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Source / Izvornik: *Collegium antropologicum*, 2006, 30, 49 - 54

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:184:847253>

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Download date / Datum preuzimanja: **2024-06-18**



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# Age-Related and Gender-Related Differences between Human Vertebral and Iliac Crest Bone – A Histomorphometric Study on the Population of the Mediterranean Coast of Croatia

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## ABSTRACT

*In this study, osseous tissue was examined in normal adult population that has inhabited areas by the Croatian Adriatic Sea. The most of such studies have shown that women are prone to lose bone connectedness, while men are predisposed to be a stronger constitution and they start with greater bone mass, though. Bone samples from two different anatomic sites were analyzed. The crista iliaca and the lumbar vertebra represent functionally different organs too. We wanted to consider whether the same age- and gender-related changes affect these two organs due to normal aging. Static histomorphometry was used to quantify involution changes in the trabecular bone. Results showed that involution process more severely affects women than men. Age-related structural changes were more prominent in lumbar vertebra than in iliac crest bone. Severe structural changes in lumbar vertebra could subsequently lead to a dysfunctional and deformed vertebral column. Therefore, iliac crest bone biopsies could hardly explain involution process that affects lumbar spine.*

**Key words:** bone histomorphometry, vertebral body, iliac crest, aging, gender

## Introduction

Normal age-related changes affect both, cortical and trabecular bone with minor differences in respect to different sexes<sup>1</sup>. According to some data, distance between horizontal trabeculae was bigger in women than in men, when vertebral bone structure was analyzed<sup>2</sup>. These data suggest that involution process develops through different mechanisms of bone remodeling, which lead to different patterns of bone loss in men or women<sup>3</sup>. Foremost, Parfitt has given some evidences for preferential lost of thinner or horizontal trabeculae in elderly women<sup>4</sup>. There are some evidences, which relate decrease in mean trabecular width, as phenomenon occurring in men, while trabecular bone connectivity disruption is a phenomenon related only to women<sup>5,6</sup>. On the other hand Weinstein

and Hutson consider that decrease in mean trabecular width as well as disruption of thinner trabeculae are inseparable, equally represented processes in overall age-related bone loss<sup>7</sup>. Recent researches have demonstrated that histomorphometric measures in iliac crest and vertebral bodies poorly correlated between the bone volume parameters<sup>1,8</sup>.

On the microscopic anatomy level, these two skeletal elements disclose two different principles in their inner organization. *Isotropic principle* reveals itself in the crista iliaca, while *anisotropic principle* dominates in lumbar vertebra. One could describe the structure of iliac bone as *quiet trabecular bone*, which is characterized by arch-formed plates reaching between the inner and outer

cortices<sup>9</sup>. In contrast, the spongy bone in lumbar vertebra shows certain dynamics which predominantly reveals itself through at least three regions – dependent qualities of three – dimensional network, mainly consisted of thick columns, which are supported with thinner horizontal plates<sup>10,11</sup>. The postulate *form follows function* leads us to the key evidence, which to some extent explains the entirely different inner organization at these two skeletal sites<sup>12</sup>. The spine and the hip joint are the most common anatomic sites, which are seized by osteopenia and osteoporosis onset<sup>13,14</sup>. Transiliac bone biopsy and following assessment of the bone status through quantification by histomorphometric parameters has become necessary in diagnostic procedures. It is also frequently used method in therapeutic follow-up evaluations such are resistant, non-compliant osteoporosis.

Naturally, the question arises whether similar close relationship exists between the iliac bone histomorphometric parameters and those from other anatomic sites, particularly vertebral trabecular bone.

For the past couple of decades such studies have demonstrated that there is a need for large regional researches to provide database of normal morphometric results for a specific population. Clearly, this is a regional study, which involves adult population of the Mediterranean Coast in Northern Croatia, with an average of 2100 solar hours per year.

## Materials and Methods

The material for this study comprised 46 human lumbar vertebral bodies and 46 transiliac bone samples. Bone samples were obtained from 23 men and 23 women who before deceased inhabited the area of the Northern Croatian Adriatic Coast (the Cities of Rijeka and Pula). Individuals were divided in separate gender related groups and subsequently men or women were divided in three separate age-related groups: 41 to 50 years (7 men and 7 women), 51 to 70 years (7 men and 7 women) and 71 to 85 years (9 men and 9 women). The first lumbar vertebral body (L1) was removed by sawing the vertebral arches at the autopsy. Bone specimens were taken from the standard biopsy site in the cristae iliaca (CI) of the same individuals. The samples were cleaned from the surrounding tissue, washed in saline solution and frozen on –20 degree of C. For each individual, age and gender were noted and samples from individuals with known metabolic and muscular-skeleton diseases were excluded.

The local ethics committee approved this study.

### Bone histomorphometry

From vertebral body, a vertical cylindrical bone sample was obtained by drilling the constant point at the central part of the vertebral body, using the bone biopsy trephine (8 mm). Samples were embedded undecalcified in MMA, cut on 5  $\mu\text{m}$  slices and stained in toluidin blue and Goldner trichrome. The Goldner trichrome straining technique showed good contrast between unmineralized

(red stained) and mineralized bone area (stained in green). Image analysis was performed by using semiautomatic image analyzer – Issa software (VAMS, Zagreb, Croatia). Histomorphometric parameters were derived from the standard formulas, proposed by the *American Society of Bone and Mineral Research*<sup>15</sup>.

Bone volume (BV/TV, %) corresponds to the amount of trabeculae bone within the spongy space. BV/TV was derived from two-dimensional (2D) measurements of bone area (B.Ar) and trabecular tissue area (T.Ar) using Parfitt's formula, as follows:<sup>15</sup>

$$\text{BV/TV} = 100 \text{ B.Ar} / \text{T.Ar}$$

Trabecular bone surface (BS/TV, in /mm) was then calculated from the values for the perimeter of trabecular bone (B.Pm) and the area of trabecular bone (B.Ar):<sup>15</sup>

$$\text{BS/TV} = \text{B.Pm} / \text{B.Ar}$$

In addition, three more values were calculated to evaluate the inner structure of trabecular bone:

(1) Trabecular thickness (Tb.Th, in mm) was derived from measurements of the trabecular perimeter (B.Pm) and bone area (B.Ar) according to Parfitt's formula:

$$\text{Tb.Th} = (\text{B.Ar} / \text{B.Pm}) \times (\pi / 2).$$

(2) Trabecular number (Tb.N, in /mm) was derived from measurements of the trabecular perimeter (B.Pm) and total tissue area (T.Ar) according to the formula:<sup>15</sup>

$$\text{Tb.N} = (\text{B.Pm} / \text{T.Ar}) \times 10$$

(3) Trabecular separation (Tb.Sp, in  $\mu\text{m}$ ) was derived according to the formula:<sup>15</sup>

$$\text{Tb.Sp} = (1000 \times \text{T.Ar} - \text{B.Ar}) / \text{B.Pm}$$

### Statistical Analyses

After the data were tested for normal distribution, student t test for independent samples was used to explore differences between the vertebral and iliac crest bone parameters for different age and gender groups.

## Results

### The first lumbar vertebra

Trabecular bone volume declined in women and in men. The volume of trabecular bone from women 41 to 50 years of age (14.05%) significantly decreased at ages 51–70 years (18% of reduction) and 71 to 82 years (37% of reduction) (Table 1). In the same manner, trabecular bone volume decrease from men 41 to 50 years of age has reached significance at ages 51 to 70 years (27% of reduction) and 71 to 82 years (33% of reduction) (Table 3). With regard to trabecular bone structure, trabecular thickness and number of trabeculae decreased, while trabecular separation increased with age. Specifically, at ages 51 to 70 years men showed significantly thicker trabeculae (100.51  $\mu\text{m}$ ) compared to women (91.58  $\mu\text{m}$ ). Men have started with thicker trabeculae than women and overall decrease in this population came to 26% re-

**TABLE 1**  
STATIC HISTOMORPHOMETRIC VETREBRAL BONE PARAMETERS DATA IN WOMEN OF DIFFERENT AGE

Parameter	41 to 50 years (X±SD)	51 to 70 years (X±SD)	71 to 82 years (X±SD)
BV/TV (%)	14.05±3.06	11.57±3.27*	8.82±2.98*#
BS/TV (/mm)	2.68±0.49	2.64±0.72	2.03±0.69*#
Tb.Th (μm)	106.14±22.08	91.58±27.42*	89.34±21.68*
Tb.N (/mm)	1.34±0.24	1.32±0.36	1.00±0.34*#
Tb.Sp (μm)	665.80±142.7	726.08±232.33	1032.37±386.83*#

p<0.005, BV/TV – trabecular bone volume, BS/TV – trabecular bone surface, Tb.Th – trabecular thickness, Tb.N – trabecular number, Tb.Sp – trabecular separation

\*vs. age group of women in range 41 to 50 years

#vs. age group of women in range 51–70 years

**TABLE 2**  
STATIC HISTOMORPHOMETRIC ILIAC CREST BONE PARAMETERS DATA IN WOMEN OF DIFFERENT AGE

Parameter	41 to 50 years (X±SD)	51 to 70 years (X±SD)	71 to 82 years (X±SD)
BV/TV (%)	17.24±3.02	13.93±3.54	11.93±3.52*
BS/TV (/mm)	2.90±0.48	2.99±0.06	2.61±0.04
Tb.Th (μm)	119.67±19.28	94.12±19.21*	91.90±20.32*
Tb.N (/mm)	1.45±0.23	1.49±0.33	1.30±0.31
Tb.Sp (μm)	584.69±106.79	605.84±150.67	717.96±200.69

p< 0.005, BV/TV – trabecular bone volume, BS/TV – trabecular bone surface, Tb.Th – trabecular thickness, Tb.N – trabecular number, Tb.Sp – trabecular separation

\*vs. age group of women in range 41 to 50 years

**TABLE 3**  
STATIC HISTOMORPHOMETRIC VERTEBRAL BONE PARAMETERS DATA IN MEN OF DIFFERENT AGE

Parameter	41 to 50 years (X±SD)	51 to 70 years (X±SD)	71 to 82 years (X±SD)
BV/TV (%)	15.31±3.83	11.08±3.54*	10.15±3.60*
BS/TV (/mm)	2.45±0.56	2.40±0.72	2.15±0.67*
Tb.Th (μm)	128.16±31.21	100.51±27.56*	96.18±24.65*
Tb.N (/mm)	1.28±0.27	1.16±0.36*	1.08±0.34*
Tb.Sp (μm)	695.00±148.63	853.841±250.61	936.25±309.29*

p< 0.005, BV/TV – trabecular bone volume, BS/TV – trabecular bone surface, Tb.Th – trabecular thickness, Tb.N – trabecular number, Tb.Sp – trabecular separation

\*vs. age group of men in range 41 to 50 years

duction at ages 71 to 82 years. In women, trabecular thickness decrease came to 14% reduction at the same those ages 71 to 82 years (Table 5). Trabecular number decrease was more appreciable in women than in men. While the decrease in number of trabeculae was evenly distributed in men between given age related groups with an overall 16% reduction of single trabeculae, women showed abrupt decrease of 24% reduction between ages 51 to 70 years and 71 to 82 years. Trabecular separation from men at ages 41 to 50 years was greater than in women, but at ages 71 to 82 years women showed relatively greater trabecular separation than men did. Trabecular separation increase from women between those

ages 51 to 70 years (726.08 μm) and 71 to 80 years (1032 μm) was significant (Table 5).

#### *The iliac crest bone and its relation to vertebra*

The volume of trabecular bone decreased in crista iliaca with age: at ages 41 to 50 years men had significantly greater values of bone volume than women did (Table 4). The relative decrease in bone volume was very similar at ages 51 to 70 years (19% reduction for both sexes), but in overall elderly women showed 33% of the iliac crest bone volume reduction, while elderly men showed 22% of the trabecular bone volume reduction in cristae iliaca (Table 2 and 4).

**TABLE 4**  
STATIC HISTOMORPHOMETRIC ILIAC CREST BONE PARAMETERS DATA IN MEN OF DIFFERENT AGE

Parameter	41 to 50 years (X±SD)	51–70 years (X±SD)	71 to 82 years (X±SD)
BV/TV (%)	20.05±3.73	16.17±2.83*	15.73±2.66*
BS/TV (/mm)	3.37±0.87	3.27±0.55	3.15±0.45#
Tb.Th (μm)	118.81±33.38	105.22±23.43	99.59±20.01*
Tb.N (/mm)	1.64±0.27	1.59±0.38	1.45±0.18*
Tb.Sp (μm)	509.79±92.98	555.36±128.20	590.41±131.60

p < 0.005, BV/TV – trabecular bone volume, BS/TV – trabecular bone surface, Tb.Th – trabecular thickness, Tb.N – trabecular number, Tb.Sp – trabecular separation

\*vs. age group of men in range 41 to 50 years

#vs. age group of men in range 51 to 70 years

**TABLE 5**  
COMPARISON OF STATIC HISTOMORPHOMETRIC PARAMETERS IN THE FIRST LUMBAR VERTEBRA AND ILIAC CREST BONE

		41 to 50 years		51 to 70 years		71 to 82 years	
		W (X±SD)	M (X±SD)	W (X±SD)	M (X±SD)	W (X±SD)	M (X±SD)
BV/TV (%)	L1	14.05±3.06*	15.31±3.83*	11.57±3.27*	11.08±3.54*	8.82±2.98*	10.15±3.60*
	CI	17.24±3.02#	20.05±3.73	13.93±3.54	16.17±2.83	11.93±3.52	15.73±2.66
BS/TV (/mm)	L1	2.68±0.49	2.45±0.56*	2.64±0.72#	2.40±0.72*	2.03±0.69*	2.15±0.67
	CI	2.90±0.48	3.37±0.87	2.99±0.06	3.27±0.55	2.61±0.63	3.15±0.45
Tb.Th (μm)	L1	106.14±22.08	128.16±31.21	89.34±21.69#	100.51±27.56	91.58±27.42	96.18±24.65
	CI	119.67±19.28	118.81±33.38	94.12±19.21	105.22±23.43	91.90±20.32	99.59±20.01
Tb.N (/mm)	L1	1.34±0.24	1.28±0.27*	1.32±0.36#	1.16±0.36*	1.00±0.34*	1.08±0.34*
	CI	1.45±0.23	1.64±0.27	1.49±0.33	1.59±0.3	1.31±0.31	1.45±0.18
Tb.Sp (μm)	L1	665.80±142.76	695.00±148.63	726.08±232.33#	853.84±250.61*	1032.37±386.83*	936.25±309.29*
	CI	584.69±106.79	509.79±92.98	605.84±150.67	555.36±128.20	717.96±200.69	590.41±131.60

p < 0.005, W – women, M – men, CI – iliac crest bone, L1 – the first lumbar vertebra, BV/TV – trabecular bone volume, BS/TV – trabecular bone surface, Tb.Th – trabecular thickness, Tb.N – trabecular number, Tb.Sp – trabecular separation

\*the first lumbar vertebra vs. iliac crest bone within the given age and gender group

#women vs. men within the given age

When compared to lumbar vertebra, significant difference in bone volume was observed within all given ages. General trend showed trabecular thickness decrease in the iliac crest bone. Women and men started with similar values of trabecular thickness and while the overall lost in men amounts 16% of reduction, decrease was much more pronounced in women with 23% of trabecular thickness reduction (Table 5). Trabecular number decrease from women between ages 51 to 70 years (1.49/mm) and 71 to 82 years (1.30/mm) was relative. When compared to vertebra, than significantly greater values in trabecular number were measured in iliac crest bone of all men, but only from women at age 71 to 82 years. Trabecular separation has increased in iliac crest bone and increase was more pronounced in women. However, iliac crest trabecular bone showed significantly smaller trabecular separation than the lumbar vertebra did. The latter was observed in men at ages 51 to 70 years and in both sexes at ages 71 to 82 years. Overall increase

of trabecular separation came to 16% rise in iliac crest and almost double, 31% rise in the first lumbar vertebra.

## Discussion

Our study has provided histomorphometric data of the normal population, which inhabited regions by the Mediterranean Coast of the Northern Croatia. These results, at least to some extent, involve specific features of our sunny climate, as well as specific ways of living. Climate and yield habits have influenced bone metabolism of our population, with age. Therefore this study could be compared to any such a regional study worldwide: British, French, South African, Northern European or American.

### Lumbar vertebra

When we consider all the bone parameters together, the most impressive is fall in bone volume which declined



to 42% less in women and 36% less in men. The fall being greater in women was observed in some other studies too<sup>3,4,16</sup>. Our study revealed that between ages 51 to 70 and 71 to 82 years, women showed the major reduction in bone volume, while the major loss in men has occurred earlier (between ages 41 to 50 and 51 to 70 years). Men start with relatively greater values of bone volume, compared to women. Such a gender peculiarity was also found in another European study<sup>1</sup>. By the parameters that describe structure of the trabecular bone, we are comparable with the study of Thomsen et al. These investigators find no gender-related differences in any of the age-related changes for the vertebral body<sup>1</sup>. The results of our study showed that women and men differed significantly by all three parameters that describe the inner organization of the trabecular bone. Such differences became apparent at ages 51 to 70 years when these inner changes were summarized as: thinner trabeculae in women, but fewer trabeculae and greater separation in men. The turn has occurred at ages 71 to 82 years when women have developed greater separation and fewer trabeculae, compared to men. Although the other study proved that men and women differed between structural parameters, they varied from our data because their women had continuously smaller Tb.N and Tb.Th and greater Tb.Sp than men<sup>3</sup>. With regard to thickness of trabeculae, our results showed that an overall lost for women was 38%, while men have lost 26% of trabecular thickness. This finding corresponds to British study, but is at variance with study which showed no age related decline in trabecular thickness or even Tb.Th increase with age<sup>4,17</sup>. Men start with thicker trabeculae, thus at ages 41 to 50 years they showed for 17, 2% greater trabecular thickness than women. The results of our study revealed that the inner organization of trabecular bone is gender dependent. As men are stronger muscular-skeletal constitution, they have developed thicker trabeculae. Greater separation between trabeculae could be ascribed to a relatively bigger vertebral body in men<sup>9</sup>. Relatively higher number of trabeculae and smaller separation argues for more delicate bones and smaller vertebral bodies in women. Structural deterioration between ages 51 to 70 and 71 to 82 years is probably not an accidental, since these findings in women are followed by menopause onset (usually between ages 50 to 55 years). If so, we believe that the bone volume and the bone structure are dramatically reduced in postmenopausal women due to deficient hormonal actions within the bone remodeling units<sup>9,18</sup>.

### *Iliac crest bone*

The results of our study showed that the volume of trabecular bone significantly differed between iliac crest and lumbar vertebra. Men and women showed 12% and 6% greater values of the iliac crest bone volume, compared to vertebral bone volume, respectively.

Bone volume declined equally in crista iliaca between ages 51 to 70 years and 71 to 82 years, with approximately 19% of reduction in men or women. British study showed that decline in bone volume was first apparent in women after 40 years, while South African study showed increase in women bone volume up to the age 60<sup>3,19</sup>.

The latter changes went along with major reduction in thickness of trabeculae, which was more pronounced in women, though. When compared to women men had higher number of trabeculae and smaller separation at ages 41 to 50 years. The latter finding differs from the lumbar vertebra at the same those ages 41 to 50 years. Likewise vertebra, crista iliaca showed more pronounced structural changes in women (16% of Tb. N decrease and 19% of Tb.Sp increase) than in men (12% of Tb.N decrease and 14% of Tb.Sp increase). The results of our study showed that lumbar vertebra or crista iliaca underwent involution processes. Specifically, vertebrae reached almost the half of the initial bone mass (37% of reduction), while the volume of trabecular bone was reduced for 28% in cristae iliaca. Our study also showed that gender-related structural changes were more prominent in lumbar vertebra. Thus, women between 51 to 70 and 71 to 82 years showed 24% of Tb.N reduction and 30% of Tb.Sp increase in their vertebrae, while cristae iliaca of the same those women showed almost halved: 13% of the Tb.N reduction and the 16% of Tb. Sp increase, though. The similar was observed in men between ages 41 to 50 and 51 to 70 years. We believe that age-related differences between the lumbar vertebra and the crista iliaca are priory due to different functional demands that are put upon these organs. As a part of the axial skeleton lumbar spine is subjected to daily loadings and its inner architecture corresponds to such demands. Inner structure of the crista iliaca reflects its more quite position in the human body. Likewise Parfitt we believe that two different mechanisms of the bone loss are underlying huge differences between these skeletal organs<sup>18</sup>. Therefore the iliac crest biopsy does not always reflect the involution changes in the lumbar vertebra.

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## **USPOREDBA TRUPA SLABINSKOG KRALJEŠKA I GREBENA BOČNE KOSTI KOD MUŠKARACA I ŽENA RAZLIČITE DOBI – HISTOMORFOMETRIJSKA ANALIZA NA ISPITANICIMA PRIOBALNOG DIJELA SJEVERNE HRVATSKE**

### **S A Ž E T A K**

Ovo je regionalna studija kojom je ispitivano koštano tkivo zdrave populacije primorskog i dijela istarskog kraja Republike Hrvatske. Slične studije su pokazale kako su žene više sklone strukturnim promjenama u trabekularnoj kosti, dok muškarci kao konstitucijski jači spol, startaju s većom koštanom masom. Analizirani su koštani uzorci s dva različita anatomska mjesta, a razmatrano je da li se jednake involucijske promjene odvijaju u ovim koštanim elementima, s obzirom na dob i spol ispitanika. Korištene je metoda statičke histomorfometrije kako bi se kvantificirale promjene u trabekularnoj kosti. Rezultati su pokazali kako su involucijske promjene više izražene kod žena nego kod muškaraca iste dobi. Dobne razlike u strukturi trabekularne kosti više su izražene u slabinskom kralješku, nego u bočnoj kosti. I na kraju zaključujemo da se analiza bioptičkih uzoraka iz bočne kosti nije pokazala vjerodostojnom u praćenju promjene koje tijekom starenja zahvaćaju humanu slabinsku kralježnicu.