Region-Specific Sex-Dependent Pattern of Age-Related Changes of Proximal Femoral Cancellous Bone and Its Implications on Differential Bone Fragility

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Abstract Despite evident interest in age-related bone changes, data on regional differences within the proximal femur are scarce. To date, there has been no comprehensive study on site-specific age-related changes in the trabecular architecture of three biomechanically important femoral subregions (medial neck, lateral neck, and intertrochanteric region) for both genders. In this study we investigated age-related deterioration in the trabecular architecture of those three subregions of the femoral neck for both genders. The research sample included 52 proximal femora (26 males, 26 females; age range, 26–96 years) from Forensic Department at University of Belgrade. Bone sections from the three regions of interest were scanned by micro-CT at University of Hamburg. The study revealed that proximal femoral microarchitecture cannot be perceived as homogeneous and, more importantly, that the aging process is not uniform. Besides the initial intersite differences, microarchitecture changed differently with increasing age, maintaining significant differences between the regions. In addition, we observed a different aging pattern between genders: deterioration was most significant in the intertrochanteric region in women, while the lateral neck was most affected in men. This finding supports epidemiological data about the differential occurrence of cervical vs. trochanteric fractures in aging males and females. In conclusion, the aging process in the proximal femur cannot be regarded as a simple function of quantitative bone loss but, rather, as an alteration of specific architecture that may degrade bone strength.

Keywords Proximal femur · Trabecular bone · Regional differences · Microstructure · Aging

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Although the risk of age-related femoral fracture cannot simply be determined by the amount of bone tissue, the strength of its trabecular bone has traditionally been explained by bone quantity, represented as bone mineral density, or bone volume. Aging results in marked cancellous bone loss accompanied by a significant reduction in bone strength and its resistance to fracture [1–6]. However, bone loss is not uniform throughout the skeleton or in the subregions of the proximal femur, which causes differential deterioration in the bone internal architecture. In a radiographic study Singh et al. [7] suggested that the bone loss occurred first in the superior and then in the inferior portion of the femoral neck. Lundeen et al. [8] explored differences in bone volume fraction between the superior and the inferior aspect of the femoral neck in a sample of females of different ages and demonstrated that the inferior femoral neck volume fraction showed the lowest age-related decline. Although they reported that bone loss in subregions of proximal femur was selective [8] (contrary to Kawashima and Uhthoff [9]),

they suggested that the observed preferential bone loss was not characteristic for an entire population and should rather be regarded as specific to individuals with still unknown predisposing factors. Lai et al. [10] scanned the midneck of femora and reported that the inferior midneck had higher anisotropy than its superior part. Meta et al. [11] used vQCT analysis of female proximal femora and reported that the trabecular bone of the femoral neck (not subdivided) showed higher age-related loss than the midtrochanter region, with the suggestion that muscles inserting at the greater trochanter prevented higher trochanteric bone loss. In their research on regional variations in trabecular microstructure Nazarian et al. [12] reported densitometric, morphometric, and mechanical differences between different sites in the femoral neck and trochanter, without considering the relations of these differences to age. In a study of microstructural parameters that compared different regions within the proximal femur in a sample of 27 male femora, Cui et al. [13] reported significant differences between the superior and the inferior neck region. Recently, Lochmüller et al. [14] investigated microstructural properties of different skeletal sites and also evaluated age-related changes in two regions of the femur (neck and trochanter). They reported differences between the genders and between these two regions, which showed no correlation with age in males (except for the increase in BV/TV in the trochanter region), while in females several parameters in the femoral neck displayed significant age-related changes [14]. Li et al. [15] identified the femoral neck, trochanter, and femoral head as the regions of proximal femur most strongly associated with hip fracture and suggested that fracture type is related to the spatial distribution of bone tissue in these areas.

This study aimed to explore the variation of differential trabecular architecture in the proximal femur in relation to age, gender, and subregion: medial (inferior) femoral neck, lateral (superior) femoral neck, and intertrochanteric region. We discussed the hypothesis that there is a different pattern of age-related changes of trabecular bone parameters depending on the femoral region, as well as the hypothesis that observed microarchitectural differences between genders could explain the differential susceptibility to hip fractures of males and females in senescence. To investigate the potential influence of body size on trabecular microarchitectural parameters, we compared the age-related changes before and after adjusting for body height and weight.

Materials and Methods

Specimen Selection

Fifty-two right proximal femora were obtained from Caucasian human donor cadavers collected at the Forensic Department of the School of Medicine in Belgrade. Ethics approval for collection of the sample was granted by the Ethics Committee of the School of Medicine, University of Belgrade. The sample comprised 26 females (27–94 years of age; mean, 59.69 ± 18.58 years) and 26 age-matched males (aged 26–96 years; mean, 60.73 ± 19.72 years), whose main causes of death, as obtained from autopsy reports and clinical diagnosis, included cardiac arrest, stroke, motor vehicle accidents, and other sudden, traumatic injuries. Any individuals with macroscopic pathological changes or a history of musculoskeletal diseases were excluded from this study. In further analysis, the samples of both males and females were divided into four age categories: I (<39 years; 5 individuals of each gender), II (40–59 years; 7 individuals), III (60–79 years; 10 individuals), and IV (>80 years; 4 individuals). The specimens were then screened radiographically to exclude individuals with evidence of metastatic disease, previous fracture, or other diseases evident radiographically. Bone samples were stored in 70% ethyl alcohol for a minimum of 2 weeks and were then manually cleaned of adherent soft tissue. The regions of interest investigated in the study represent medial *s.* inferior and lateral *s.* superior regions of the neck, and the intertrochanteric region. The position of the regions was standardized by using two reference lines: (A) transversal, passing through the middle of the neck; and (B) passing through the tip of the lesser and greater trochanters (Fig. 1). Bone slices were obtained by using a water-cooled diamond saw (Exakt, Germany), providing three cancellous bone specimens per femur: the medial (inferior) neck section, the lateral (superior) neck section, and the intertrochanteric section.

Micro-CT Imaging

Each bone sample was attached to a sample holder with a consistent proximal–distal orientation and scanned by micro-computed tomography (Scanco Medical μCT 40; Switzerland) at the Center for Biomechanics & Skeletal Biology, University Medical Center Hamburg-Eppendorf, Germany. Images were obtained at 70 kV and 114 μA, at a resolution of 36 μm, isotropic, 1024×1024 pixels per slice. The integration time per each projection was 300 ms. The microarchitecture of the trabecular bone was automatically evaluated using the built-in program (Conebeam Conv./Backpr.) of the micro-CT with direct 3D morphometry. The following microarchitectural parameters were determined: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), structure model index (SMI), connectivity density (Conn.D), and degree of anisotropy (DA).
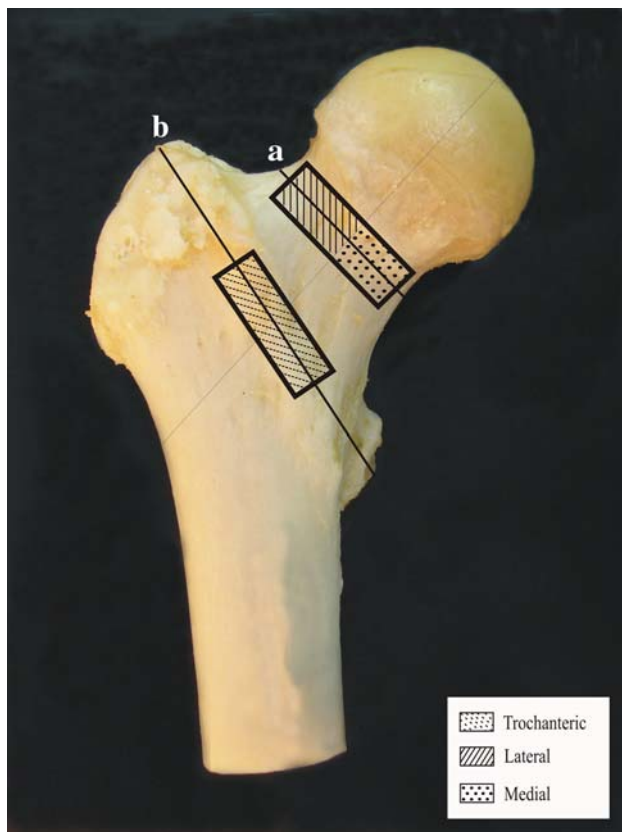
Fig. 1 (**a**) Transversal line, passing through the middle of the neck; (**b**) line passing through the tip of the lesser and greater trochanter; (1) lateral (superior) neck section; (2) medial (inferior) neck section; (3) intertrochanteric section

Statistical Analysis

The Kolmogorov–Smirnov test was used to verify normality of the distribution of the structural characteristics. The age dependence of the microstructural parameters in each investigated region of the proximal femur was determined by using linear regression analysis, separately in males and females. In further analysis, both the male and the female samples were divided into four age categories: I (<39 years), II (40–59 years), III (60–79 years), and IV (>80 years), and one-way analysis of variance (ANOVA) was used to examine the significance of the differences of microstructural parameters between each of these age groups. Since it is not known whether trabecular microarchitectural parameters are dependent on body size, we compared the obtained results before and after adjusting for body height and weight. To compare the micro-CT parameters between different sites of the proximal femur, we applied the analysis of variance for repeated measurements. The Bonferroni test was used in these multiple-comparison procedures at a significance level of 0.05/number of comparisons, i.e., 0.008. All analyses were conducted using SPSS statistical software (version 12.0) and the results were considered statistically significant at the 0.05 level.

Results

Age Dependence of Trabecular Microstructural Properties

With increasing age, bone volume fraction of males significantly decreased in the lateral neck (Fig. 2) and intertrochanteric (Table 1, Fig. 3) regions. One-way analysis of variance, used to compare the bone volume fractions in the four age categories, demonstrated significant differences only in the lateral neck region, between the first and the fourth age group (Table 2). In females, the decrease in BV/TV with increasing age reached a significant level in the intertrochanteric and medial neck regions (Table 3), although no significant differences were found between age groups in any of the regions (Table 4).
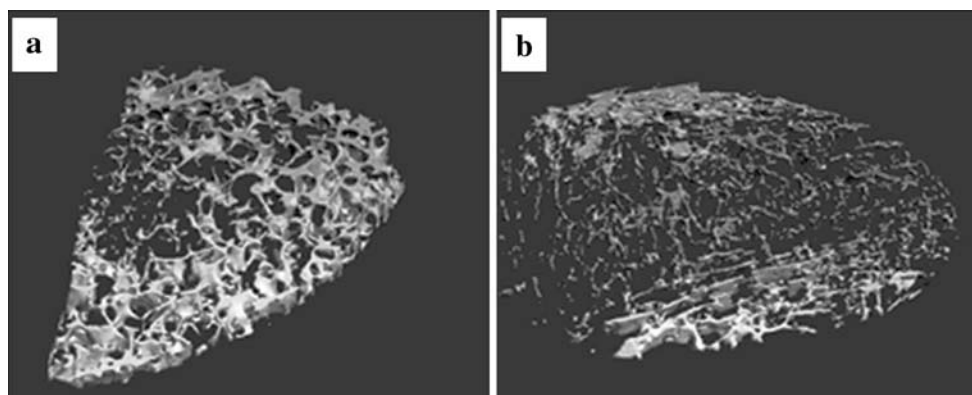

Fig. 2 Three-dimensional micro-CT reconstruction of the trabecular bone in the lateral neck region in a 46-year-old male (**a**) and a 94-year-old male (**b**)

Table 1 Correlation coefficient r values from linear regression analysis for regional variations in femoral microstructural parameters with age in males

Microstructural parameter	Lateral neck	Medial neck	Intertrochanteric
BV/TV	–0.540**	–0.203	–0.431*
Conn.D	–0.613**	–0.668**	–0.594**
SMI	0.440*	0.123	0.270
Tb.N	–0.288	–0.437*	0.111
Tb.Th	0.037	0.217	0.131
Tb.Sp	0.498*	0.505*	0.410*
DA	–0.033	0.352	0.022

Note: * $p < 0.05$; ** $p < 0.01$

In males, connectivity density decreased significantly in all regions with increasing age (Table 1, Fig. 4); moreover, significant differences between pairs of mean values in different age groups were found in all regions (Table 2). As in males, connectivity density in females decreased significantly in all regions with increasing age (Table 3, Fig. 5). However, significant differences between age groups were found only in the medial neck and intertrochanteric regions (Table 4).

In males, the SMI showed a significant increase with age in the lateral neck region (Table 1), but the comparisons between age groups showed no significant differences in mean values (Table 2). In females, a significant relationship between SMI and age was observed only in the medial neck region (Table 3), without significant differences between the mean values of each age category (Table 4).

Only in the medial neck region of males did the number of trabeculae significantly decrease with age, while the trend in the lateral neck was also to decrease, but significance was not attained (Table 1). Additionally, age categories showed no significant differences (Table 2). The decrease in the trabecular number in females with increasing age reached a significant level in the medial

neck and intertrochanteric regions (Table 3), and these same regions showed differences between different age categories (Table 4).

In both genders, significant changes in trabecular thickness with increased age were not found in any of the investigated regions (Tables 1 and 3), nor did particular age groups differ significantly (Tables 2 and 4).

Trabecular separation in males increased significantly with age in all three regions (Table 1, Fig. 6), but only the medial neck region displayed a significant difference between age groups (Table 2). Likewise, in females Tb.Sp increased with age in all regions (Table 3, Fig. 7), while age categories differed significantly in the medial and intertrochanteric regions (Table 4).

In males, the trend for DA was to increase with age in the medial neck, although not reaching statistical significance (Table 1) and without any significant differences between individual age groups (Table 2). In females, the DA of the medial neck increased significantly with advancing age (Table 3), and the same region also demonstrated a significant difference between age groups (Table 4).

After adjusting for body height and weight, all microstructural parameters showed the same pattern of age-related changes as before the adjustment.

Analysis of Intersite Differences

Analysis of variance for repeated measurements was used to determine the differences in micro-CT parameters between the various sites within the proximal femur. Multiple-comparison procedures revealed that the majority of parameters displayed significant intersite differences. In particular, in both sexes and for all parameters, the medial neck was significantly different ($p < 0.01$) from the lateral and intertrochanteric regions, while differences between the latter two regions reached significance ($p < 0.05$) for BV/TV of males and for Conn.D, Tb.N, and Tb.Sp in both sexes.

Fig. 3 Three-dimensional micro-CT reconstruction of the trabecular bone in the intertrochanteric region in a 27-year-old female (**a**) and an 83-year-old male (**b**)
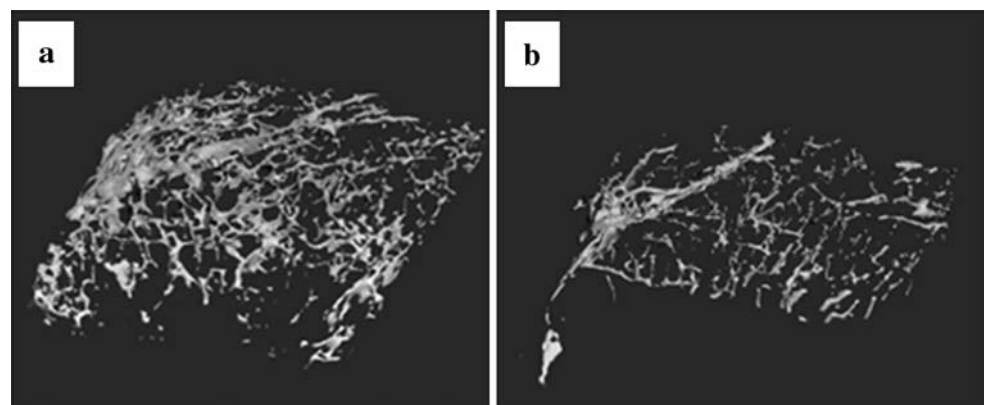
Table 2 Differences of trabecular microstructural parameters between age categories at three femoral regions for males

Microstructural parameter	Age group			
	1 (<39 yr)	2 (40–59 yr)	3 (60–79 yr)	4 (>80 yr)
Lateral neck				
BV/TV[a]	0.12 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.05 ± 0.02
Conn.D[a]	1.46 ± 0.69	0.97 ± 0.50	0.75 ± 0.49	0.29 ± 0.11
SMI	1.98 ± 0.36	2.45 ± 0.26	2.36 ± 0.60	2.76 ± 0.15
Tb.N	1.30 ± 0.05	1.28 ± 0.07	1.28 ± 0.13	1.22 ± 0.01
Tb.Th*	190 ± 20	180 ± 40	210 ± 30	180 ± 20
Tb.Sp*	800 ± 40	820 ± 50	840 ± 50	880 ± 20
DA	1.70 ± 0.15	1.65 ± 0.17	1.72 ± 0.54	1.63 ± 0.19
Medial neck				
BV/TV	0.18 ± 0.06	0.18 ± 0.03	0.16 ± 0.09	0.14 ± 0.08
Conn.D[a,b]	1.73 ± 0.60	1.47 ± 0.38	0.88 ± 0.46	0.68 ± 0.25
SMI	1.57 ± 0.44	1.99 ± 0.15	1.64 ± 0.99	1.92 ± 0.51
Tb.N	1.35 ± 0.09	1.35 ± 0.08	1.28 ± 0.09	1.26 ± 0.05
Tb.Th	220 ± 30	250 ± 30	260 ± 60	260 ± 90
Tb.Sp[d]	750 ± 70	740 ± 50	810 ± 60	830 ± 40
DA	1.98 ± 0.21	2.33 ± 0.14	2.29 ± 0.29	2.46 ± 0.61
Intertrochanteric				
BV/TV	0.11 ± 0.06	0.09 ± 0.03	0.06 ± 0.04	0.06 ± 0.04
Conn.D[d]	0.84 ± 0.30	0.78 ± 0.42	0.42 ± 0.26	0.32 ± 0.13
SMI	1.98 ± 0.59	2.37 ± 0.45	2.32 ± 0.57	2.38 ± 0.44
Tb.N	1.26 ± 0.06	1.24 ± 0.06	1.26 ± 0.12	1.23 ± 0.04
Tb.Th	200 ± 40	210 ± 40	190 ± 80	190 ± 60
Tb.Sp	850 ± 50	860 ± 50	880 ± 20	890 ± 10
DA	1.66 ± 0.30	1.63 ± 0.15	1.62 ± 0.18	1.66 ± 0.15

Note: * Trabecular thickness and trabecular separation are expressed in micrometers

[a] Significant difference between first group and fourth group; ANOVA, $p < 0.05$

[b] Significant difference between first group and third group; ANOVA, $p < 0.05$

[d] Significant differences between all groups but not between particular groups; ANOVA, $p < 0.05$

Table 3 Correlation coefficient r values from linear regression analysis for regional variations in femoral microstructural parameters with age in females

Microstructural parameter	Lateral neck	Medial neck	Intertrochanteric
BV/TV	–0.380	–0.552**	–0.439*
Conn.D	–0.419*	–0.656**	–0.531**
SMI	0.383	0.490*	–0.037
Tb.N	–0.332	–0.629**	–0.412*
Tb.Th	–0.036	0.126	0.099
Tb.Sp	0.420*	0.639**	0.462*
DA	–0.138	0.509**	0.252

Note: * $p < 0.05$; ** $p < 0.01$

Discussion

Microstructural Parameters Related to Bone Quantity vs. Microstructural Parameters Reflecting Internal Architecture

Changes in bone volume fraction represent the most illustrative parameter of bone loss in aging [10, 16–19], and its documented correlation with clinical measurements of bone density contributes to its importance. The overall decrease in BV/TV in the proximal femur with age has been recognized in previous studies [8, 13, 14, 20]. Our study revealed specific significant differences between the regions, i.e., although all three regions displayed a decrease in BV/TV with increasing age, this decrease was significantly correlated with age in the lateral neck and intertrochanteric regions of males, and in the medial neck and intertrochanteric regions of females. A recent study, focused exclusively on male femora [13], also suggested the smallest decline in BV/TV in the medial neck with age. Lochmüller et al. [14] reported a significant BV/TV decrease in the neck region of female individuals (though they did not subdivide into superior and inferior neck regions), while the observed increase in BV/TV in the trochanteric region of males was explained as a consequence of the predominance of very old individuals in their sample.

The distribution of BV/TV in relation to age group and gender demonstrated that in males, the lowest BV/TV was recorded in the lateral neck in the elderly group, while in females the lowest values were in the intertrochanteric

Table 4 Differences in trabecular microstructural parameters between age categories at three femoral regions for females

Microstructural parameter	Age group			
	1 (<39 yr)	2 (40–59 yr)	3 (60–79 yr)	4 (>80 yr)
Lateral neck				
BV/TV	0.11 ± 0.02	0.08 ± 0.04	0.09 ± 0.03	0.06 ± 0.07
Conn.D	1.37 ± 0.72	0.72 ± 0.74	0.65 ± 0.35	0.58 ± 0.97
SMI	2.42 ± 0.18	2.61 ± 0.43	2.49 ± 0.36	2.92 ± 0.62
Tb.N	1.31 ± 0.08	1.25 ± 0.05	1.24 ± 0.03	1.25 ± 0.10
Tb.Th*	200 ± 30	180 ± 30	210 ± 40	180 ± 60
Tb.Sp*	790 ± 60	840 ± 40	850 ± 20	850 ± 80
DA	1.73 ± 0.29	1.57 ± 0.15	1.74 ± 0.31	1.58 ± 0.22
Medial neck				
BV/TV	0.20 ± 0.08	0.20 ± 0.06	0.16 ± 0.04	0.10 ± 0.06
Conn.D[a,b]	2.20 ± 1.08	1.51 ± 0.51	0.89 ± 0.67	0.70 ± 0.50
SMI	1.86 ± 0.64	1.76 ± 0.51	2.18 ± 0.34	2.47 ± 0.55
Tb.N[a,b]	1.43 ± 0.13	1.34 ± 0.07	1.28 ± 0.07	1.25 ± 0.06
Tb.Th	250 ± 40	270 ± 40	290 ± 60	210 ± 40
Tb.Sp[d]	700 ± 90	740 ± 50	810 ± 60	820 ± 60
DA[b,c]	1.75 ± 0.24	2.33 ± 0.27	2.34 ± 0.27	2.21 ± 0.23
Intertrochanteric				
BV/TV	0.09 ± 0.05	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.02
Conn.D[d]	0.93 ± 0.60	0.55 ± 0.38	0.37 ± 0.18	0.19 ± 0.15
SMI	2.95 ± 1.53	2.59 ± 0.32	2.46 ± 0.33	3.26 ± 0.26
Tb.N[d]	1.27 ± 0.06	1.21 ± 0.03	1.21 ± 0.02	1.19 ± 0.03
Tb.Th	190 ± 40	200 ± 20	180 ± 20	210 ± 70
Tb.Sp[d]	830 ± 50	880 ± 30	890 ± 20	900 ± 10
DA	1.50 ± 0.12	1.58 ± 0.08	1.68 ± 0.17	1.58 ± 0.16

Note: * Trabecular thickness and trabecular separation are expressed as micrometers

[a] Significant difference between first group and fourth group; ANOVA, $p < 0.05$

[b] Significant difference between first group and third group; ANOVA, $p < 0.05$

[c] Significant difference between first group and second group; ANOVA, $p < 0.05$

[d] Significant differences between all groups but not between particular groups; ANOVA, $p < 0.05$
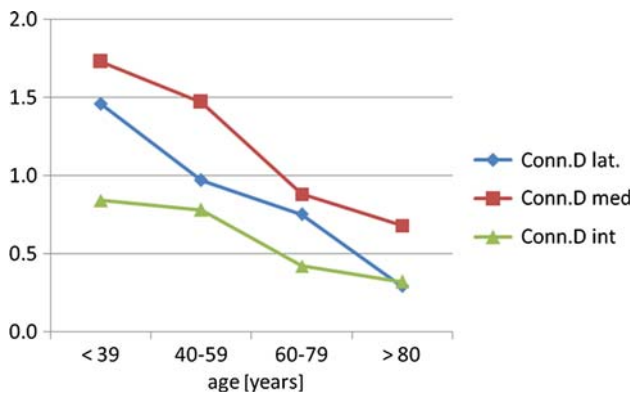

Fig. 4 Connectivity density in males of different ages

Fig. 5 Connectivity density in females of different ages

region in the elderly. The observed gender-dependent pattern could contribute to our understanding of the changes in the relative prevalence of cervical vs. trochanteric fractures with increasing age in males and females that were reported in epidemiological studies [21–26].

Two other microstructural parameters predominantly related to bone quantity, trabecular number and trabecular thickness, demonstrated changes with age in both genders
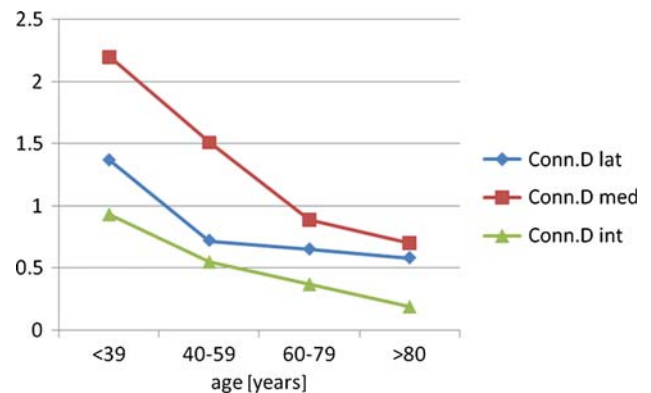
less frequently. In particular, trabecular thickness maintained significant initial regional differences with increasing age, with the thickest trabeculae being in the medial neck. Contrary to some other studies [13, 27], trabecular thickness did not show any significant trend with aging. Trabecular number also displayed significant differences between all three regions, with the highest number in the medial neck. The highest values of trabecular number in
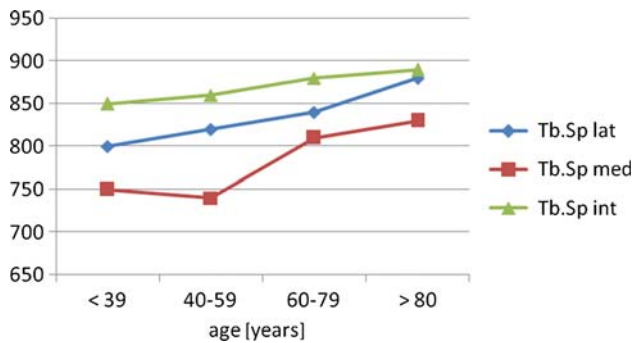
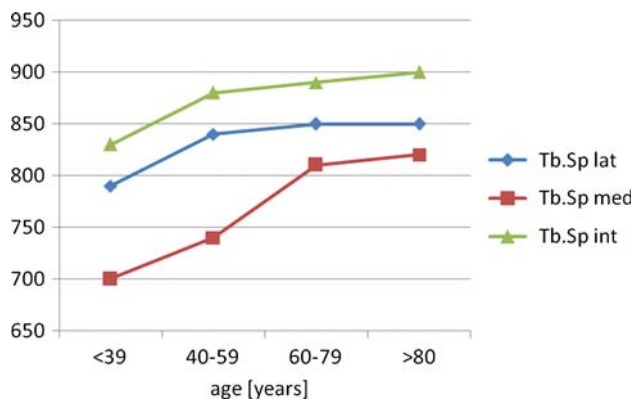**Fig. 6** Trabecular separation in males of different ages

Fig. 7 Trabecular separation in females of different ages

the medial neck region are compatible with those in other studies [10, 12, 13]. In our study, however, in both genders a significant age-related decrease in trabecular number was noted in the medial neck as well as in the intertrochanteric region of females.

The study also revealed that microstructural parameters predominantly related to spatial relationships and internal arrangement seem to play an even more important role in the aging of trabecular bone. Although some authors reported that BMD and BV/TV were better correlated with age than other more complex microstructural parameters [14, 28], in our study trabecular separation and connectivity density were actually the only parameters which changed significantly with age in all three regions and both sexes.

Connectivity density decreased dramatically with age in all regions and in both genders, being particularly low in the lateral neck region of elderly males and the intertrochanteric region of elderly females. This could reflect a loss of connectivity between aged trabeculae, which contributes to decreased bone strength and stiffness in isolated trabecular cores [29].

The mean values of trabecular separation in different femoral regions demonstrated significant intersite

differences and a significant increase with age in all three regions and both genders. The lowest separation value was in the medial neck of younger males and females, while the highest values were recorded in the intertrochanteric region of elderly females. This deterioration could be of significance to the biomechanical competence of trabecular bone in the intertrochanteric region of elderly women, which seems to be weak and therefore often subject to fractures. This is supported by the findings from a study regarding vertebral fragility [30], which reported that trabecular separation was greater in the fracture group than in the controls.

Significantly higher values of SMI in the lateral neck and intertrochanteric regions vs. the medial neck in this sample indicate that the medial neck tends to maintain its plate-like structure. It is considered that the SMI indicates the type of trabecular structure that significantly influences the trabecular bone mechanical properties [31, 32], and that a low-density rod-like structure (high SMI) develops in regions with low mechanical stresses, while a high-density plate-like structure (low SMI) predominates in the regions with high stress [32, 33]. In that context, the lowest SMI in the medial neck could reflect the highest stress in that region, with the major loads transferred through the primary compressive group of trabeculae in a normal femur [34, 35]. On the other hand, high values of SMI in the lateral neck and intertrochanteric regions (particularly in the elderly) could correspond to the weakened mechanical properties of these regions (due to the perforation of plates and the formation of rods) and their significance in age-related fractures.

In females, the degree of anisotropy displayed a significant increase with age in the medial neck, while in males it tended to increase most obviously in the medial neck, although not reaching statistical significance. This would seem to indicate that trabeculae are more aligned to one direction, corresponding to a principal stress direction in terms of the "bone functional adaptation" law [36–38]. Anisotropy probably increases most in the medial neck because this region is the most loaded with high compressive stresses during habitual loading [34, 35]. Ciarelli et al. [29] found that as the bone mass decreases, patients who experience hip fracture seem to preferentially preserve femoral neck trabecular elements parallel to the primary load axis, allowing those transverse to the load axis to be resorbed. The thesis of resorption of transverse trabeculae with aging could be supported by our findings, i.e., in the medial neck, the increase in DA was accompanied by a decreased Tb.N. However, such a mechanism could actually be disadvantageous, given that, as stated by Ciarelli et al. [29], although anisotropic specimens would be stronger and stiffer when loaded along the direction of dominant trabecular orientation, they would be weaker when loaded along an axis where the trabeculae are less oriented, which occurs during a fall.

Although it is known that the macroscopic bone geometry is dependent on body size (i.e., larger individuals need larger bones) [54, 55], it is not known whether trabecular microarchitectural parameters are size dependent. In our study, adjustment of microarchitectural parameters for body height and weight did not affect the observed age-related changes, indicating that the values and changes of these parameters with age could not be regarded as the consequence of differences in body size.

Site Differences in Aging: Biomechanical Context

The study revealed that femoral microstructure is not uniform in all regions, and that the aging process does not uniformly affect all sites within the proximal femur. Such regional heterogeneity can be explained in biomechanical terms by different loading conditions. Namely, it is widely accepted that bone adapts its structure to changes in its mechanical environment [11, 12, 36, 37, 39]. The traditional point of view, which considered that the principal compressive group of trabeculae (the medial or inferior neck) was loaded in compression and that the principal tensile group (the lateral or superior neck) was loaded in tension, was recently shown to be inaccurate. As Lovejoy [40] stated, our potent abductor muscle apparatus eliminates a significant portion of the tensile stress. It is documented that ligaments, muscles, and joint capsule forces also reduce tension in the superior neck [35, 38, 41]. Furthermore, a very low strain signal in the cortical bone of the superior neck was reported by Kalmey and Lovejoy [42]. All data indicate that the superior neck experiences low mechanical strain energies. Since mechanical load is essential for maintaining and reinforcing bone trabeculae [38], underloading of the superior neck leads to bone loss and atrophic changes in that region [15, 43, 44]. This could explain why the superior neck in our study showed lower baseline values and a high rate of structural deterioration. Contrary to low stress in the superior aspect of the femoral neck, a higher level of compressive stresses under habitual loading conditions in the inferior (medial) neck [15] could explain why this region showed the lowest decline in trabecular bone volume fraction with age in males in our study. Moreover, in both sexes, the medial neck preserved the highest number of trabeculae (although that number decreased significantly with age), as well the thickest trabeculae without a significant change with age, and the highest values of connectivity density. The significantly lower values of SMI found in the medial neck indicate a high level of preservation of its plate-like structure, which is believed to be more favorable for support in the high-stress region. Additionally, the increased anisotropy in this region suggests that the trabeculae are mostly aligned with the principal stress direction which maintains bone strength.

In addition, asymmetry between the superior and the inferior aspect of femoral neck may reflect trabecular eccentricity, which has been proposed as a bone adaptive response [45]. Apart from a biomechanical explanation, it is also possible that genetics contributes to the site specificity of age-related bone loss. Some of the recent research on mice concerning primarily disuse osteopenia suggests that there is a genetic influence on bone responsiveness to changes in its mechanical environment [46]. It is also suggested, in general, that genetic modulation of bone loss is strongly site specific [46].

Possible Relation of Microstructural Parameters to Fracture Occurrence

It was suggested that the superior neck is critical for hip fractures and represents the fracture-initiating site during a fall onto the greater trochanter as it undergoes the greatest stress during the fall [12, 13, 34]. The results of this study demonstrated that microstructural properties of the superior neck deteriorate significantly with aging (particularly in males; Fig. 2), which certainly leads to impairment of the mechanical integrity of this region even more during the impact of a fall.

Although in our study the medial neck maintained notably higher values of microstructural parameters than in the other two regions, possibly due to a high load-bearing stimulus, it was not "immune" to the aging process. Given that a larger proportion of the load on the femoral neck trabeculae is borne by the medial group, its deterioration with aging could weaken the bone and, as also suggested by Nazarian et al. [12], resorption of trabeculae of the inferior (medial) neck would predispose individuals to weight-bearing fractures of the hip. However, even though it also deteriorates with aging, we suppose that "good" medial neck baseline values could represent a "safety factor" for age-related losses, allowing the medial neck to keep its strength more than the other two regions.

The intertrochanteric region also deteriorated with aging, representing one of the "weak spots" of the proximal femur, particularly in females (Fig. 3). Namely, in the intertrochanteric region in females, aging was associated with a significant decrease in bone volume fraction (average values in the fourth age category were more than 50% lower than in the first group), a decrease in trabecular number, and a decrease in connectivity density (with nearly 80% lower values in the oldest age category in comparison with the youngest females), as well as higher values of SMI (although without a significant change with age). As recognized by Ciarelli et al. [29], lower bone volume fraction, lower trabecular number, and decreased connectivity are important determinants of hip fracture, since they were found in cases of hip fracture compared to the control

group. Such deterioration found in the female intertrochanteric region could be related to reported data about the progressive increase in proportion of trochanteric fractures in females with advanced age [21, 22, 25, 26, 47] and more prevalent trochanteric fractures in females [48, 49], while males tend to have more common cervical fractures with increasing age [21, 47].

Concerning tracing microstructural changes with age, a possible limitation of this study is that it is of a cross-sectional nature, i.e., it notes the structure of femora originating from people of different ages at death and thus does not have the opportunity to follow the changes in the same person as he or she ages. In addition, this study deals with unfractured femora, and although it suggests wider conclusions, they cannot be fully extrapolated to the cases with fractures.

In conclusion, the study demonstrates that overall age-related changes in observed regions of the proximal femur display a different pattern between genders, i.e., females generally tended to deteriorate most in the intertrochanteric region while males did so in the lateral neck.

Another significant contribution of this study is that it documented that the medial (inferior) neck region maintained more *favorable* values of microstructural properties with age than the other two sites did, which could be a consequence of its continuous reinforcement in response to a weight-bearing stimulus. In addition, underloaded regions such as the lateral (superior) neck displayed extensive deterioration, particularly in elderly males, while the aging process in female femora seemed to markedly affect the intertrochanteric region, which could be related to an increase in trochanteric fractures in elderly women [21, 22, 25, 26, 50].

It should be borne in mind that bone fragility is not conditioned only by features of trabecular bone, but also by cortical properties. However, it is not yet fully understood how much each of the two bone compartments contributes to bone strength [5, 51–53]. Nevertheless, our study demonstrated that neither initial values of trabecular microstructural parameters nor the aging process in the proximal femur are uniform, and this could be an important determinant contributing to age-related differential bone fragility. Regarding age-related changes in micro-CT parameters in the proximal femur, this study also revealed that, apart from the bone volume fraction (as a more quantitative indicator of bone loss), the parameters that more closely reflect bone internal organization and arrangement (particularly connectivity density and trabecular separation) change significantly with age. This observation suggests the view that the proximal femur aging process cannot be regarded exclusively in terms of quantitative bone loss, but should also be regarded as a change of architecture, arrangement, and trabecular connections.

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