### ORIGINAL ARTICLE

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# **Regional variations in microstructural properties of vertebral trabeculae** with aging

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Abstract The aim of this study was to identify regional variations in the three-dimensional microstructure of vertebral cancellous bones, and their relative differences with respect to aging. Ninety trabecular specimens were obtained from six normal L4 vertebral bodies of six male cadaver donors in two age groups, three aged 62 years and three aged 69 years; (n = 45, each). In each vertebral body, five trabecular columns, each of  $8 \times 8 \times 25 \,\mathrm{mm^3}$ , were cut from the anterior, posterior, central, right, and left regions. These columns were scanned, using high-resolution microcomputed tomography (µCT), three times, to obtain superior, middle, and inferior layers. Fifteen regions were obtained for each vertebral body. For all 90 trabecular specimens the bone volume fraction (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th), as well as the three radii of the mean intercept length (MIL) ellipsoid (H1, H2, and H3) were determined. Regional variations in different transverse layers and vertical columns within and between the two age groups were then analyzed. The results showed significant differences in BV/TV, Tb.N, DA, and H2/H3 between the two age groups. The BV/TV and Tb.N were decreased, while the anisotropic parameters were increased significantly with age, increasing from age 62 to 69. Change in Tb.Th was not statistically significant, although the average was slightly smaller in the 69-year group. Each microstructural parameter followed its own pattern of regional variation within each group, suggesting both mechanical and age-related adaptation. This is the first study that has provided microstructural data of the vertebral body

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in a Chinese sample. These data may help us to gain more insight into the mechanism of the occurrence of lumbar osteoporosis and the related regional fracture risks, and may provide a reference for better enhancement of fracture repair.

**Key words** Vertebral body · Trabecular bone · Microstructural properties · Regional variations · Aging · MicroCT

# Introduction

Bone tissues may widely vary in architecture, from dense and compact – cortical bone – to highly porous structures – trabecular bone. They can grow, adapt, and repair themselves, enabling them to last a lifetime. Osteoporosis, a result of an imbalance in bone metabolism, is a condition of excessive bone loss, usually combined with deterioration of bone architecture. The inferior quality of osteoporotic bone leads to an increased risk of bone fractures. Therefore, osteoporosis has become an epidemic public health issue (Riggs and Melton [1]), especially with the aging of our society (Ettinger [2], Hamerman [3]).

Quantification of bone is necessary for better prediction of osteoporosis and related fractures. Bone mineral density (BMD) is often used to quantify bone quality in the clinical setting. However, studies show that BMD, only, cannot represent the mechanical properties of bone accurately (Goulet et al. [4]), while the three-dimensional (3D) microarchitecture provides better information for understanding bone quality (Goulet et al. [4], Homminga et al. [5], Kabel et al. [6], Keaveny et al. [7], Van Rietbergen et al. [8]). Reconstructions of high-resolution micro-computed tomography ( $\mu$ CT) images allow an assessment of the bone architecture in detail. The 3D microarchitectural information often includes the bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and trabecular number (Tb.N) (Whitehouse [9], Parfitt et al. [10], Hildebrand and Rüegsegger [11]). The orientation of trabecular structure is another important factor in

the anisotropic mechanical properties of bone structure. The quantification of orientation and architectural anisotropy can be evaluated from the mean intercept length (Harrigan and Mann [12]), or from 3D volume-based measures, such as volume orientation, star volume distribution, and star length distribution (Odgaard et al. [13]).

Studies have been conducted to determine changes of the 3D microstructure in central and peripheral trabecular bone under different mechanical environments caused by aging. Ding and Hvid [14] quantified the age-related changes in the structure model type and trabecular thickness of human tibial cancellous bone, and found that the structure model type changed towards more rod-like characteristics in the elderly and that trabecular thickness declined significantly after age 80 years. Later, Ding et al. [15] studied the age-related variations in the microstructure of human tibial cancellous bone. The microstructural parameters under investigation were BV/TV, the degree of anisotropy, mean marrow space volume, bone surface-to-volume ratio, mean trabecular volume, and bone surface density and connectivity. It was found that, except for connectivity, all the other parameters either increased or decreased significantly with aging.

It is understood that the bone structure is regiondependent. To obtain the microstructural parameters using micro-CT, the specimens used are often cubes or cylinders, with a volume of about 4–10 mm<sup>3</sup>. This can only provide data for small localized bone volume, which may differ from that in other regions, and these data from such a small specimen cannot be used to determine the overall bone strength or fracture risk. On the other hand, larger specimens can provide average structural and mechanical properties of a piece of bone, but may not reflect regional structural variations, where failure may occur (Ulrich et al. [16]). A thorough understanding of the regional variations in the microstructural properties of lumbar vertebral trabeculae is crucial for the diagnosis and treatment of the age-related degeneration of the lumbar vertebral body, and may help us to gain more insight into the mechanism of the occurrence of lumbar osteoporosis and the related fracture risks.

Simpson et al. [17] identified variations in vertebral cancellous bone architecture in the sagittal plane at increasing states of intervertebral disc disorganization of the T12-L5 lumbar spine, using 2D histoquantification. Each sagittal vertebral bone slice was divided into nine sectors, with superior/central/inferior regions representing horizontal layers of three sectors, to investigate the regional variations within the whole vertebral body. This was a 2D microstructural study and the trabecular specimens from the left and right sides of the vertebral body were not included. However, the 3D quantification of regional variations in the microstructural parameters of vertebral trabecular bone need to be investigated.

Accordingly, the aims of this study were: (1) to identify regional variations in the 3D microstructure of cancellous bone of the L4 vertebral body, with specific emphasis on bone BV/TV, Tb.N, architectural anisotropy, and Tb.Th; and (2) to investigate the effect of aging on these parameters.



Fig. 1. Schematic diagram of micro-CT scanning preparation procedure. The superior layer was 1 mm below the superior endplate, the inferior layer was 1 mm above the inferior endplate, and the middle layer was between the superior and inferior layers. Superior, middle, and inferior layers were obtained by grouping the trabecular cubes that formed the same layer, but came from different columns and different specimens in the same age group. In the same way, the column data sets were obtained in the anterior, central, right, left, and posterior columns. The three layers and five columns constituted 15 different regions altogether in a vertebral body. A, anterior; P, posterior; C, central; L, left; R, right

#### Methods

Six L4 vertebral bodies from six male cadaver donors, three aged 62 years (62-year group) and three aged 69 years (69-year group) were obtained for this study. They were examined, using X-ray, to rule out potential pathological changes affecting bone structural evaluation. For each vertebral body with the superior and inferior endplates, five square columns, each of  $8 \times 8 \text{ mm}^2$ , with a height of about 25 mm, were cut surgically from the anterior, posterior, right, left, and central regions of the trabecular bone without damaging the trabeculae. The cortical shell was not included in each column (Fig. 1).

micro-CT system (µCT40; Scanco Medical, А Bassersdorf, Switzerland) was used to evaluate the microstructure of the trabecular columns. Each column was placed, aligned to the inferosuperior (IS) axis, into a cylindrical sample holder and then filled with 75% ethanol. The column was secured with foam board to avoid shifting during scanning. The spatial resolution for specimen scanning was set to 20µm. Each column was scanned thrice to obtain three 5-mm-thick transverse layers, i.e., the superior layer, 1 mm below the superior endplate; the inferior layer, 1 mm above the inferior endplate; and the middle layer, between the superior and inferior layers (Fig. 1). During each scanning, the column was scanned continuously with increments of 20-µm thickness for 250 slices. The voxel size was  $20 \times 20$  $\times 20 \mu m^3$ . After scanning, the micro-CT images of 15 different regions of six vertebral bodies were obtained. Subsequently, the region of interest (ROI) was chosen as a  $5 \times 5$  $\times$  5 mm<sup>3</sup> cube in the center of each region, to exclude boundary artifacts. All the ROIs were segmented using the same threshold, corresponding to the Archimedes-based volume fractions, resulting in a total of 90 vertebral trabecular cubes.

The architectural parameters of trabeculae were evaluated by a direct method in the system for a 3D model; namely, BV/TV, Tb.N, Tb.Th, and the three radii of the mean intercept length (MIL) ellipsoid (H1, H2, and H3, with H1 > H2 > H3). The degree of architectural anisotropy (DA) was a relative measure of orientation within the trabecular cubes. The higher the DA, the more the bone is aligned in the principal direction (on-axis direction) relative to other directions. The DA was calculated as the ratio between the maximal and minimal radii of the MIL ellipsoid (Harrigan and Mann [12]), i.e., H1/H3. The relative architectural difference between the two off-axis directions (i.e., directions other than the primary direction of trabeculae) was found by determining H2/H3. An H2/H3 value close to 1.0 means a near transversely isotropic architecture. Each trabecular cube was represented by a set of these above parameters. A 3D reconstruction of each cube was also generated, using the built-in program of the micro-CT (Fig. 2).

Statistical analyses were done on all the parameters for all 90 trabecular cubes. In each age group, the microstructural parameters of the trabecular cubes from the same layers were pooled to form layer data sets. The superior, middle, and inferior layers were obtained by grouping the trabecular cubes that formed the same layer but came from different columns and different specimens in the same age group. In the same way, column data sets were obtained in the anterior, central, right, left, and posterior columns. The three layers and five columns constituted 15 different regions altogether in each vertebral body. Each individual trabecular cube was labeled according to its position for both its layer and column, such as superior-anterior specimen. One-way analysis of variance (ANOVA) was used to compare the microstructural parameters for the layers or columns in each group. If the F-test showed significance, Student-Newman-Kuels comparison of the means was done to find differences between the layers or columns. Student's t-test was performed for comparisons between the two groups regarding age. The significance level of P was chosen to be 0.05.

# Results

Table 1 shows the mean values of the microstructural parameters for each age group and the differences between groups. Each value (mean  $\pm$  SD) was obtained from 45 data sets grouped in 15 regions for three subjects. Statistical analyses showed significant differences in BV/TV, Tb.N, DA, and H2/H3 between the two age groups. The BV/TV and Tb.N were decreased, while the anisotropic parameters, DA and H2/H3, were increased significantly with age increasing from 62 to 69. The change in Tb.Th was not statistically significant, although the mean value was slightly lower in the 69-year group.

From 62-year group



From 69-year group

1.0 mm

Fig. 2. Typical trabecular microstructures of different columns in the two age groups, (a) central column, (b) anterior column, (c) posterior column, (d) right column, and (e) left column. The decrease in BV/TV in the central column with aging was relatively small compared with that in the peripheral columns. The decrease in Tb.N was more significant in the off-axis directions. BV/TV in the anterior column decreased significantly with aging, compared with the central column, and there was not as much preferential preservation of trabecular elements parallel to the primary load axis as there was in the central column. For the posterior column, the deterioration of rods in the off-axis direction with aging was remarkable, and, in particular, the trabecular elements parallel to the primary load axis were preserved. BV/TV decreased most in the right column with aging, which was mainly caused by the transformation from plates to rods and the thinning of trabeculae, but the decrease of Tb.N was not significant. The decrease of BV/TV in the left column was caused by the decrease of Tb.N in all directions

Regional variations in the three layers within and between the two age groups

Table 2 lists the mean values for the microstructural parameters in the three layers in the two age groups, and the differences between layers and between age groups. In this analysis, all data from different regions for the same layers of each group were grouped together. For BV/TV, no significant difference was found between any layers within either group, although the BV/TV value in the inferior layer was greater than the values for the other two layers in both groups. However, BV/TV values in all three layers from the 69-year group were significantly lower than those from the 62-year group.

There were significant differences in Tb.N between layers. The middle layer had the lowest Tb.N and the inferior layer had the highest, for both age groups. Tb.N in all three layers from the 69-year group was significantly lower than the Tb.N values in the same layers from the 62-year group.

For DA, there were no significant differences between layers within each group, although DA in the inferior layer was slightly lower than in the other two layers in both groups. However, DA in all three layers from the 69-year group was significantly higher than the DA values from the 62-year group.

Table 1. Microstructural parameters in the two age groups

Group	62-Year	69-Year	Difference (%)
BV/TV (%) Tb.N (1/mm) DA (-) H2/H3 Tb.Th (μm)	$\begin{array}{c} 7.49 \pm 1.79 \\ 1.17 \pm 0.19 \\ 1.43 \pm 0.17 \\ 1.16 \pm 0.10 \\ 113.23 \pm 12.74 \end{array}$	$5.43 \pm 1.47 \\ 1.03 \pm 0.17 \\ 1.60 \pm 0.26 \\ 1.21 \pm 0.17 \\ 109.69 \pm 19.00$	-27.50* -3.42* 11.89* 4.31* -3.13

\*P < 0.05

Values are means ± SD

In the 62-year group, H2/H3 in the inferior layer was significantly lower than that in the superior and middle layers, while in the 69-year group only the difference between the middle and inferior layers reached significance. The middle layer had the highest H2/H3 in both groups. H2/H3 in all three layers from the 69-year group was higher than these values in the same layers from the 62-year group, but only the difference between the inferior layers reached significance.

For Tb.Th, significant differences between the three layers were seen within the 62-year group, but no significant differences were seen between the three layers within the 69-year group. The middle layer had the highest Tb.Th in both groups. However, there was no difference between the superior and inferior layers in either group. Tb.Th values in all three layers from the 69-year group were slightly lower than those from the 62-year group.

Regional variations in the five columns within and between the two age groups

The microstructural parameters in the different columns of each age group, and their relative differences, are listed in Table 3. In this analysis, the three layers of each column were grouped together. For BV/TV, there were no significant differences between the five columns within each group. However, BV/TV values in all five columns from the 69-year group were significantly lower than those in the same columns from the 62-year group. The relative difference between the central columns of the two groups was smaller than the relative differences between the peripheral columns.

There was no significant difference in Tb.N between the five columns within each group. Tb.N values in all five columns from the 69-year group were lower than those in the same columns from the 62-year group, but only the differ-

Table 2. Microstructural parameters of the three layers in the two age groups

	Layer	62-Year group	69-Year group	Relative difference (%)
BV/TV (%)	Superior Middle Inferior	$7.37 \pm 1.70$ $7.28 \pm 1.46$ $7.81 \pm 2.22$	$5.13 \pm 1.11$ $5.18 \pm 1.47$ $5.99 \pm 1.72$	$-30.40^{4*}$ $-28.91^{4*}$ $-23.25^{4*}$
Tb.N (1/mm)	Superior Middle	$\begin{array}{c} 7.81 \pm 2.22 \\ 1.23 \pm 0.11^{2*} \\ 0.98 \pm 0.14^{3*} \end{array}$	$\begin{array}{c} 5.99 \pm 1.72 \\ 1.04 \pm 0.12^{1*} \\ 0.89 \pm 0.13^{3*} \end{array}$	$-25.25^{4}$ $-15.66^{4*}$ $-9.27^{4*}$
DA (-)	Inferior Superior Middle	$\begin{array}{c} 1.30 \pm 0.12 \\ 1.47 \pm 0.15 \\ 1.46 \pm 0.17 \end{array}$	$1.15 \pm 0.16$ $1.63 \pm 0.28$ $1.67 \pm 0.30$	$-11.78^{4*}$ $11.03^{4*}$ $14.05^{4*}$
H2/H3 (-)	Inferior Superior Middle	$\begin{array}{l} 1.36 \pm 0.17 \\ 1.16 \pm 0.08^{3*} \\ 1.22 \pm 0.12^{3*} \end{array}$	$\begin{array}{l} 1.51 \pm 0.15 \\ 1.19 \pm 0.16 \\ 1.29 \pm 0.21^{3} \ast \end{array}$	10.85 <sup>4</sup> * 2.13 5.89
Tb.Th (µm)	Inferior Superior Middle Inferior	$\begin{array}{r} 1.08 \pm 0.04 \\ 110.15 \pm 8.96^{2*} \\ 120.49 \pm 15.73^{3*} \\ 109.07 \pm 9.87 \end{array}$	$\begin{array}{c} 1.15 \pm 0.08 \\ 108.43 \pm 19.12 \\ 114.10 \pm 20.88 \\ 106.55 \pm 17.34 \end{array}$	$5.88^{4*}$ -1.56 -5.30 -2.31

Values are means  $\pm$  SD

<sup>1</sup>\* Significantly different from middle and inferior layer; P < 0.05; <sup>2</sup>\* significantly different from middle layer; P < 0.05; <sup>3</sup>\* significantly different from inferior layer; P < 0.05; <sup>4</sup>\* significant difference between 62-year group and 69-year group; P < 0.05

Table 3. Microstructural parameters of the five columns in the two groups

	Column	62-Year group	69-Year group	Relative difference (%)
BV/TV (%)	Central	$7.51 \pm 1.53$	$6.26 \pm 0.70$	-16.65***
	Anterior	$7.30 \pm 2.60$	$7.07 \pm 1.39$	-30.58***
	Posterior	$6.93 \pm 1.29$	$5.17 \pm 1.54$	-25.42***
	Right	$7.96 \pm 1.71$	$5.02 \pm 1.47$	-37.02***
	Left	$7.74 \pm 2.23$	$5.66 \pm 1.93$	-26.89***
Tb.N (1/mm)	Central	$1.20 \pm 0.13$	$1.10 \pm 0.16$	-9.52
	Anterior	$1.22 \pm 0.18$	$0.98 \pm 0.15$	-19.46***
	Posterior	$1.12 \pm 0.23$	$1.03 \pm 0.17$	-8.53
	Right	$1.15 \pm 0.22$	$1.05 \pm 0.19$	-8.63
	Left	$1.17 \pm 0.19$	$0.97 \pm 0.21$	-16.53***
DA (-)	Central	$1.33 \pm 0.16*$	$1.50 \pm 0.23$	12.49***
	Anterior	$1.50 \pm 0.14$	$1.55 \pm 0.09$	3.39
	Posterior	$1.32 \pm 0.14$	$1.69 \pm 0.33$	27.82***
	Right	$1.49 \pm 0.16$	$1.65 \pm 0.36$	10.81
	Left	$1.52 \pm 0.15$	$1.63 \pm 0.22$	7.42
H2/H3 (-)	Central	$1.11 \pm 0.07$	$1.08 \pm 0.06^{**}$	-2.98
	Anterior	$1.13 \pm 0.06$	$1.17 \pm 0.07$	3.58
	Posterior	$1.16 \pm 0.11$	$1.31 \pm 0.24$	13.22
	Right	$1.18 \pm 0.12$	$1.26 \pm 0.18$	7.49
	Left	$1.21 \pm 0.12$	$1.22 \pm 0.13$	1.30
Tb.Th (μm)	Central	$116.67 \pm 13.25$	$114.57 \pm 13.34$	-1.80
	Anterior	$106.10 \pm 5.74$	$106.77 \pm 11.39$	0.63
	Posterior	$115.48 \pm 19.36$	$117.13 \pm 21.92$	1.43
	Right	$115.26 \pm 10.00$	$106.26 \pm 29.38$	-7.81
	Left	$112.67 \pm 11.35$	$103.74 \pm 13.53$	-7.92

Values are means  $\pm$  SD

\*Significantly different from right column; P < 0.05; \*\*significantly different from posterior column; P < 0.05; \*\*\*significant difference between 62-year group and 69-year group; P < 0.05

ences between the anterior columns and between the left columns in the two groups reached the significance level. The relative difference between the two groups was greatest for the anterior columns.

For DA, a significant difference was found only between the central and right columns in the 62-year group, and no significant difference was found between any columns within the 69-year group. DA values in all five columns from the 69-year group were higher than those in the same columns from the 62-year group, but only the differences between central columns and between posterior columns reached the significance level.

For H2/H3, no significant difference was seen between any columns within the 62-year group. The differences in H2/H3 between the same columns from the two groups followed two different patterns: H2/H3 in the central column in the 69-year group was slightly lower than that in the 62-year group, and H2/H3 in the other four columns in the 69-year group was higher than the H2/H3 values in the 62-year group, in which the difference between the posterior columns from the two groups was greatest, but none of these differences reached the significance level.

For Tb.Th, there was no significant difference between any columns within each group. The difference between the two groups was smallest for the anterior columns, while the differences between the left columns and right columns from the two groups were relatively large, although none of these differences reached the significance level.

# Discussion

Variations in spinal BMD have been extensively reported in terms of exploring their association with regional mechanical strength and better enhancement of fracture fixation (Zheng et al. [18]). However, bone material quality is better explained by its structure (Amling et al. [19], Goulet et al. [4]), Homminga et al. [5], Kabel et al. [6], Keaveny et al. [7], Van Rietbergen et al. [8]). For example, Amling et al. [9] have reported that the overall bone volumes of osteoporotic specimens may be within the range of normal subjects, as measured by 2D and 3D histomorphometric analysis, but the selective loss of structural elements was the main variable resulting in reduced load-bearing capacity, suggesting the importance of bone structural evaluation in the research on osteoporosis.

In agreement with the investigation of iliac crest biopsy samples in ovariectomized goat, done with MicroCT and peripheral quantitative (pQ)CT by Siu et al. [20], it appears that BV/TV and Tb.N may be more sensitive to alterations in the trabecular microstructure than Tb.Th as a consequence of aging, as well as in osteoporosis. Similar findings about the changes of Tb.Th with aging were reported by Ding and Hvid [14], who showed that Tb.Th in human tibial cancellous bone was not reduced significantly until age 79 years.

Our study has been the first to use high-resolution microCT to obtain microstructural data for the vertebral body in a Chinese. The findings of our evaluation are in accordance with those in other ethnic groups, showing that BV/TV in the vertebral body decreases with aging (Mosekilde [21]). The uniqueness of the present study was, however, that it provided quantitative BV/TV data for the whole vertebra, each layer as well as each column, comprising 15 individual positions within a vertebral body, and the regional variations in different transverse layers and vertical columns, within and between two age groups, were analyzed. The difference in BV/TV between the 62-year and 69-year groups reached the significance level. But the changes of microstructural parameters with aging may follow their own specific patterns, suggesting both mechanical and age-related adaptation. Vertebral trabecular architecture was shown to be closely related to the state of the intervertebral discs and to age (Keller et al. [22], Simpson et al. [17], Adams et al. [23]). The intervertebral discs of our six L4 vertebral bodies did not show macroscopic signs of severe degeneration, and could be classified as normal or only slightly degenerated (Ferguson and Steffen [24], Keller et al. [22], Hansson and Roos [25]). The data we obtained about the differences in microstructural parameters between the two groups depended on mainly their different ages, as both groups had normal intervertebral discs.

Figure 2 shows the typical structures in different columns of the two age groups, and the differences in microstructures can be seen directly.

The decrease in BV/TV in the central column with aging was relatively small compared with that in the peripheral columns. This changing pattern of BV/TV with aging can be mainly attributed to adaptation to the loads exerted by the intervertebral discs. As the intervertebral discs were not severely degenerated, the loads were distributed such that the stresses through the nucleus region of the disc were higher than those through the annulus region, for both groups (Adams et al. [23]). For the central column, only the difference in DA between the two age groups reached the significance level. The decreases in Tb.N were more significant in the off-axis directions (i.e., directions other than the primary direction of the trabeculae) than in the on-axis direction (i.e., the primary direction of the trabeculae).

BV/TV in the anterior column decreased significantly with aging compared with the central column, and this was mainly caused by the decrease in Tb.N and the breaks of rods in all directions. In the 69-year group, in the anterior column, there was not as much preferential preservation of trabecular elements parallel to the primary load axis as in the central column, because DA increased little in this group, which may be attributed to the lower stress delivered by the intervertebral disc to the anterior column compared with that on the central column (Adams et al. [23]).

The decrease of BV/TV with aging in the posterior column may be caused mainly by the remarkable deterioration of the rods in the off-axis directions. Because the decrease of Tb.N in the posterior column was the least among the five columns, removal of the entire bone structural elements did not happen frequently. In the 69-year group, the relative difference between the secondary and tertiary trabecular direction was 31%, which means that the structure in the tertiary direction was severely deteriorated compared with trabeculae in the primary and second directions. Such directional deterioration also caused the significant increase of DA with aging, and trabecular elements parallel to the primary load axis, in particular, were preserved.

The degree of architectural anisotropy increased with aging in each layer, as well as in each column. Among the five columns, the increases in the central and posterior columns reached the significance level. The fast deterioration and disconnection of trabeculae in the off-axis directions may be a trigger for fractures. The functions of the off-axis trabeculae may be two-fold: bearing off-axis loads and preventing the on-axis trabeculae from buckling (Borah et al. [26]). So preserving the off-axis trabeculae may be crucial for preserving vertebral strength and reducing the risks of fracture as a consequence of aging, as well as in osteoporosis. Borah et al. [26] showed that risedronate treatment significantly preserved off-axis trabeculae in the vertebra of calcium-deficient ovariectomized minipigs. Further experimental study is needed to determine the effect of risedronate on the age-related changes in bone microstructure.

In the anterior and posterior columns, there was a slight increase in Tb.Th with aging. This phenomenon was in accordance with the idea of "removal-compensatory thickening" proposed by Ding and Hvid [14]. They found that this process, primarily, caused thinning of the horizontal struts in the load-bearing vertebral trabecular network and, secondarily, caused osteoclastic perforations, thus keeping the mean trabecular thickness unchanged or slightly increased.

Concerning the layer difference with aging, the decrease in BV/TV in the superior and inferior layers may be caused mainly by the deterioration of rods and the decrease in Tb.N. In the middle layer, the decrease in Tb.N in the offaxis directions was greater than that in the other two layers. The trabeculae in the middle layer were more aligned to the primary direction, and the decrease in Tb.Th was greater than that in the other two layers.

Simpson et al. [17] reported the distribution of microstructural parameters in the sagittal plane of T12-L5 lumbar vertebrae at increasing states of intervertebral disc disorganization. The distributions of Tb.Th and Tb.N they reported in the three layers were similar to those obtained in this study, while the distribution of BV/TV in their study in the anterior, central, and posterior columns differed from ours. Our specimens were all from male cadavers, and the mechanisms of bone loss in men and women may be different, i.e., trabecular plate loss is the dominant mechanism in women, whereas trabecular thinning occurs largely in men (Aaron et al. [27], Parfitt et al. [28]).

Because of the limited availability of donors, we only studied L4 vertebral bodies from two age groups, of 62 and 69 years. Lin et al. [29] found no correlation between the material properties of the lumbar disc and the segment level. For lumbar vertebral trabeculae, Hansson et al. [30] also suggested that the compressive properties of lumbar vertebral trabeculae were not dependent upon the segment level. So the data generated from this study may be taken as typical microstructural data for 62- and 69-year-old vertebral bodies with normal intervertebral disks, and may serve as a reference for sex and ethnic comparisons. Of course the fracture risks at different levels are not the same. Lower thoracic and upper lumbar (T10-L2) vertebrae are more susceptible to fracture than other vertebral bodies, which may be related to their anatomical sites. They are located at the turning point of the physiological spinal curvature. Everyday movements, such as stooping or lifting, will increase loading on the spine, and this loading has more impact on the lower thoracic and upper lumbar vertebrae. Fracture risks are much higher for osteoporotic vertebrae. Regional variations in the microstructural properties of osteoporotic vertebral trabeculae deserve further study if such specimens are available.

In summary, this study offered an insight into the distribution and variation of microstructural parameters within a vertebral body. The data obtained may help us to gain more insight into the mechanism of the occurrence of lumbar osteoporosis and the related regional fracture risks, and may provide a reference for better enhancement of fracture repair. The regional variations in mechanical responses with aging, as well as in osteoporosis, should be the subject of further studies to increase our understanding of osteoporosis-related fractures.

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