Image Anal Stereol 2001;20:35-39
Original Research Paper

EFFECT OF HISTOMORPHOMETRIC PARAMETERS ON COMPRESSION STRENGTH OF VERTEBRAL BODIES

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(Accepted March 7, 2001)

ABSTRACT

Computer aided image analysis was applied to elaborate an automatic method of histomorphometric analysis of trabecular bone samples. Transverse sections of decalcified vertebral bodies were examined using optical microscopy and digital image acquisition system. Further analysis was done by means of a general purpose image analysis package. The same algorithm was applied to all the images tested, thus enabling obtainment of objective and repeatable results. High efficiency in measurements and evaluation of parameters not accessible for manual methods makes this method an interesting alternative for classical histomorphometric analysis. The results obtained demonstrated that assessment of bone mineral density is not sufficient for evaluation of compression strength of vertebral bodies. In contrast, mechanical properties correlate well with histomorphometric parameters. As a consequence it was postulated that compression strength of vertebral bodies is controlled by trabecular structure rather than bone mineral density.

Keywords: bone mineral density (BMD), compression strength, histomorphometry, image analysis, vertebral bodies.

INTRODUCTION

Continuous growth of life expectancy, as well as other civilization factors, lead to the increasing number of bone fractures, connected with poor mechanical properties of the bone rather than overloading conditions. The number of such cases reported is well over millions per year (Lyritis, 1991). In view of that, appropriate diagnostic and treatment methods are required and searched for. One of the best known, however not the unique one, example of such deteriorating changes in bone properties is osteoporosis, detected primarily in elderly women. Evaluation of the bone mineral density (BMD) is used as the main tool in diagnosis of osteoporosis, as the loss in BMD is postulated to be an excellent indicator of the internal changes in bones (Ebbesen *et al*., 1998; Kanis *et al*., 2000). Consequently, it is assumed that the bone strength is approximately proportional to BMD. Other methods, like ultrasonography or magnetic resonance are also applied to test the bone structure but they are either extremely expensive and not suitable for routine examination or their sensitivity is not sufficient for reliable results (McBroom *et al*., 1985; Riggs and Melton, 1995).

To summarize, BMD seems to be the best currently available tool for estimation of the bone strength or its susceptibility to fracture in vivo. However, some independent reports reveal results which are in clear disagreement with the above statement. Both, unexpected fractures in bones with high BMD and lack of them in heavily loaded bones with very low BMD, are observed (Jensen *et al*., 1990; Myers and Wilson, 1997; Prafitt, 1992). Simultaneously, materials engineering has undoubtedly demonstrated that all the material properties (not only the mechanical ones) are a function of material microstructure understood as spatial properties and arrangement of constituent phases, discontinuities, etc. Consequently, the aim of this work is to investigate whether the bone microstructure, characterized by means of histomorphometric parameters of trabecular bone, can better describe its strength than a single BMD parameter.

MATERIAL AND METHOD

Vertebral bodies taken from deceased patients were chosen for experiments. This choice was caused by the following factors:

- vertebral bodies undergo mainly compression forces and therefore we can relatively easily model these loading conditions when testing mechanical strength,
- short length of individual bones assures the lack of significant changes in the trabecular bone along the loading axis, thus enabling more precise analysis of the structure-property relationship,
- unidirectional loading simplifies testing of the bone structure as only transverse sections should be decisive for the final properties.

As a material for further experiments, 18 pairs of lumbar vertebral bodies L1 and L2 were taken from deceased patients. The available documentation excluded bone tumours or similar diseases which could have an extra effect on the bone strength. Vertebral bodies were taken from 7 women and 11 men of age from 17 to 90 years (average of 55.4 years). Just L1 and L2 vertebral bodies were chosen as the fractures are observed predominantly in this part of the spine.

BMD was tested on L2 vertebral bodies, using quantitative computer tomography (QCT) The Somaton DRH apparatus with the Osteo-CT software was applied. Next, all the L2 vertebral bodies underwent compression tests on the Tiratest tensile-compression machine equipped with special grips for bone testing.

Histomorphometric analysis was performed on L1 vertebral bodies, prepared as decribed below.

MICROSCOPE SLICES PREPARATION

Whole vertebral bodies taken at autopsy were preserved and stored in a 4% formaldehyde buffer solution. The body was cut in three basic planes, which allowed us to obtain fragments comprising 1/8 of the total vertebral body volume. For decalcification the fragments were placed in a 7.5% nitric acid (HNO_3) solution. Because of the size of the fragments a long-term (10 – 14 days) decalcification period was needed. During this time the solution was exchanged 4-5 times. The material was then rinsed under running water for about 2 hours, and then in ethyl alcohol for 1-2 days. Intermediate solutions such as 75% alcohol, 96% alcohol, absolute alcohol and a mixture of diethyl ether/absolute alcohol (in a ratio of 1:1) were used (one day in each solution). The fragments were then immersed in increasing celluloid solutions (Fluka 81680) at concentrations of 2%, 4%, 8%, 10% in a mixture of ether/alcohol for about 1 week in each.

After evaporation of the solvent and the formation of blocks the material was sectioned using a sliding microtome in the vertical plane of the body at a thickness of approx. 20 μm. The fragments were stained in a 1% eosin solution. After exposure of the fragments in xylene they were then mounted and secured by Canadian balsam. The fragments obtained were about ¼ of the area of the vertebral body. Because the fragments were derived from differing vertebral body heights, the average number of trabeculae was characteristic for the whole thickness of the bone. In each specimen 13-16 fields comprising the whole area of the analyzed vertebral body were evaluated.

IMAGE ACQUISITION AND PROCESSING

The samples prepared were observed on the NIKON ECLIPSE E400 research microscope with the objective of magnification x1 and the images were digitized into the PC computer memory using the CCD color camera Panasonic GP-KR222E, coupled to the MATROX METEOR frame grabber. All the images, of size 768×576 pixels, were stored in 24-bit RGB mode. The image resolution was 8,5 μm per pixel which corresponds to 32 mm^2 per image. Depending on the slice quality, between 11 and 16 images were recorded from each L1 vertebral body. The subsequent processing was performed using a PC computer and the APHELION v.2.4 software for image analysis.

Application of automatic image analysis methods are not very widely used in medicine as manual ones are usually preferred. Consequently, appropriate examples are not available. Fortunately, image analysis methods are very flexible and some experience based on materials engineering can be easily adopted (Wojnar, 1998). All the images underwent the following image processing steps:

- the initial color image was split into the RGB components and the B component was rejected, mainly due to the high noise level
- the remaining R and G components (compare Fig. 1) were taken for further analysis. Trabecular structure is best visible in the R component (white regions) but the same shade have many artifacts and fat particles. Fortunately, the unwanted features are clearly visible as the darkest elements in the negative of the G component
- multiplying the R component and negative of the G component one obtains a new image, suitable for further processing. First step of this processing was median filtering which removes lot of the noise without significant loss of details (Fig. 2)
- due to the changes in optical density, the background of Fig. 2 is clearly uneven (much darker at the left side). In order to remove this effect, appropriate shade correction procedure was applied
- the corrected image was binarized using the hysteresis threshold technique that enables detection of gray objects under condition that they contain bright details (Fig. 3)
- the final processing consisted of four steps: median filtering smoothing the image, closing which removes small discontinuities, hole filling and small opening that removes the remaining defects. Fig. 4 contains the final result of image processing
- additional processing, including skeletonization and partial removal of branches leads to a generalized image, suitable for counting the number of branches, knot points, evaluation of mean boundary length, etc. The resulting smoothed skeleton, overlying the binary image, is shown in Fig. 5.

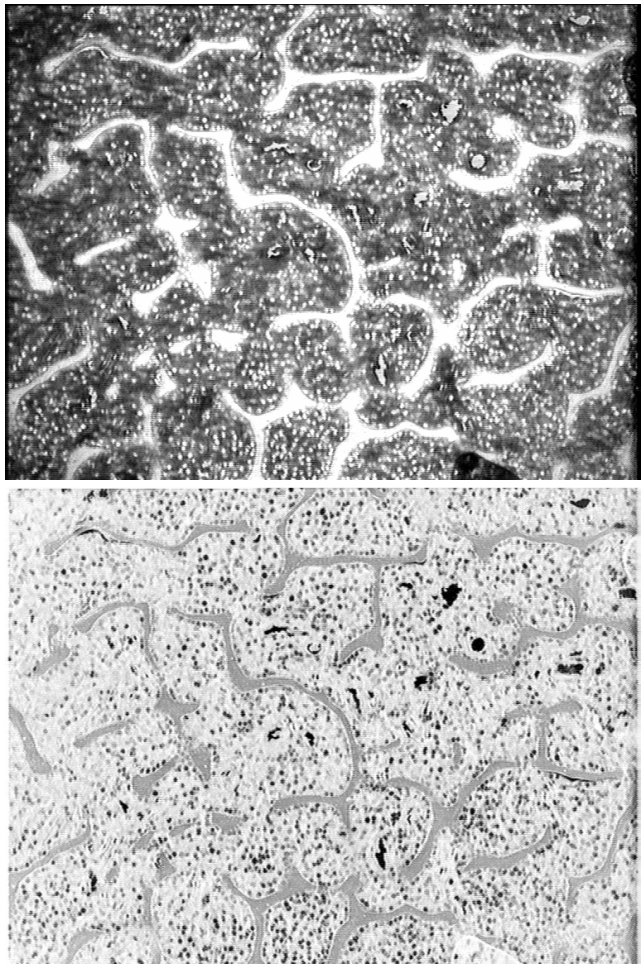

Fig. 1. *R (up) and G (down) components of the initial color image, used for subsequent analysis. Note that the G component is displayed as inversion (negative).*

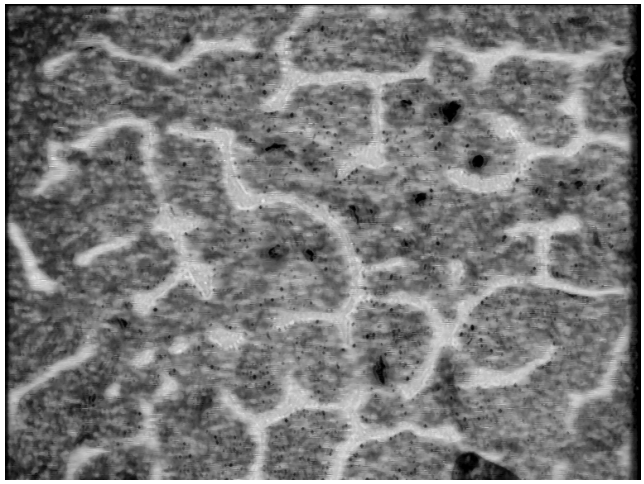

Fig. 2. *Median filtering of the product of R and G (negative) components.*

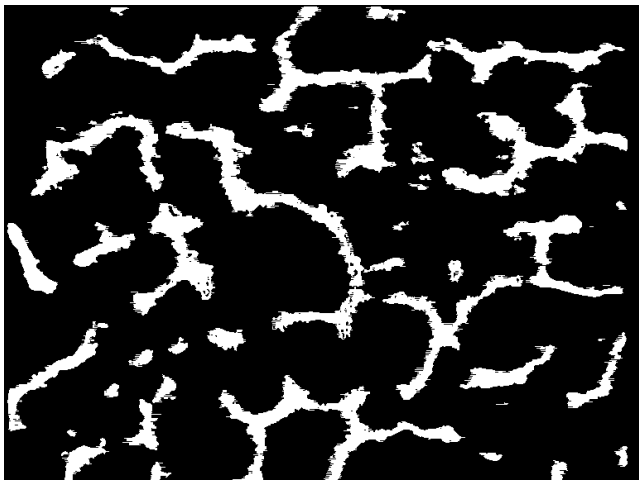

Fig. 3. *Initial binary image.*

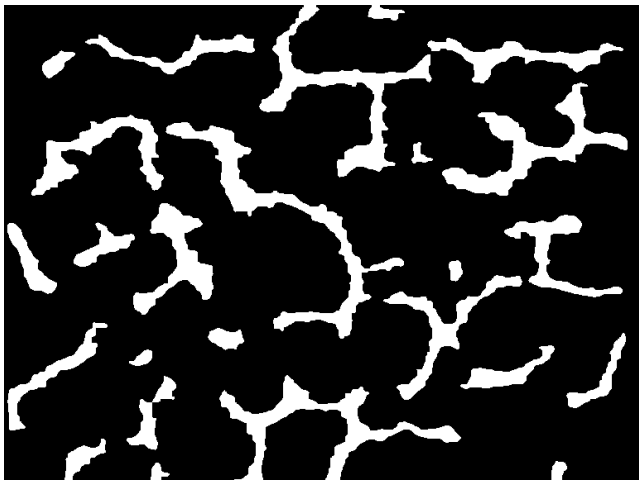

Fig. 4. *The final result of image processing.*

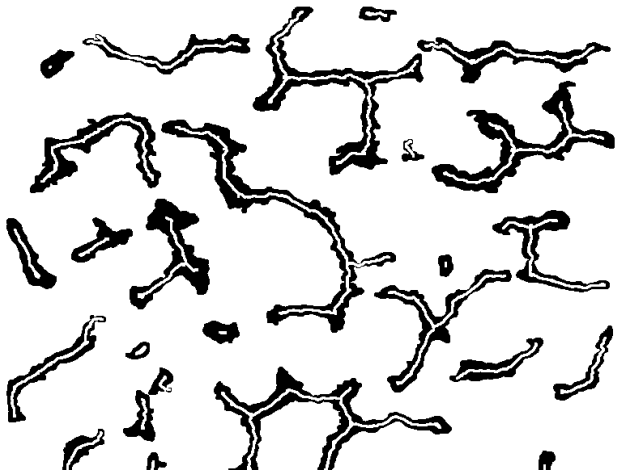

Fig. 5. *The resulting smoothed skeleton, overlying the binary image.*

RESULTS

If we plot the bone compression strength (R_C) data versus BMD (Fig. 6) we clearly see that there is only very loose relation between these two components, as the square of correlation coefficient reaches only the value of 0.23. This supports strongly the thesis that knowing only BMD is not sufficient for estimation of R_C.

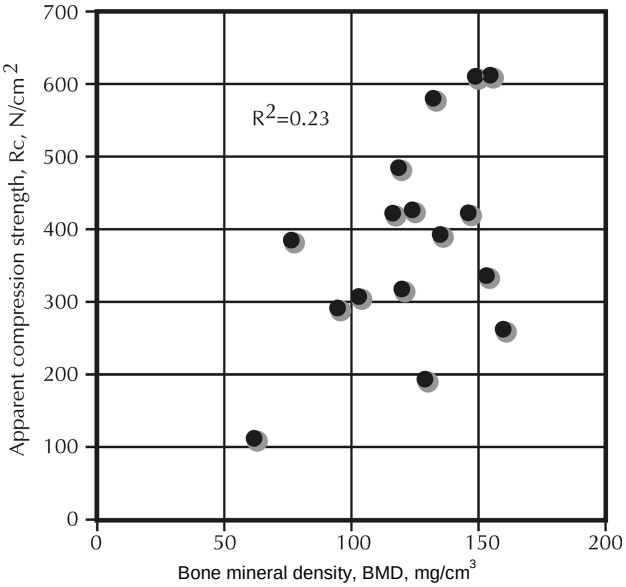

Fig. 6. *Compression strength versus BMD.*

So, we will try to look for possible correlation between the histomorphometric parameters and R_C. The following parameters turned out to be most valuable (Kurzydlowski and Ralph, 1995; Wojnar, 1998):

- area fraction of trabecular bone (proportion between the surface area of detected trabeculae and the surface area of the image), A_A,
- mean free path between trabeculae, λ [mm],
- mean length of branches, l [mm] and
- mean number of knots (triple points) in as single trabecula, n.

Naturally, this was expected relatively high correlation between area fraction of trabeculae and BMD. Surprisingly, this relation was very week, with correlation coefficient, $r = 0.13$. Significant correlations, better than between *Rc* and BMD, were found between *Rc* and A_A, λ or l. The best results, however, were observed when more complex relations were taken into account, as shown in Table 1.

Table 1. *The best structure – strength relations.*

N°	Relation	correlation coeff.
1	$ln(Rc) = 0.813 + 7.2548*l + 12.495*A_A$	0.91
2	$ln(Rc) = 3.8232 + 6.3248*l + 0.6747*\lambda$	0.82
3	$ln(Rc) = -0.655 + 11.479*l + 0.2914*n$	0.83

Rc – compression strength, N/cm^2, A_A - area fraction of trabecular bone, λ - mean free path between trabeculae, [mm], *l* - mean length of branches, [mm], *n* - mean number of knots (triple points) in a single trabecula

This is clear that the best fit is achieved when taking into account two parameters: area fraction and mean length of branches (relation 1). Comparison between the R_C value computed using this relation and the apparent compression strength is shown in Fig. 7. One can observe that the trend line can be characterized as the best possible fit and only some statistical scatter affects this line. Taking into account more complex models, based on three parameters, does not lead to significant improvement in the accuracy of relationships based on linear models.

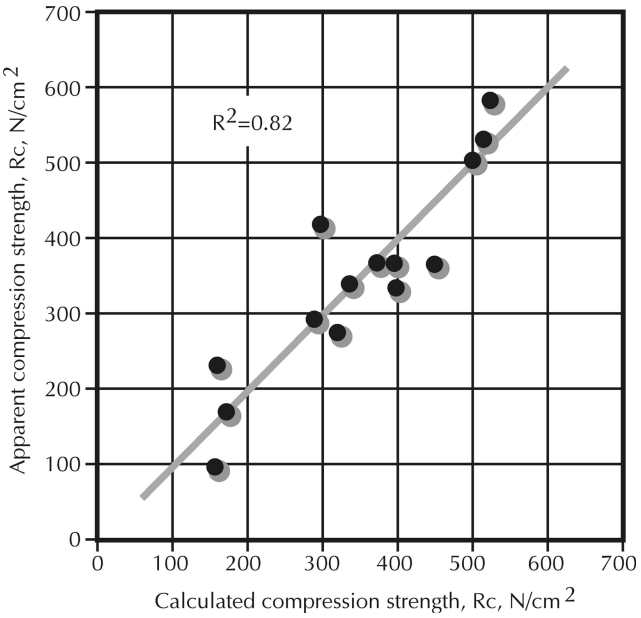

Fig. 7. *Apparent compression strength versus compression strength calculated from histomorphometric parameters.*

CONCLUSIONS

1. No significant correlation was found between the bone compression strength and bone mineral density (BMD).
2. Significant correlations between bone compression strength and several histomorphometric parameters were documented. The highest correlation was noted when area fraction of trabecular bone and mean length of branches were taken into consideration. This suggests that the bone is more resistant to compression forces when its structure is built with high volume of trabeculae with long and thin branches.
3. The use of image analysis enables efficient estimation of various histomorphometric parameters. Some of these parameters cannot be evaluated during manual inspection.
4. In order to improve accuracy of diagnostics one should look for *in vivo* methods of evaluation of histomorphometric parameters.

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