UNIVERSIDADE DE SÃO PAULO
SCIENTIA VINCES

Orthopedicians 2017
Kuala Lumpur, Malaysia

EESC · USP
Escola de Engenharia de São Carlos
Universidade de São Paulo

Nanoindentation of trabecular bone in human vertebrae classified as normal, osteopenic and osteoporotic by ultrasonometry of the calcaneus

- The evaloution of microarchitecture resistance of the trabecular bone may contribute in determining the risk and preventing fractures associated to osteoporosis

Motivation

Do the mechanical properties of trabecular bone microstructure allow us to indicate the bone quality of human vertebrae?

- Is it possible to indentify (Mechnically) osteoporosis in one single trabecula?

Objective

Evaluate the **“quality"** of trabecular bone microarchitecture **of vertebrae** (human cadavers)**,** classified as **normal, osteopenic and osteoporotic** by **ultrasonometry** technique of the calcaneus, **through nanoindentation test.**

Introdution → Osteoporosis

- **Loss of bone mass (density); Increased porosity and fracture risk;**
- Specifically in trabeculae the **reduction of connectivity, number, thickness and deterioration of plate to rod format.**

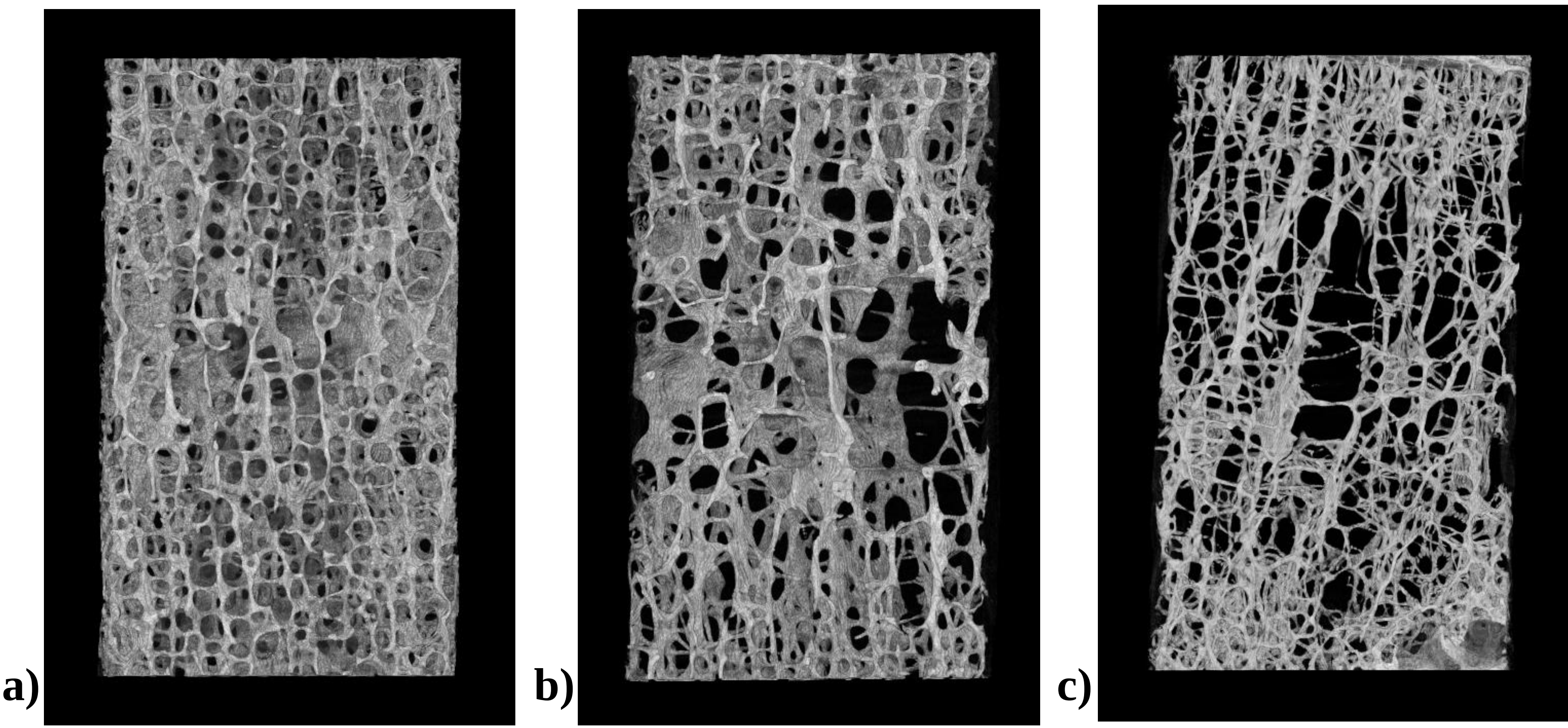


FIG. 1 - Trabecular bone of human vertebrae classified as: a) normal; b) osteopenic; c) osteoporotic

Introdution → Trabecular bone

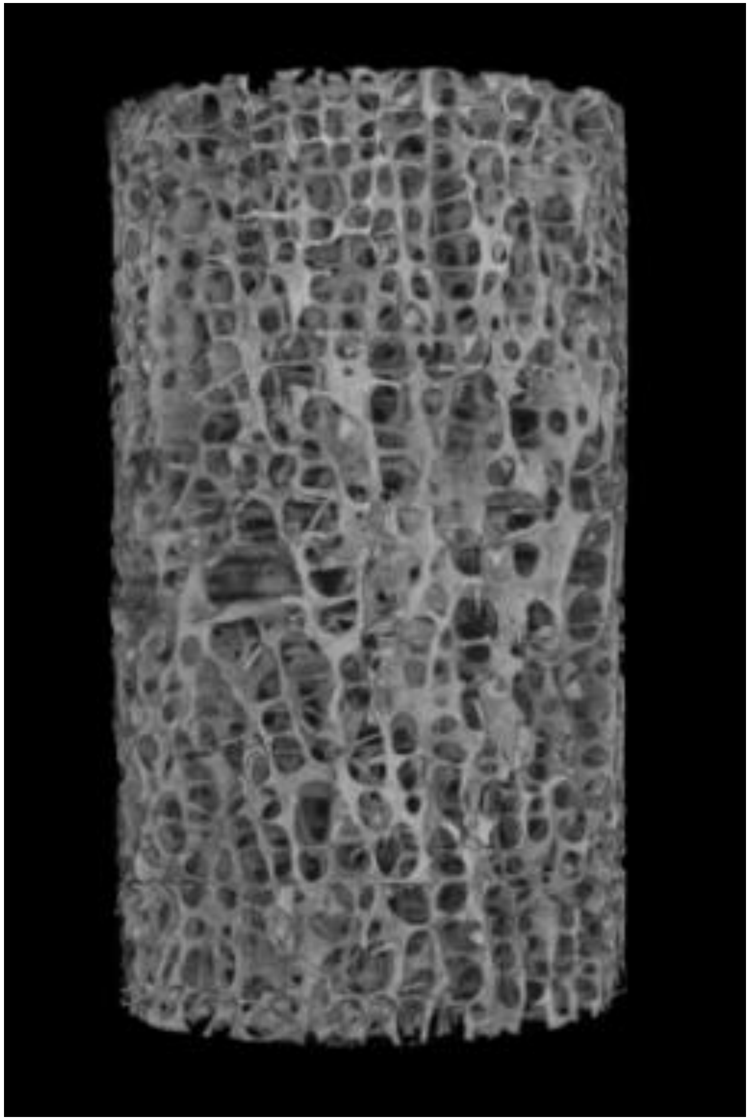


FIG. 2 – Trabecular bone of human vertebrae classified as normal

- Composite
- Heterogeneous
- Orthotropic
- Anisotropic
- Viscoelastic

Classification of calcaneus ultrasonometry

a) 

b) 

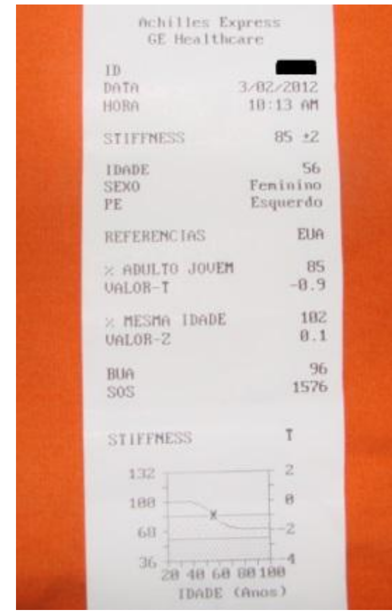
c) 

FIG. 3 – Procedure for ultrasonometry analysis of calcaneus: a) Hygiene treatment with isopropyl alcohol; b) leg coupling in the Achilles Insight equipment; c) Results of an individual considered as normal (T-score -0.9) in function parameters such as gender, ethnicity, weight, age and bone stiffness of the calcaneus (elastic module).

Materials and Methods

Samples

- A total of 90 human **trabecular** vertebrae were dried (dehydrated), distributed proportionally by the regions $\mathbf{T_{12}}$, $\mathbf{L_1}$ and $\mathbf{L_4}$ in a total of 30 vertebrae segments of each normal osteopenic and osteoporotic group extracted from individuals (human cadavers of Brazilian nationality).

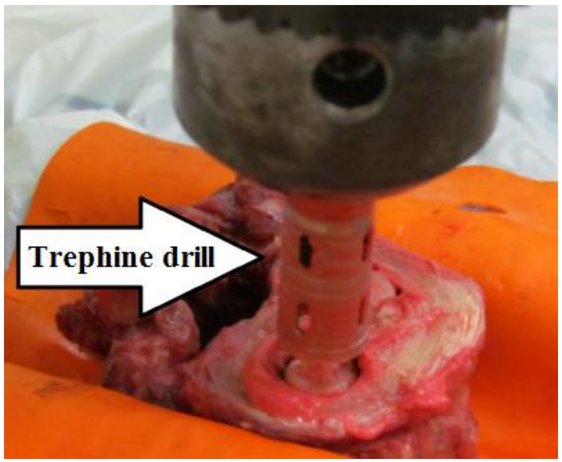
Materials and Methods

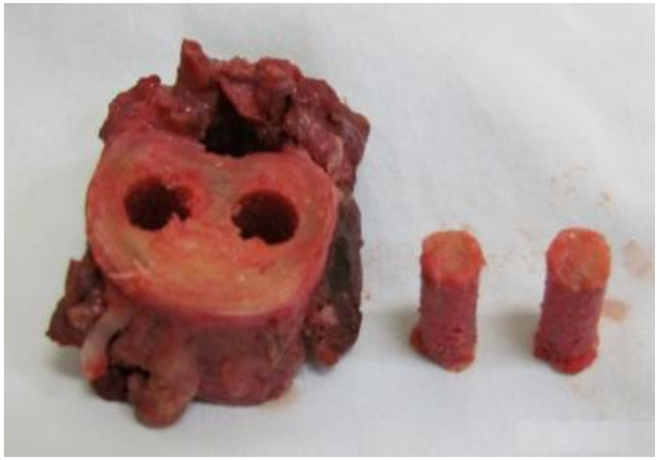
Samples

- They are classified trough bone quality index (BQI) as normal, osteopenic and osteoporotic bones through the ultrasometry of the calcaneous bone.

Casuistic, Materials and Methods

Preparation of samples

a) 

b) 

c) 

FIG. 04 – a) Vertebral body penetration through the trephine drill; b) Cylindrical specimens of trabecular bone; c) Proof bodies (20 by 10) mm without bone marrow of L_1 region, classified as normal, osteopenic and osteoporotic

Bone marrow removal with physiological saline soluction (NaCl 0.9%)

Casuistic, Materials and Methods

Preparation of samples

FIG. 05 - Test samples without bone marrow, built into acrylic resin L1 region, classified as normal, osteopenic and osteoporotic

Casuistic, Materials and Methods

- The nanoindentation technique allowed the evaluation of the elastic module (E) and nanohardness (H) in one single trabecula in each respective group: Normal, Osteopenic and osteoportic.

Casuistic, Materials and Methods

Trabecular Bone Test Bodies Mecanical Resistence Analysis When Subjected To Punctual External Loads

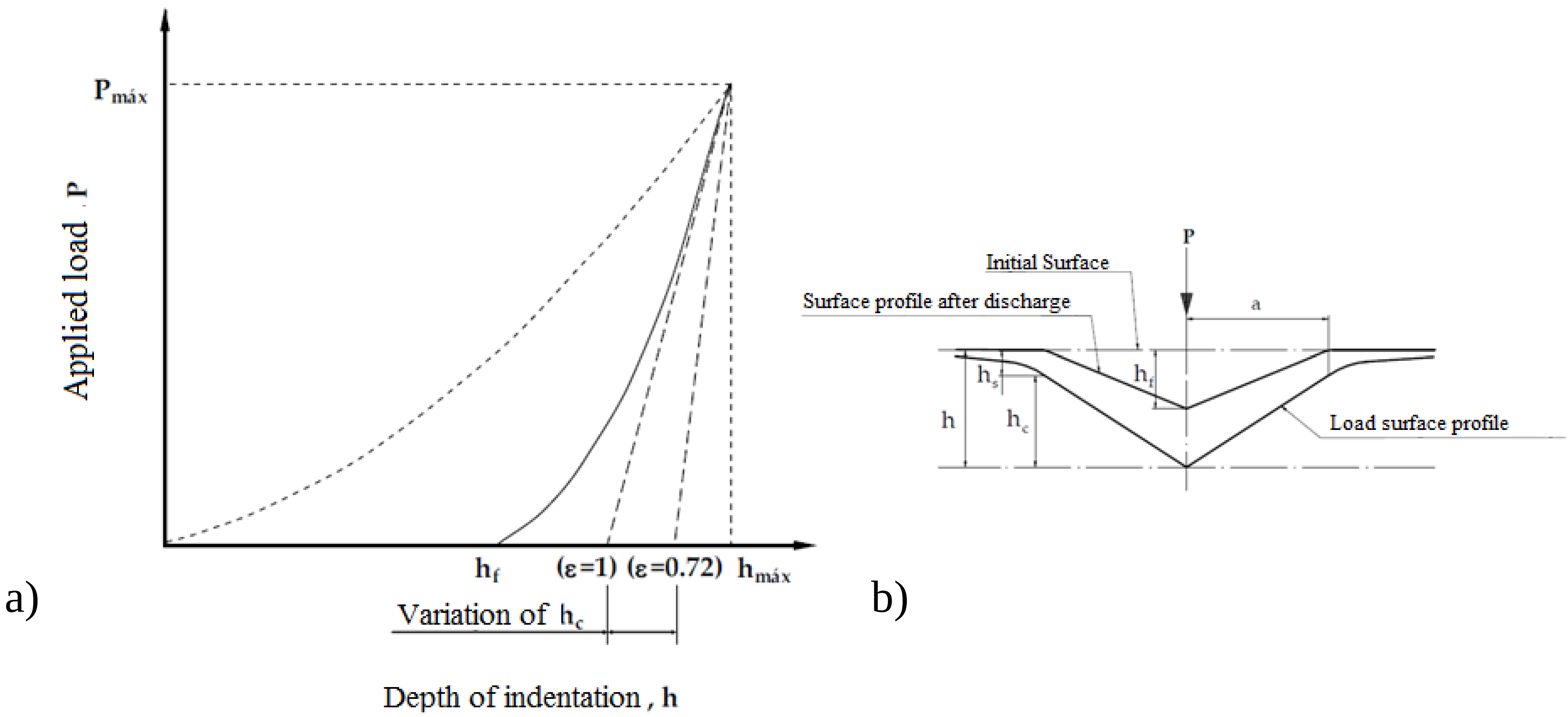
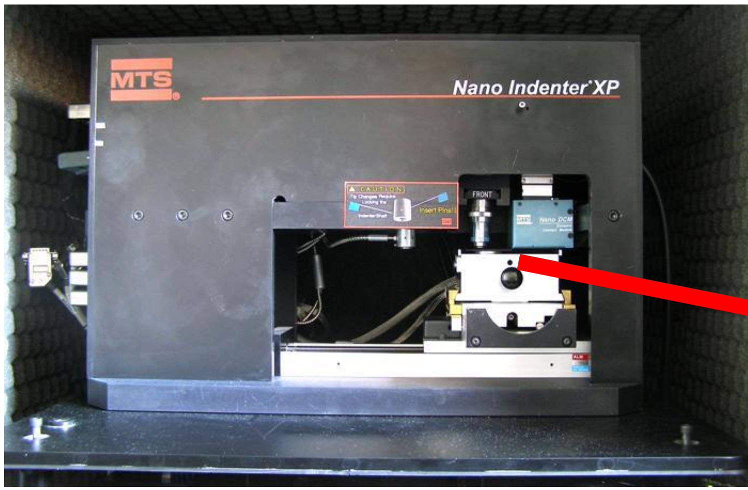
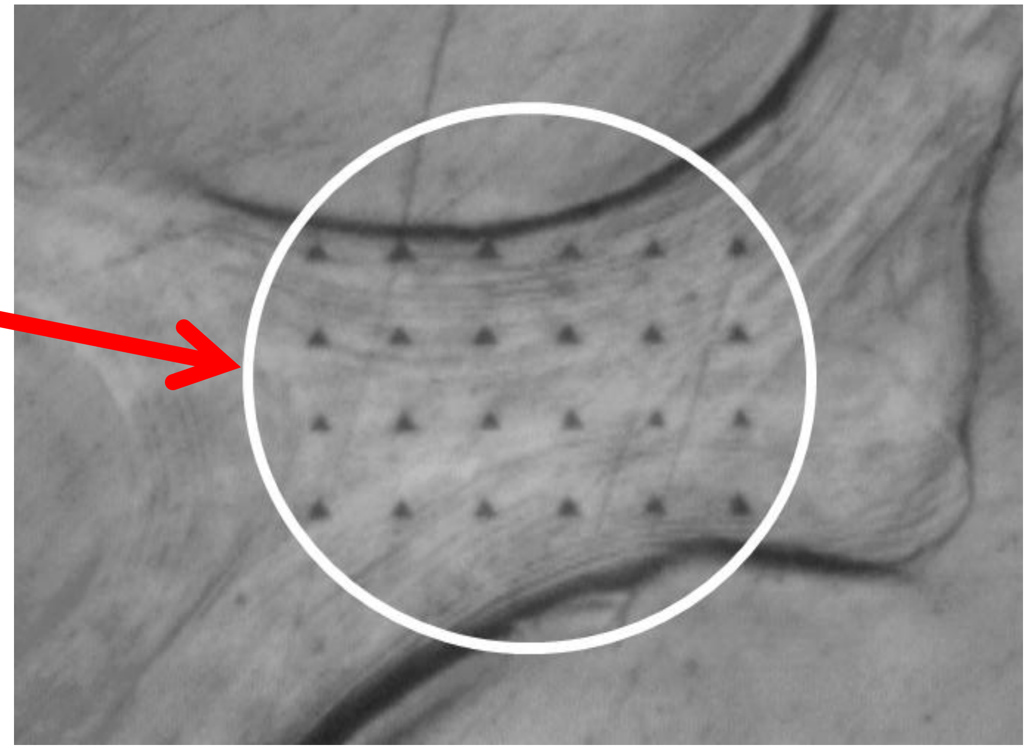


FIG. 6 - a) Typical nanoindentation curve; b) The surface profile and geometric parameters of an indented sample.

Casuistic, Materials and Methods

Nanoindentation test





Speed : **400MN/s;**
Load cell : **50MN (5gf);**
Resolution : **500nN**

FIG. 07 – a) Nanoindentation System (Nano Indenter XP)**, b)** Realization of 24 indentations (6x4) in trabecular bone of the T12 region of the normal group.

Casuistic, Materials and Methods

The vertebrae of the regions **T_{12}** and **L_1**, were chosen beacause they have a higher rate of fractures and the **L_4** has a better distribuition of trabeculae and axial alignment to the cranial-caudal axis in the vertebrae body.

Casuistic, Materials and Methods

The minimum numbes of samples (N = 8.64) was determined as function of the quantitative variables for an infinite population and is defined by Equation.

$$N = \left(\frac{Z_{\alpha/2} SD}{SE} \right)^2 \rightarrow N = 8.6$$

Were $Z_{\alpha/2}$: critical value for the desired degree of confidence (usually: 1.96 (95%); SD: standard deviation of the average; and SE: standard error of the average (SD = 1.80 MPa, SE = 1.2 MPa). The adopted sample size was ten (N = 10) for each group: normal, osteopenic and osteoporotic, totalizing thirty (30) subjects.

Casuistic, Materials and Methods

Statistical method

- ✓ Descriptive statistics: **Mean** (M), **Median** (Md), **Standard Deviation** (SD), **Standard Error** (SE), **Minimum Value** (Min), **Maximum Value** (Max), **Variational Coefficient** (VC) and **number of samples** (N).

- ✓ **Normality test by Shapiro-Wilk method**

- ✓ Analysis of **comparisons by ANOVA methods** and **multiple comparisons by Tukey HSD Kramer**

Casuistic, Materials and Methods

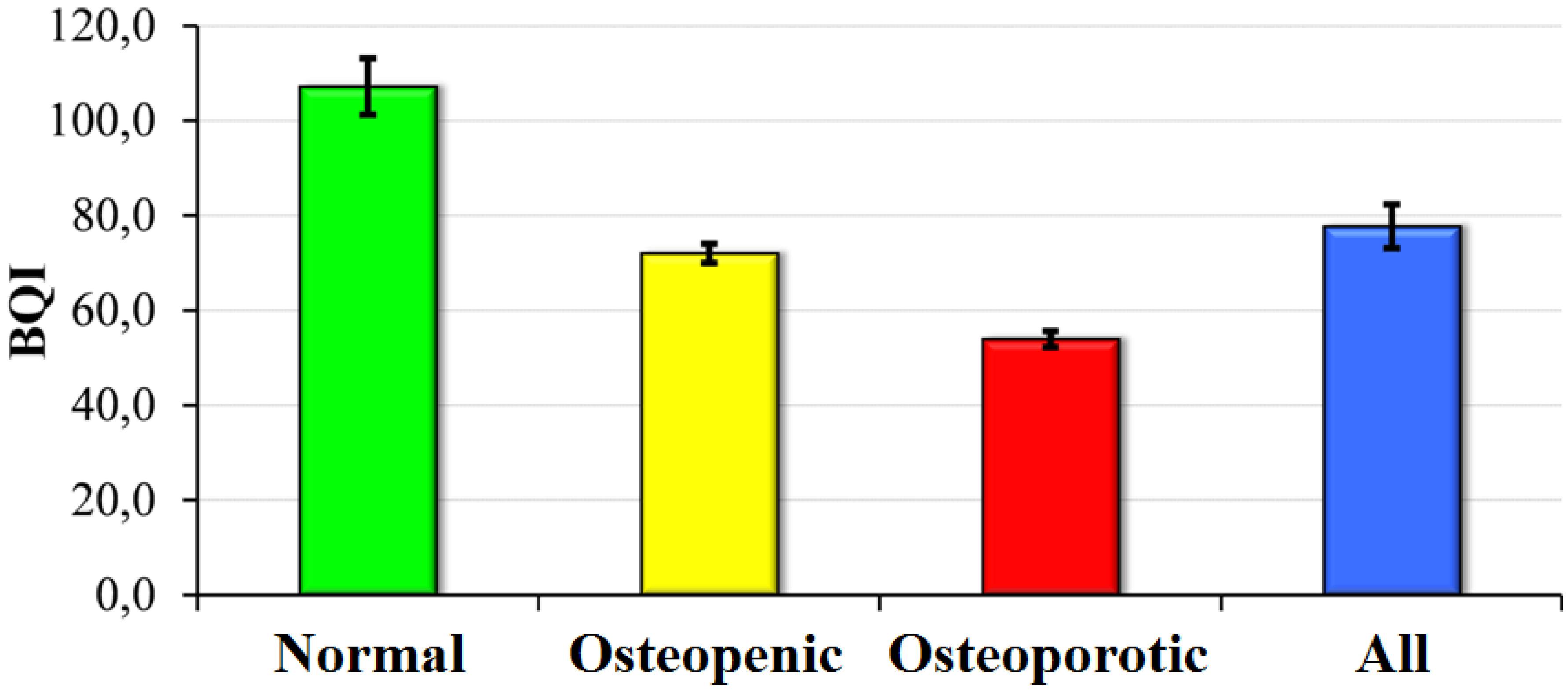
Statistical method

- Descriptive statistics of **BQIs** of **30 individuals** classified as normal, osteopenic and osteoporotic bone by the ultrasonometry of the calcaneus.

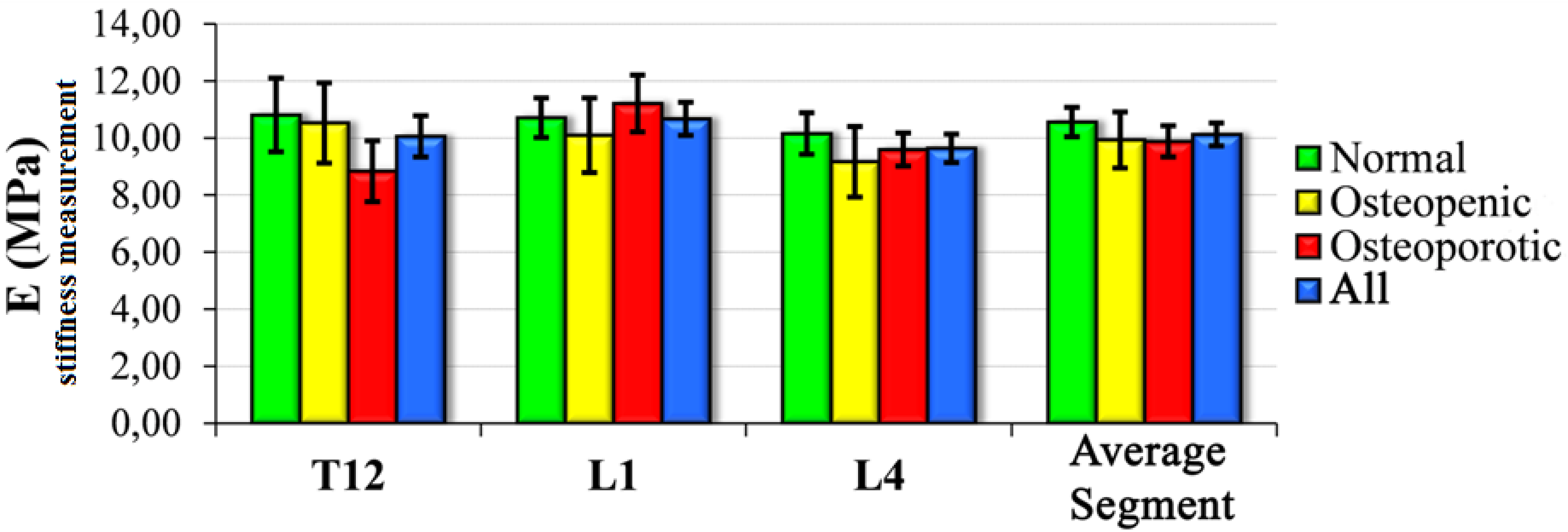
- Descriptive statistics of **mean E** of trabecular bone between the regions T_{12}, L_1 and L_4 of **30 human vertebral segments**, determined by mechanical testing of nanoindentation and classified as normal, osteopenic and osteoporotic bone by the QUS.

- Descriptive statistics of **mean H** of trabecular bone between the regions T_{12}, L_1 and L_4 of **30 human vertebral segments**, determined by mechanical testing of nanoindentation and classified as normal, osteopenic and osteoporotic bone by the QUS.

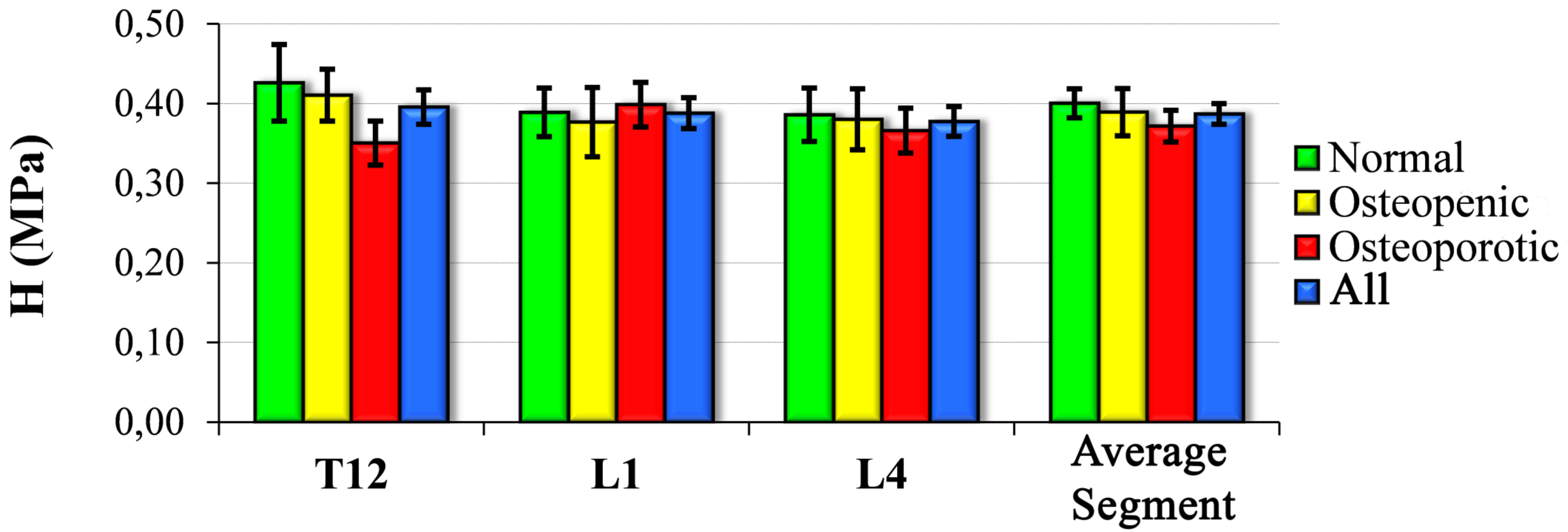
Results → Ultrasonometry of calcaneous



Results → Nanoindentation



Results → Nanoindentation



Results → Nanoindentation

TAB. 1 – Correlation of bone quality index (**BQI**), modulus of elasticity (**E**) and mechanical compression test USC (Ultimate Compressive Strength to Trabecular Bone = 2.48 ± 1.8 MPa) **maximum load supported by the bone** by the Spearman test.
The value of r <than 0.5 → rejected.

Variable	Correlation	r
BQI	Age (years)	-0.501
	E (MPa)	0.499
	Ultimate Compressive Strengh To Trabecular Bone UCS = 2.48 ± 1.8 MPa	0.508
E	Age (years)	-0.584
Ultimate Compressive Strength To Trabecular Bone UCS = 2.48 ± 1.8 MPa	Age (years)	-0.684

Conclusion

- The nanoindentation test of vertebrae proof bodies (human cadaver) allowed the analysis of the mechanical properties in a single dehydrated trabecular The suggested hypothesis was not confirmed, there were no significant differences between the normal osteopenic and osteoporotic groups.

Conclusion

- The tests demonstrated that the fracture resistance associated with osteoporosis is more closely linked to the architecture (macroscopic condition) of the set of trabeculae, than to the bone matrix (microscopic condition) of a single trabecula, because there were no significant differences between groups.

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