the fact that the experimental data are in very good agreement with relation (5), we get similar SDF values for various vertebrae. In spite of the small variation in this parameter, it can significantly affect the final results. Statistical analysis of the data leads to the following formula:

$$R_c = 91.98519 \cdot V_V - 0.81438 \cdot N_V(junctions) - 17.0406 \cdot SDF + 10.66137 \quad (9)$$

Equation (9) also suggests that degradation of the trabecular network decreases the compression strength. This result is in good agreement with the intuitive interpretation of the effect of microstructure on mechanical properties. A comparison of the results against measured values is shown in Fig. 8. Values of the correlation coefficient and relative errors for this function are shown in Table 3. The results presented in Table 3 show that adding SDF to the list of parameters improves the results of the estimation.

Fig. 9 shows a comparison of all the results. In 74% of cases, the application of volume fraction, number of nodes, and the degradation coefficient return the best or second-best result and the result is worst in only 2 cases (9%). By contrast, application of age gave the worst result in 11 cases (48%).

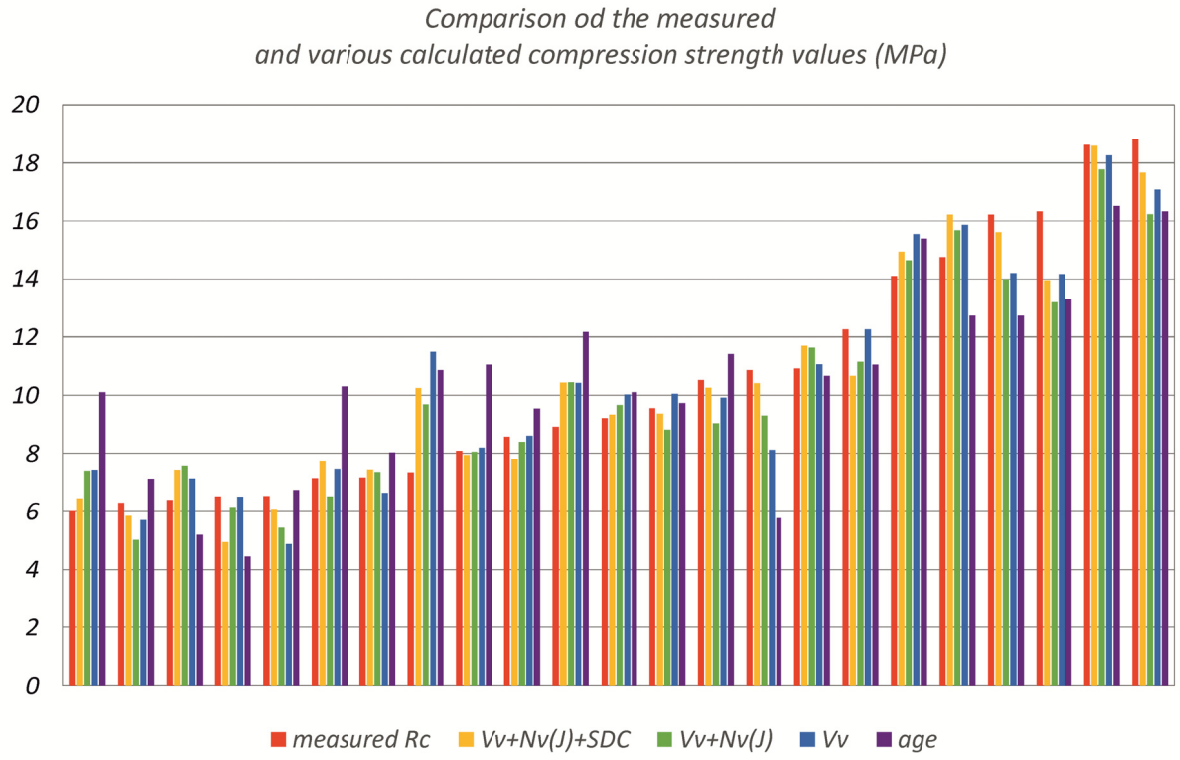

Fig. 9. Comparison of the measured R_c values (denoted as Rc), with values estimated on the basis of volume fraction, density of nodes and structure degradation factor (Vv+Nv(J)+SDC), volume fraction and density of nodes (Vv+Nv(J)), volume fraction only (Vv) and age (age).

Equation (9) can be recalculated for normalised values of the parameters. This means that the minimum value of any parameter is expressed as 0, and the maximum is expressed as 1. The formula for normalised parameters is as follows (additional index n denotes normalised value):

$$R_c = 16.26298 \cdot V_{Vn} - 4.13698 \cdot N_{Vn}(junctions) - 2.44923 \cdot SDFn + 7.115283 \quad (10)$$

On the basis of the coefficients in equation (10), we can say the following: the calcium content in bone, described by volume fraction, is responsible for approximately 70% of bone strength; the density of the trabeculae is responsible for approximately 20% of compression strength; the quality of the spatial network is responsible for approximately 10% of the compression strength of vertebrae.

The effect of age on the values of the parameters discussed above is illustrated in Fig. 10. Both volume fraction and number of junctions (nodes) decrease with age. However, no such clear tendency is observed in

the case of the degradation factor (or coefficient). This may suggest that other factors (for example, physical activity) may affect the structure of vertebrae.

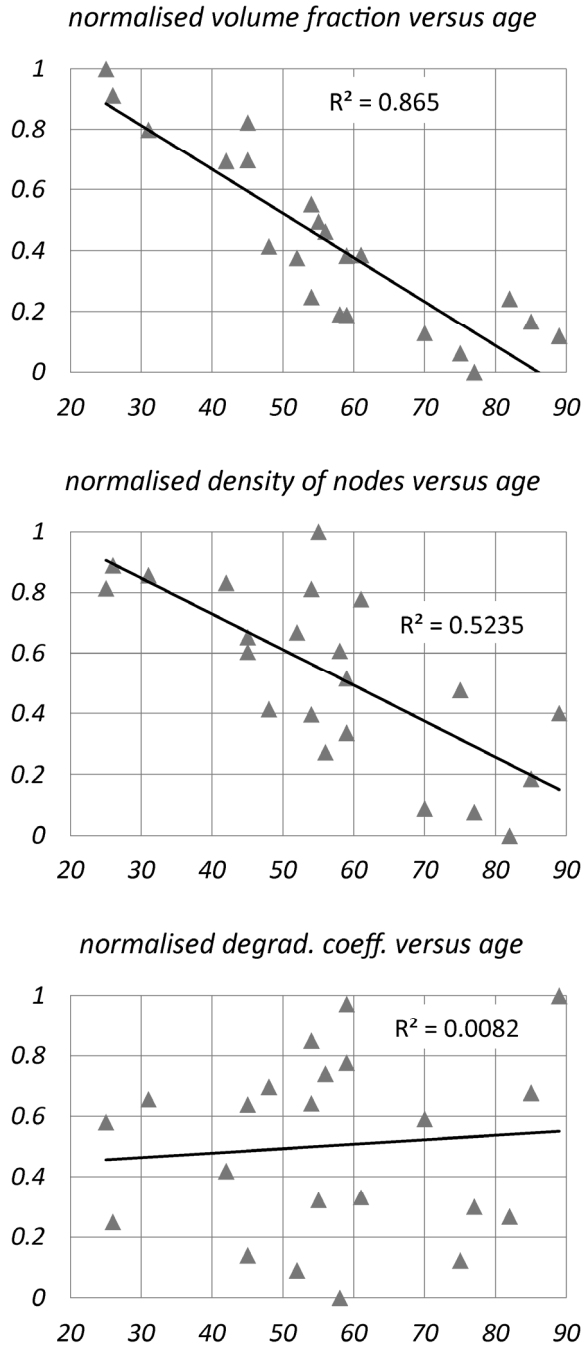

Fig. 10. Effect of age on various structural parameters. Their values were normalised for better comparison of changes (min=0, max=1).

Only some parameters measured and presented in Table 2 were used for analysis. This was a result of PCA (Principal Component Analysis), which proved that the rest of the parameters can be omitted as they have no significant effect on the estimation of compression strength. This part of the analysis is not documented in this paper. However, this is an important observation as it demonstrates the role of careful preparation of the experiment. Usually, collecting as many quantitative parameters as possible does not lead to good results; it is more effective to select the limited list of parameters which describe a model of the process. Such a practice should be recommended in all quantitative analyses using stereological and image analysis methods.

The results presented in this paper allowed evaluation of several correlation equations that provide an insight into the role of microstructure in defining the compression strength of vertebrae. Unfortunately, all these equations can only be interpreted qualitatively. In other words, they have no value as predictive tools, especially for in vivo patients. The reasons for this are at least twofold.

First, microtomographs could not be used for the in vivo experiments due to the size of our specimens. The resolution of tomographs used for diagnostic purposes is too low to obtain sufficiently precise microstructural information. All the vertebrae analysed in this paper were also examined with the use of classical medical tomography, but the microstructure recorded was not suitable for any successful analysis. Therefore, these results are not included in this paper.

Second, even the resolution of microtomography is insufficient for quantitative analysis that is sufficiently precise to build good predictive models. The voxel size in the images used in this experiment was 34.6 μm. Consequently, the diameter of a single branch was usually 6 voxels. All the images were recorded with identical parameters, and the same image processing, including binarization, was applied. The slightly different apparatus or parameters used in microtomography and subtle variations in image processing may lead to a seemingly small change in branch diameter, for example, only 2 voxels (only a single voxel symmetrically on each side of the object). Consequently, the average branch diameter will be equal to 8 voxels and the measured volume fraction will more than double. Such results would be completely incomparable. To make things worse, we have no “gold standard” for verification of the results of detection. In other words, we have no alternative method which gives results that are commonly accepted as correct and exact and can be applied in checking the correctness of image processing.

CONCLUSIONS

The compression strength of vertebral bodies is an effect of their microstructure. Some changes in this microstructure are typical in patients of different ages, therefore a rough correlation between age and compression strength is observed. The significantly better predictive potential of microstructural data indicates that age is not a good predictor of the mechanical properties of the spine.

Compression strength of vertebral bodies is predominantly affected by the volume fraction of the vertebrae. However, an important role is played by the spatial structure of branches; there is possibly an optimal microstructure of this structure.

Only a very limited number of microstructural parameters is worth analysing. In this study it was demonstrated that the application of only three of them is sufficient: volume fraction, density of nodes and degradation factor. The list of measured parameters should be carefully selected prior to experiments as this can save time and money during research and further simplify analysis.

Quantitative microstructure analysis gives important indicators which are decisive for the mechanical properties of bone. Unfortunately, the limited resolution and specimen size of the currently available microtomography devices does not allow very precise quantitative prediction of the properties and the risk of fracture of bones.

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