

APPLICATIONS BULLETIN

Intrinsic bone tissue quality is altered in osteoporosis and selectively influenced by its treatments

Bone regeneration presents a major challenge to orthopedic medicine. Current methods for the treatment of massive bone loss are essentially dependent on artificial prostheses. Prostheses cannot be used in every case due to the limitation of movement and biocompatibility issues. Similarly, the prosthesis can fail in the long term and result in the loss of function and possibly morbidity.

New nanoscale analysis applied in bones and other mineralized biological materials enable a new window into the fine details of mechanical behaviour at extremely small scales. Micro and macroscopic analysis, which yield averaged quantities over larger length scales, may not be sensitive enough to identify the underlying differences between two similar samples. Hence, nanoscale studies are desirable for the resolved characterization of these complex materials. In addition, nanoscale methodologies are useful when the volume of material available is too small for larger scale analyses, for example with tissue engineered bone formation in critical-sized defects and rat models. The accuracy of biomechanical properties reduced using traditional engineering beam theory applied to whole bone bending tests on mouse bone has also been questioned.

Nanoindentation analysis has focused differences between cortical and trabecular bone, anisotropy, time-dependent plasticity, variations as a function of distance from the osteonal center through the femoral cortex, viscoelasticity and variations due to mineral content.

In order to evaluate the role of intrinsic bone tissue quality in bone strength, a nanoindentation test was performed at the level of the vertebral cortex of adult rats after various dietary and hormonal manipulations known to markedly influence bone strength of an intact skeletal piece.

The nanoindentation technique assesses both hardness and elasticity of dry and wet bone tissue with a high spatial resolution. Nanoindentation has also been proved to be a reliable method to assess the intrinsic mechanical properties of single bone structural units (BSU). The local elastic properties of bone structural units were found to vary significantly among individuals, anatomical locations, the type of bone (interstitial, osteonal, and trabecular), and trabecular orientation.

The results of the present study indicate that besides geometry and microarchitecture, intrinsic bone tissue property is an important determinant of the mechanical competence of rat vertebrae after dietary OVX treatment and low protein intake.

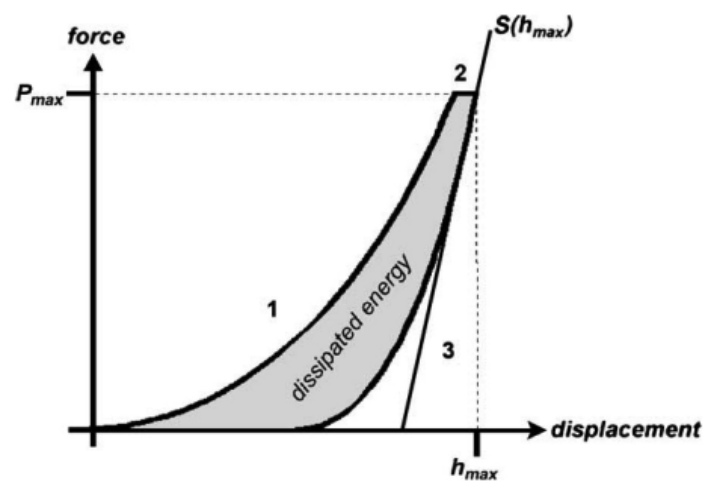
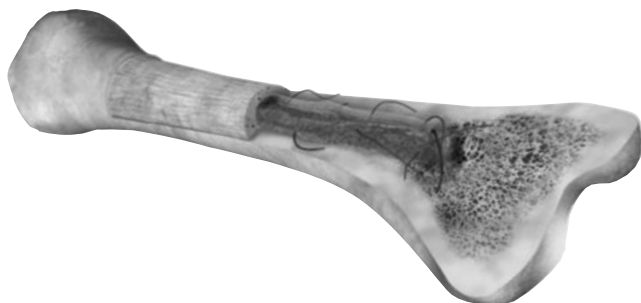


Fig. 1. Force-displacement curve of a nanoindentation test: loading (1), holding (2), unloading (3) of an indenter tip. The third part leads to elastic recovery of the material and its initial slope is used to derive the elastic indentation modulus. The hysteresis represents the dissipated energy.

Nanoindentation represents a test of the intrinsic mechanical properties of bone tissue. This technique acquires force-displacement data of a pyramidal diamond indenter that is pressed into a material. Fig. 1 shows the resulting curve that consists of three parts. In part 1, the indenter tip is loaded onto the sample that results in a complex combination of elastic and postyield deformation.

At maximum force, the load is held constant leading to creep of the material below the tip. When the force on the tip is released, the elastic response of the material is detected (Part 3). The slope at the point of initial unloading is considered to derive the elastic properties of the sample. The unloading slope has with:

$$S(h_{\max}) = \frac{dP}{dh}(h_{\max}) = \frac{2}{\sqrt{\pi}} E_r \sqrt{Ac}(h_{\max}) \quad (\text{equ 1})$$

a direct relationship with the contact area $Ac(h_{\max})$ and the reduced modulus E_r . The contact area is the projected area of contact between the pyramidal indenter and the sample and represents a calibration parameter.

The reduced modulus represents a sum of the compliance of the material and the diamond indenter.

$$\frac{1}{E_r} = \frac{1 - \nu_{\text{specimen}}^2}{E_{\text{specimen}}} + \frac{1 - \nu_{\text{tip}}^2}{E_{\text{tip}}} \quad (\text{equ 2})$$

The first fraction is defined as the indentation modulus and derives from the known properties of the indenter tip and the reduced modulus. The indentation modulus combines with

$$E_{\text{ind}} = \left(\frac{1}{E_r} - \frac{1 - \nu_{\text{tip}}^2}{E_{\text{tip}}} \right)^{-1} = \frac{E_{\text{specimen}}}{1 - \nu_{\text{specimen}}^2} \quad (\text{equ 3})$$

the local elastic modulus and Poisson ratio of the specimen and represents the first parameter of interest in this paper. The ratio of maximum force and the contact area supplies a second mechanical parameter, hardness:

$$H = \frac{P(h_{\max})}{Ac(h_{\max})} \quad (\text{equ 4})$$

Hardness can be interpreted as a mean pressure the material can resist.

A third output of the indentation experiment was taken into account, that is, the area of the hysteresis (see Fig. 1). This parameter has the dimension of mechanical work and represents the energy dissipated during the indentation test.

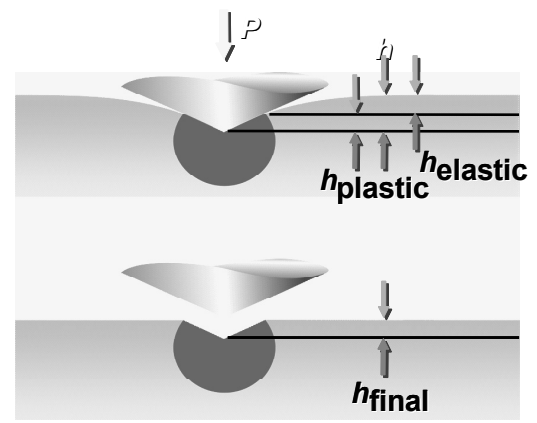


Fig. 2. Schematic representation of the elasto-plastic behaviour on bone during the nanoindentation

For the nanoindentation tests, the L_5 vertebral body of each rat was dissected at the level of the intervertebral disks. The bone specimens were kept frozen until preparation for the mechanical tests. The vertebra was cut transversally in the middle of the approximately 8 mm high body. The samples were embedded in PMMA and the face of the transverse cut was polished finishing with a 0.25- μm diamond solution. After these preparation steps, the specimen were dried for 24 h at 50°C.

The mechanical tests included 9 indents on the cortical shell of each vertebral body, 3 indents at the posterior, three at the lateral and three further on the anterior site. On each site, three indents were done on the periosteal, the central, and the endosteal location of the bone matrix (Fig. 3a) The indents were run to 900 nm maximum depth applying an approximate strain rate of $\epsilon = 0.066$ 1/s for both loading and unloading. At maximum load a 5-s holding period was employed. The limit of the maximum allowable thermal drift was set to 0.1 nm/s. The indents were performed at the center of the lamellae; indents at the edge of two lamellae were excluded. In the present study, only cortical bone was tested, since major deterioration and destruction of the trabecular structure was observed in OVX rats fed a low protein diet.

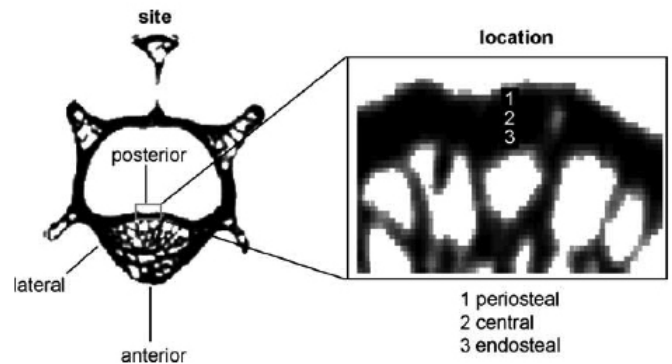


Fig. 3a. Schematic representation of the indent areas. On transversal slices of lumbar vertebral body, three sites were chosen: anterior, posterior, and lateral sites (see left figure). On each site, three locations were defined as structure of interest: the periosteal, central, and endosteal locations (see right part of the figure).

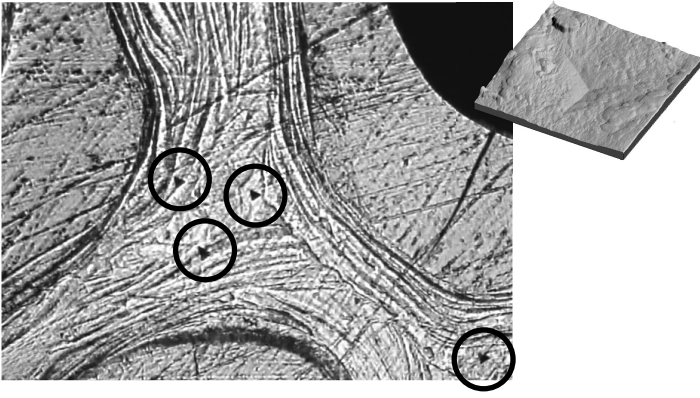


Fig. 3b. Optical micrograph of Berkovich indentation on Trabecular/cortical Nanoindentation and AFM high-resolution image. (12 x 12 x 1.5 μm)

Determinants of Bone Strength:

3D Repartition

- Geometry
- Microarchitecture

Amount of Material

Material Quality

- Mineralisation
- Matrix
- Organization

Results:

Variation of nanomechanical characteristics in vertebral body cortices

The heterogeneity of nanomechanical characteristics measured in different sites of the vertebral body cortex, (that is, anterior, posterior, and lateral) was first evaluated in controls animals. For all three mechanical parameters (indentations modulus, hardness, and the dissipated energy) lower values were detected on the anterior site. A two-way ANOVA performed with location and site as fixed effects, showed that site was highly significant for all these three mechanical parameters ($P < 0.0001$). Location (periosteal, endosteal or central), on the other hand, was not significant ($P > 0.6$).

Effect of protein intake on nanomechanical characteristics

The influence of isocaloric protein undernutrition and of essential amino acids supplements was then assessed. Three-way ANOVA for indentation modulus considering the complete data set showed again high global significance for the site (anterior, lateral, posterior) ($P < 0.001$). The factor location (periosteal, endosteal, central) was moderately significant ($P = 0.029$), and treatment was not globally significant ($P = 0.65$). However, the interaction between treatment and site was close to the significance level ($P = 0.06$). This led us to apply an individual statistical evaluation for each of the three sites. Two-way ANOVAs were performed with treatment and location as fixed effects. The influence of the treatment was not significant ($P > 0.1$). In contrast, the factor location was significant for the anterior site ($P = 0.013$) and for the posterior site ($P = 0.0002$), but not significant for the lateral site ($P = 0.2$). The nano-mechanical properties of the different locations are separately presented (Fig. 4). Comparison between the treatment groups was done for all locations (Table 1). post hoc analysis showed significant decreases of nano-mechanical properties ($P < 0.05$) at the endosteal location in OVX rats fed the low protein diet as compared with SHAM. This difference was detectable for all three nanomechanical parameters. On the central part of the posterior vertex, hardness and dissipated energy were significantly reduced ($P = 0.02$ and $P = 0.03$, respectively) in response to ovariectomy and the low protein diet. In periosteal location, significant alteration of elastic properties and energy dissipation between SHAM and OVX rats with the low protein diet was also detected ($P = 0.01$ and $P = 0.02$, respectively). The positive trend of essential amino acids supplements on indentation modulus and dissipated energy was not significant ($P < 0.1$) at the endosteal location. There was also a trend for an effect of essential amino acids supplements on hardness at the central location ($P < 0.1$). For indentation modulus at the periosteal location, the effects of essential amino acid supplements were almost significant ($P = 0.06$).

Macroscopic mechanical results versus nanomechanical tissue properties and bone mineral mass

For the correlation between nanomechanical data and macroscopic tests, which were obtained by axial compression of the vertebral body [2], the mean values of hardness and indentation modulus and dissipated energy of each rat were used (Fig. 5). Macroscopic energy to failure showed a correlation ($R^2 = 0.6$) with the dissipated energy of the indentation test. Macroscopic ultimate strength correlated moderately with hardness ($R^2 = 0.27$) and stiffness showed no correlation with the intrinsic elastic properties.

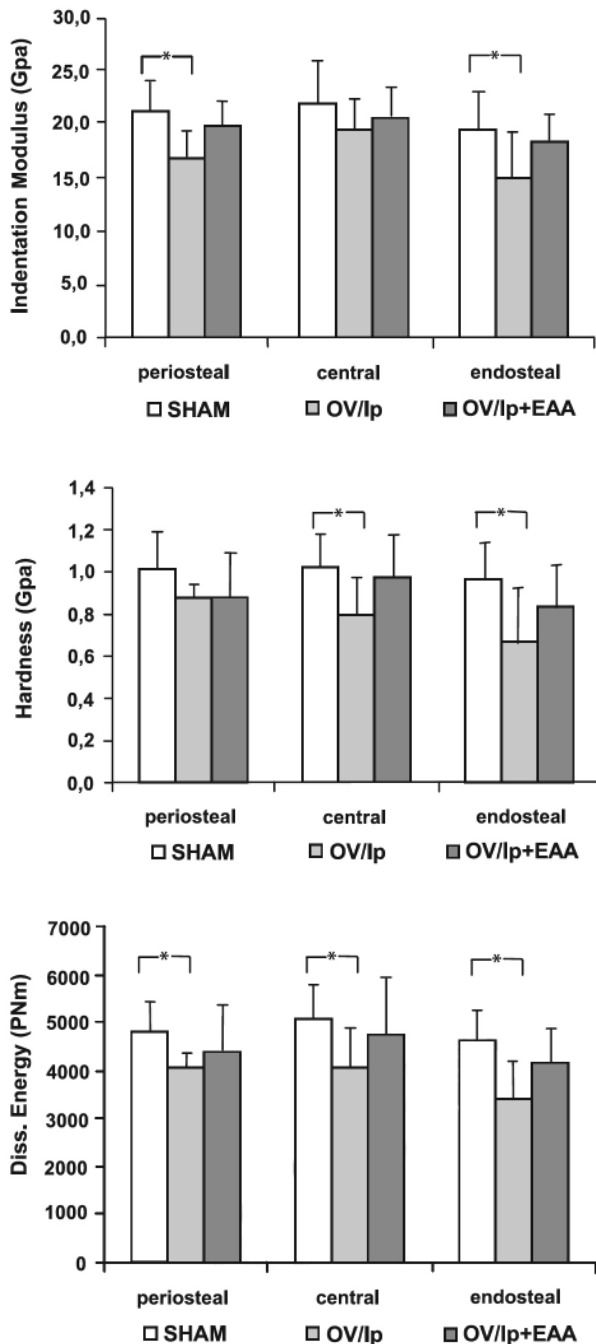


Fig. 5. Results of the nanoindentation tests at the level of the posterior part of the vertebral body for all three treatment groups (see text). *Statistically significant ($P < 0.05$).

Comparison between axial compression (old macroscopic method) and Nano-indentation (New nanometric method) are perfectly reliable but the nanoindentation give more results on tissue behaviour at low scale.

Acknowledgments

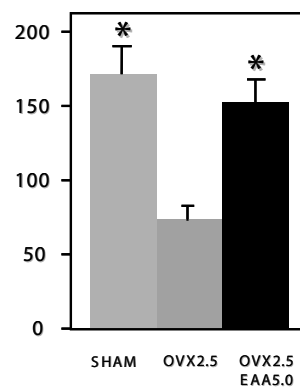
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2005, vol. 36, no1, pp. 134-141 [8 page(s) (article)] (28 ref.)

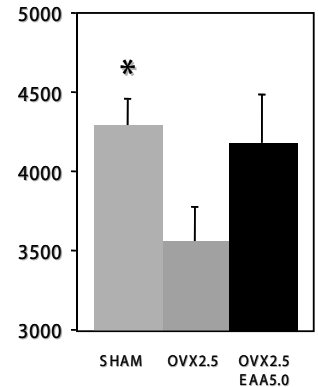
Axial Compression

Maximal Load [N]



Nano Indentation

Working Energy



* VS OVX2.5

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Fig. 6. Comparison between axial compression testing and nanoindentation

Conclusion

The present study showed a heterogeneity of intrinsic bone tissue properties of the rat vertebral body, which varied in relation to protein intake. Low protein intake associated with ovariectomy, paired with essential amino acids supplements, decreased the nanomechanical values. These results underline the capacity of the nanoindentation technique to detect changes induced by nutritional and hormonal manipulations. Correlations between macroscopic mechanical results as assessed by axial compression of the vertebral body and nanomechanical tissue properties suggest that macroscopic postelastic behavior varied strongly with material fragility detected on the tissue level. Macroscopic stiffness however was dominated by bone geometry changes and less by variations of tissue properties, as nanoindentation reveals. Other mineralized biological materials such as dentine, enamel and calcified cartilage could be studied by the nanoindentation technique.



This Applications Bulletin is published quarterly and features interesting studies, new developments and other applications for our full range of mechanical surface testing instruments.

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