ORIGINAL RESEARCH

Cortical Bone Histomorphometry in Male Femoral Neck: The Investigation of Age-Association and Regional Differences

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Abstract Low bone volume and changes in bone quality or microarchitecture may predispose individuals to fragility fractures. As the dominant component of the human skeleton, cortical bone plays a key role in protecting bones from fracture. However, histological investigations of the underlying structural changes, which might predispose to fracture, have been largely limited to the cancellous bone. The aim of this study was to investigate the age-association and regional differences of histomorphometric properties in the femoral neck cortical bone. Undecalcified histological sections of the femoral neck ($n = 20$, aged 18–82 years, males) were cut (15 μm) and stained using modified Masson-Goldner stain. Complete femoral neck images were scanned, and cortical bone boundaries were defined using our previously established method. Cortical bone histomorphometry was performed with low (×50) and high magnification (×100). Most parameters related to cortical width (Mean Ct.Wi, Inferior Ct.Wi, Superior Ct.Wi) were negatively associated with age both before and after adjustment for height. The inferior cortex was the thickest ($P < 0.001$) and the superior cortex was the thinnest ($P < 0.008$) of all cortical regions. Both osteonal size and pores area were negatively associated with age. Osteonal area and number were higher in the antero-inferior area ($P < 0.002$) and infero-posterior area ($P = 0.002$) compared to the postero-superior area. The Haversian canal area was higher in the infero-posterior area compared to the postero-superior area ($P = 0.002$). Moreover, porosity was higher in the antero-superior area ($P < 0.002$), supero-anterior area ($P < 0.002$) and supero-posterior area ($P < 0.002$) compared to the infero-anterior area. Eroded endocortical perimeter (E.Pm/Ec.Pm) correlated positively with superior cortical width. This study describes the changes in cortical bone during ageing in healthy males. Further studies are needed to investigate whether these changes explain the increased susceptibility to femoral neck fractures.

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Introduction

Bone loss occurs throughout the skeleton with ageing, and consequently, the risk of fractures increases [1, 2]. In particular, hip fractures not only result in a high level of morbidity and mortality, but also cause a heavy burden to the healthcare system [3, 4]. Skeletal failure occurs when the forces applied to the bone exceed its load-bearing capacity, and the age-dependent bone loss is usually accelerated under the influence of osteoporosis [5, 6].

Histomorphometric analysis has commonly been used to study the changes in bone remodelling and bone

structure. It is essential to understand the age-dependent variation in histological properties at the femoral neck since cortical thinning and loss of cancellous bone are both causes of age-related femoral neck fractures [7, 8]. The pioneering work by Jonathan Reeve and colleagues has authenticated the necessity of investigating changes in cortical bone histology in healthy subjects at the femoral neck [9, 10].

Although the cancellous bone is critical for stress transfer and femoral neck stiffness, the cortical bone has been regarded as the major load-bearing compartment by many previous studies [8, 11, 12]. However, it is also likely that the contribution of cortical and cancellous bone can vary greatly with age and even between individuals owing to different bone volume fractions [13–16]. In general, various features of the cortical bone in human femoral neck were assessed in previous studies to either detect structural changes explaining increased hip fracture risk [17, 18] or to investigate the mechanical properties (strength, elasticity, geometry, etc.) by applying visual or acoustic microscopy [19–22].

Histological analysis of the structural bone characteristics attributable to age-related bone loss has mostly been conducted at the iliac crest [23–25], and previous histomorphometric studies in healthy subjects have focused almost exclusively on cancellous bone [26, 27]. The understanding of cortical bone across adulthood and the studies of the underlying morphometric changes that might predispose a person to a fragility fracture of the femoral neck are limited. Furthermore, females with potential postmenopausal osteoporosis have been the subjects of most histomorphometric studies [18, 28–30]. No systematic investigation has been conducted for the histomorphometric properties of the cortical bone at the femoral neck of male subjects with respect to ageing and between femoral neck regions. This study aims to resolve this issue. We hypothesise that the structural changes in cortical bone occur with age. Since the ability of cortical bone to resist fracture deteriorates with ageing, the age-associated changes of the cortical structure may explain the susceptibility for fractures. In the present work, we studied the complete cross section of the male femoral neck using quantitative cortical bone histomorphometry. The variation of histological values with age as well as between anatomical regions was then investigated.

Materials and Methods

Subjects

Femoral neck samples were obtained from 20 cadavers (mean age, 47 ± 18.2 years, range 18–82 years, males) at

Table 1 Basic anthropometric data of the cadavers

Age (years)	Age group	Height (cm)	Weight (kg)
17	1	178	74
22	1	186	106
26	1	175	60
29	1	184	105
32	1	171	69
34	1	187	102
36	1	177	74
39	1	185	84
43	1	171	98
46	1	185	85
48	1	178	85
50	1	185	108
52	2	180	136
53	2	176	73
58	2	175	73
58	2	169	96
62	2	170	68
74	2	166	64
77	2	177	72
82	2	165	53
47 ± 18.2		177 ± 6.9	84 ± 20.4

Kuopio University Hospital, Kuopio, Finland. The subjects were divided into two sub-groups based on age (≤50 years, $N = 12$ and >50 years, $N = 8$). There was no previous history of medical conditions or use of drugs known to affect bone metabolism (Table 1).

Sample Preparation

Transverse cross sections were cut from the femoral neck with a band saw. A minor cut was marked on the inferior bone edge to enable the correct orientation of the sample during the measurements. Samples were dehydrated in ethanol for at least 48 h before embedding in polymethylmethacrylate according to standard protocols [31]. After embedding, 15-μm-thick sections were cut using a microtome (Reichert-Jung; Cambridge Instruments, Heidelberg, Germany) and stained with modified Masson-Goldner trichrome stain. The entire cross section of the femoral neck was scanned using an auto-image scanner (Particle Analyzer; Carl Zeiss, Jena, Germany) to acquire a complete histological image (×50) for histomorphometric analysis. An image programme (GNU Image Manipulation Program, version 2.0) was utilised for delineation of different histological boundaries in the images [32].
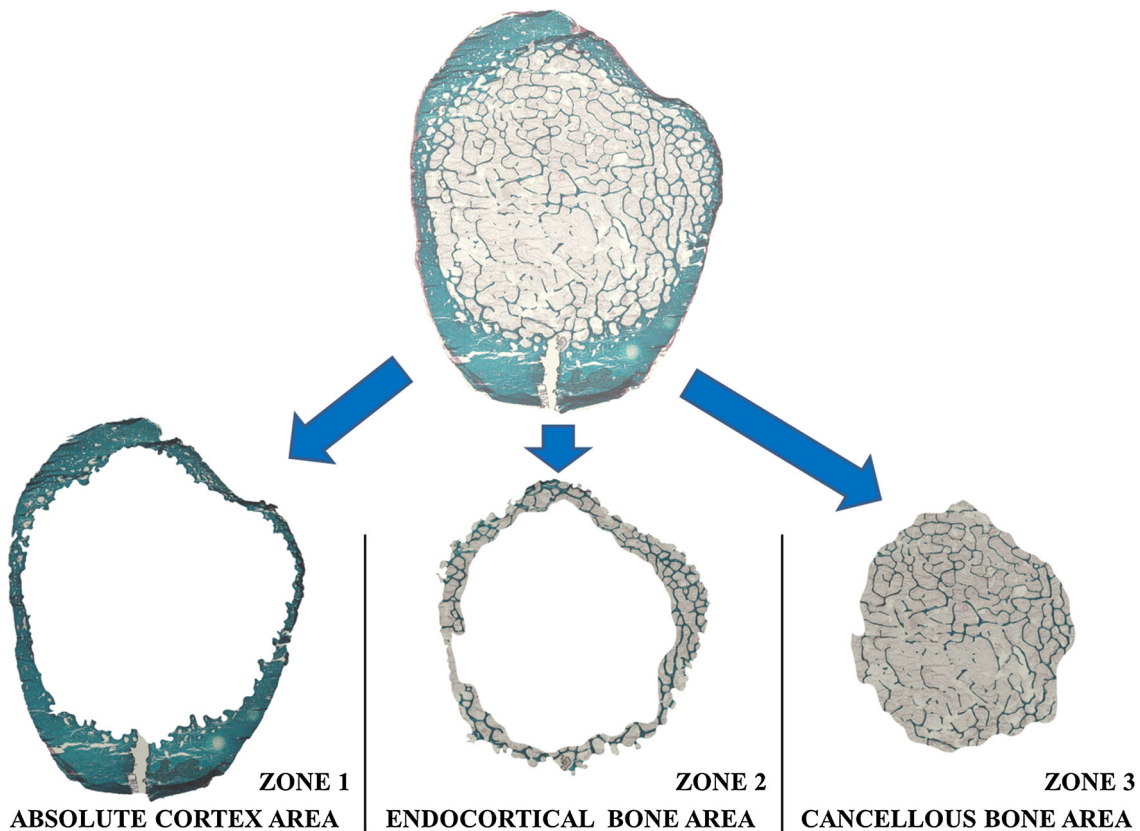
Bone Histomorphometry

Each histological image (×50) was separated into three independent zones (the absolute cortex area, the endocortical bone area and the cancellous bone area), based on our previous criteria of histological boundary definition (Fig. 1) [32].

The histomorphometric analyses of cortical bone were conducted using Bioquant Osteo II (Bioquant Image Analysis, Nashville, TN, USA). The nomenclature, abbreviations and parameters follow the recommendations by the American Society for Bone and Mineral Research (AS-BMR) [33]. First, the samples were analysed with bright light microscopy using a magnification of ×50 in images covering the complete femoral neck (low-magnification measurements). Then, the absolute cortex area was evaluated under bright light and polarisation microscopy using a magnification of ×100 (high-magnification measurements). For this analysis, the absolute cortex area was divided into eight anatomical locations. In each location, at least five fields (1.51 mm^2 for each) of view were randomly imaged and measured (Fig. 2).
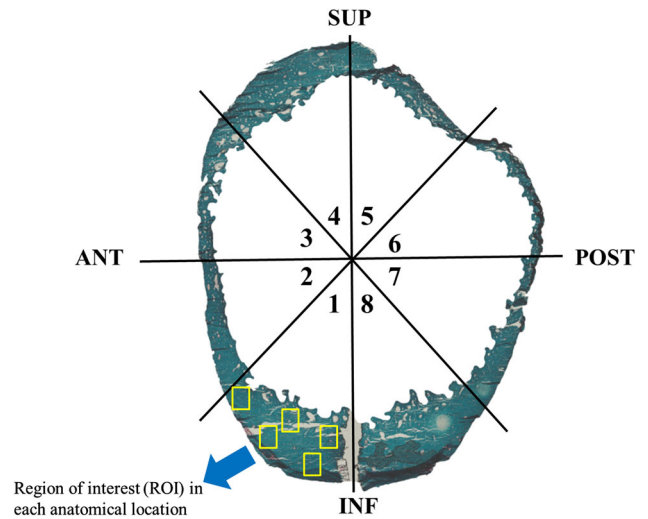
Fig. 2 Cross section of the femoral neck at the cut face showing eight anatomical locations: area *1* infero-anterior; area *2* antero-inferior; area *3* antero-superior; area *4* supero-anterior; area *5* supero-posterior; area *6* postero-superior; area *7* postero-inferior; area *8* infero-posterior. In each location, at least five fields (1.51 mm^2 for each) of view were randomly imaged and measured

Fig. 1 Schematic cross section of the femoral neck showing absolute cortical, endocortical and cancellous zones. A minor cut was marked as an orientational indicator in the inferior bone edge. The histomorphometric analyses of cortical bone were conducted within the absolute cortical zone

Based on low-magnification ($\times 50$) imaging, the area and width parameters were determined. Cortical bone area [Ct.B.Ar. (mm^2)] was measured as the tissue area between the absolute cortex boundary and the periosteum. Endo-cortical area [Ec.Ar. (mm^2)] was measured as the tissue area between the absolute cortex boundary and the endo-cortical boundary. Cancellous bone area [Cn.B.Ar. (mm^2)] was measured as the inner tissue area outside the endo-cortical boundary. The parameters of cortical bone area per tissue area [Ct.B.Ar./T.Ar. (%)], endocortical area per tissue area [Ec.Ar./T.Ar. (%)] and cancellous bone area per tissue area [Cn.B.Ar./T.Ar. (%)] were also determined. Mean cortical width [mean Ct.Wi. (mm)] was calculated as the average value of all (average 315 measurements/sample) perpendicular widths between the absolute cortex boundary and the periosteum. Similarly, the regional cortical widths at four different anatomic regions were measured, i.e. anterior cortical width [anterior Ct.Wi. (mm)], posterior cortical width [posterior Ct.Wi. (mm)], inferior cortical width [inferior Ct.Wi. (mm)] and superior cortical width [superior Ct.Wi. (mm)]. Besides, the periosteal perimeter [Ps.Pm. (mm)] and endocortical perimeter [Ec.Pm. (mm)] were further measured.

Based on high-magnification ($\times 100$) imaging, the bone tissue area was quantified per octant. The percentage of osteonal area per sampled cortical area was calculated: [On.Ar./Sa.Ct.Ar. (%)]. The mean osteonal perimeter [On.Pm. (μm)] was determined as an average of all (average 30 osteons/octant) measured osteons. Similarly, the Haversian canal perimeter [H.Pm. (μm)] was determined as the average value of all (average 30 Haversian canals/octant) measured Haversian canals. The Haversian canal area [H.Ar. (mm^2)] was determined as the sum of all (average 30 Haversian canals/octant) measured Haversian canals. The osteon number [N.On (#)], the minimum osteonal diameter [min.On.Dm. (μm)] and the maximum osteonal diameter [max.On.Dm. (μm)] were also determined. The mean wall width [W.Wi. (μm)] of osteons was calculated using all (average 12 measurements/osteon) perpendicular distances between the Haversian canal boundary and the outer edge of the circular osteon. Moreover, pore area [Po.Ar. (mm^2)] was determined as the sum of all (average 15 pores/octant) measured pores, and the porosity was calculated as follows: [Po.Ar/Sa.Ct.Ar. (%)]. The pore number [N.Po (#)] was also determined. Osteoid surfaces were recognised as unmineralised seams along the absolute cortex boundary, and the bone turnover of the endocortical surface was identified by calculating osteoid perimeter per endocortical perimeter [O.Pm./Ec.Pm. (%)]. Likewise, erosion surfaces were recognised (with or without osteoclasts) along the absolute cortex boundary by calculating erosion perimeter per endocortical perimeter [E.Pm./Ec.Pm. (%)].

Statistical Analysis

Shapiro–Wilkinson tests were used to determine whether the data were normally distributed. An independent T test was used to compare low-magnification parameters between the age groups. In addition, analysis of covariance (general linear model) was used to adjust for height. The Mann–Whitney U test was used to compare different age groups of high-magnification parameters since the data of two groups were not normally distributed. In other cases of the non-normally distributed variables, the Kruskal–Wallis test followed by multiple comparison analysis using the Mann–Whitney U test was performed to evaluate the differences of cortical width in four anatomical regions of the femoral neck and examine the areal differences of high-magnification parameters between octants. The Bonferroni correction was applied in both cases.

Spearman's correlation was conducted to investigate the possible relationships between endocortical parameters (O.Pm/Ec.Pm, E.Pm/Ec.Pm) and low-magnification parameters. All analyses were performed using SPSS Statistical software (version 20.0; SPSS, Chicago, IL, USA). P values <0.05 were considered to be statistically significant.

Results

Low-Magnification Cortical Bone Parameters

Most cortical width-related parameters (mean cortical width, inferior cortical width and superior cortical width) were negatively associated with age ($P < 0.05$) (Table 2). Similarly, the values of cortical bone area per tissue area were found to be negatively associated with age ($P < 0.01$) (Fig. 3). In contrast, the parameters of cancellous bone area and periosteal perimeter exhibited an age-related increase ($P < 0.05$). No age-association was found on either the cortical width of the anterior (anterior cortical width, $P = 0.907$) and posterior region (posterior cortical width, $P = 0.056$), or other bone area-related parameters (cortical bone area, $P = 0.096$; endocortical area, $P = 0.306$; endocortical area/tissue area, $P = 0.81$; cancellous bone area/tissue area, $P = 0.093$) (Table 2).

After adjustment for height, the statistically significant age-association in cortical width-related parameters (mean cortical width, inferior cortical width and superior cortical width) still remained. Similarly, the values of cortical bone area ($P = 0.018$) and cortical bone area per tissue area ($P = 0.003$) were found to be significantly associated with age.

There was a significant variation in cortical width in four different anatomical regions ($P < 0.001$). The inferior

Table 2 Low-magnification cortical bone parameters ($\times 50$) in two age groups (≤ 50 years, >50 years)

Parameters	Age $\leq$ 50 (N = 12)		Age > 50 (N = 8)			
	Mean	SD	Mean	SD	Diff.	P value
Ct.B.Ar (mm^2)[a]	133	27.7	112	25	21.4	0.096
Ec.Ar (mm^2)[b]	125	26.2	140	39.3	−15.4	0.306
Cn.B.Ar (mm^2)[c]	316	83.1	399	79.5	−82.5	**0.040**
Ct.B.Ar/T.Ar (%)[d]	23.3	4.2	17.3	3.8	6	**0.005**
Ec.Ar/T.Ar (%)[e]	22.4	6.7	21.7	5.9	0.7	0.810
Cn.B.Ar/T.Ar (%)[f]	54.3	8.4	61	8.1	−6.7	0.093
Ps.Pm (mm)[g]	104	7.1	112	7.2	−8.1	**0.023**
Mean Ct.Wi (mm)[h]	1.6	0.4	1.2	0.3	0.4	**0.016**
Ant. Ct.Wi (mm)[i]	1.2	0.3	1.3	0.4	−0.02	0.907
Post. Ct.Wi (mm)[j]	1.3	0.4	1.0	0.2	0.3	0.056
Inf. Ct.Wi (mm)[k]	3.1	1.3	1.9	0.6	1.1	**0.019**
Sup. Ct.Wi (mm)[l]	1	0.6	0.6	0.2	0.5	**0.032**
Ec.Pm (mm)[m]	123	10.7	136	11.2	−13.7	**0.013**

Mean values with standard deviations [SD] are shown

Bold values indicate statistical significance ($P < 0.05$)

[a] Cortical bone area (mm^2)

[b] Endocortical area (mm^2)

[c] Cancellous bone area (mm^2)

[d] Cortical bone area/tissue area (%)

[e] Endocortical area/tissue area (%)

[f] Cancellous bone area/tissue area (%)

[g] Periosteal perimeter (mm)

[h] Mean cortical width (mm)

[i] Anterior cortical width (mm)

[j] Posterior cortical width (mm)

[k] Inferior cortical width (mm)

[l] Superior cortical width (mm)

[m] Endocortical perimeter (mm)

P values were obtained with independent samples T test

cortex was the thickest ($P < 0.001$) and the superior cortex was the thinnest of all cortical regions ($P < 0.008$). There was no significant difference between the width of the anterior and posterior cortex ($P = 0.29$) (Fig. 4).

High-Magnification Cortical Bone Parameters

The osteonal structure-related parameters (osteonal area and Haversian canal area) were negatively associated with age ($P < 0.05$) (Fig. 5). There was also a negative association between pore-related parameters (pore area and porosity) and age ($P < 0.01$). There were no significant differences in other high-magnification cortical bone parameters (Table 3).

When comparing the eight anatomical areas, there was significant variation in the parameters of osteonal area ($P < 0.01$), osteonal number ($P < 0.01$), Haversian canal area ($P < 0.05$) and porosity ($P < 0.01$). For other high-magnification parameters, no significant areal differences were found (Table 4).

Specifically, osteonal area was higher in the antero-inferior area compared to the postero-superior area ($P < 0.001$), as well as higher in the infero-posterior area compared to the postero-superior area ($P < 0.002$). Similarly, osteonal number was also higher in the antero-inferior area ($P < 0.002$) and the infero-posterior area ($P = 0.002$) compared to the postero-superior area. Haversian canal area was higher in the infero-posterior area compared to the postero-superior area ($P = 0.002$). Moreover, porosity was higher in the antero-superior area ($P < 0.002$), supero-anterior area ($P < 0.002$) and supero-posterior area ($P < 0.002$) compared to the infero-anterior area (Fig. 6).

Correlations

The erosion perimeter per endocortical perimeter was positively associated with superior cortical width ($P < 0.01$). The osteoid perimeter per endocortical perimeter did not correlate with any of the low-magnification cortical parameters.

Discussion

This study provides histological analyses of cortical bone structure at the femoral neck from previously healthy male cadavers. We demonstrated that the changes in cortical bone structure are both age- and anatomical region-associated.

In the study subjects, the height correlated with age ($P = 0.01$) while the weight did not ($P = 0.213$). Therefore, low-magnification parameters were adjusted for height when these parameters were compared between the age groups.

The cortical thickness was negatively associated with age both before and after height adjustment. It has been suggested that the age-related bone loss at the hip occurs through a series of structural changes in bone. These changes tend to preserve bone structure in mechanically loaded regions (e.g. the postero-superior and antero-inferior regions of the femoral neck cortex [34]), whereas less mechanically loaded regions (e.g. the superior cortex of the femoral neck) are attenuated with age [35]. According to this theory, we found no significant age-associated loss of cortical thickness in the anterior and posterior cortex either before or after adjustment for height. However, recent
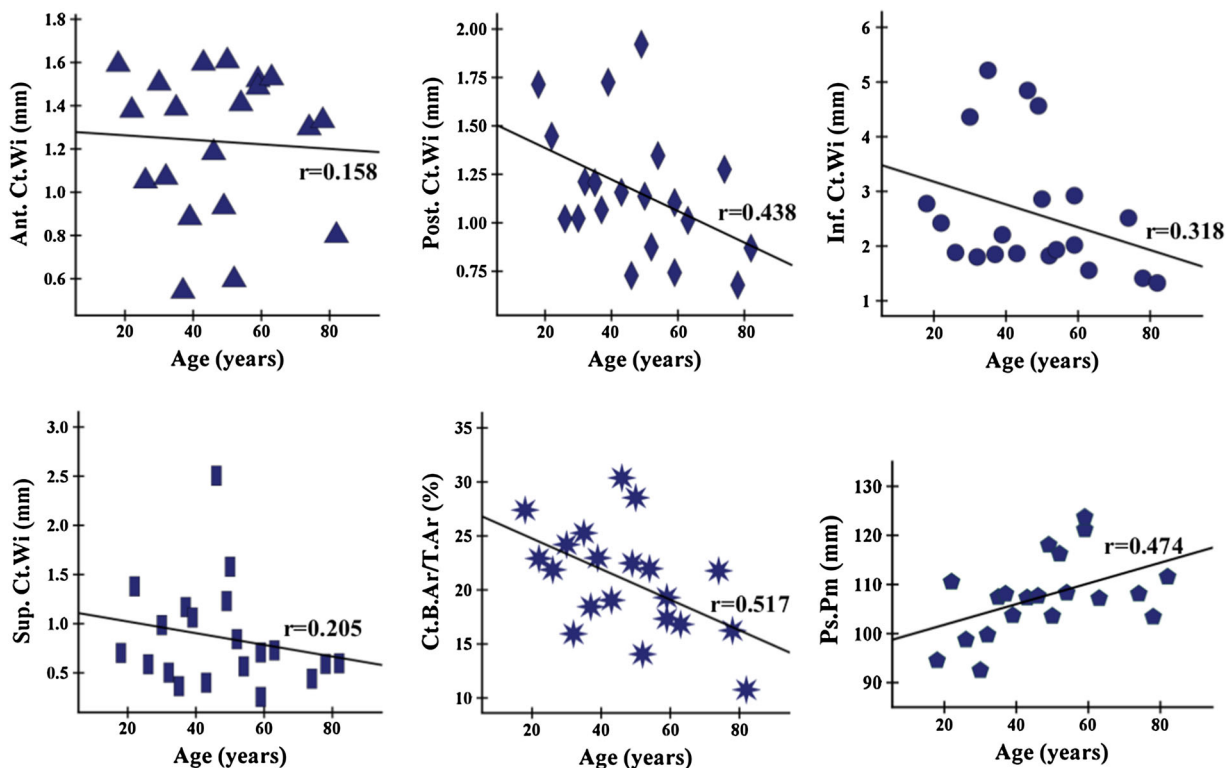
Fig. 3 Scatter plots for associations between the values of low-magnification parameters with age. The inferior, superior cortical width and values of cortical bone area were negatively associated with age ($P < 0.05$). Although the anterior and posterior cortical width also exhibited decrescent tendency, the variations were not statistically significant. In contrast, the periosteal perimeter was found to increase with ageing ($P < 0.05$). *Ant. Ct.Wi* anterior cortical width, *Post. Ct.Wi* posterior cortical width, *Inf. Ct.Wi* inferior cortical width, *Sup. Ct.Wi* superior cortical width, *Ct.B.Ar/T.Ar* cortical bone area/tissue area, *Ps.Pm* periosteal perimeter
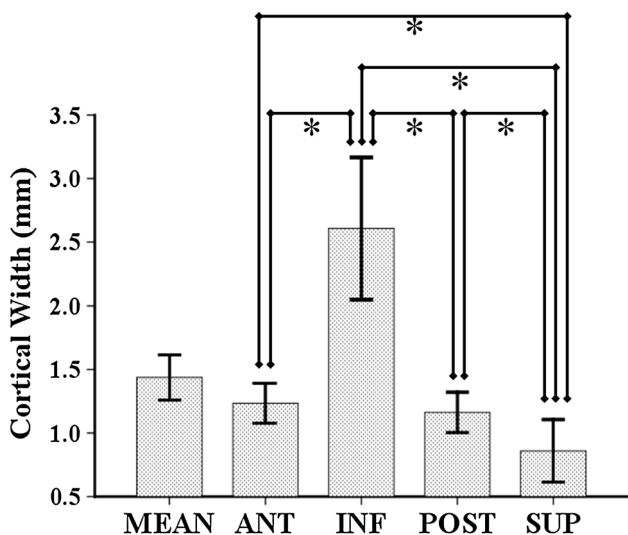
Fig. 4 Mean cortical width (SD) in four regions of the femoral neck ($n = 20$). The inferior cortex was the thickest ($P < 0.001$) and the superior cortex was the thinnest of all cortical regions ($P < 0.008$). There was no significant difference between the width of the anterior and posterior cortex ($P = 0.29$). *$*P \leq 0.008$

studies indicate that hip fractures involve a complicated set of structural deficits. Besides rarely loaded structures, regions that are relatively protected from age-related bone loss (e.g. the inferior cortex of the femoral neck [36]) may also be somehow involved [37]. Therefore, thinning of the inferior cortex of the femoral neck with age in our study was expected. The negative age-associated decreases in mean cortical width observed in this study are consistent with the data reported by others, both at the femoral neck and at other skeletal sites [23, 25].

Based on our histological criteria [32], we divided the cross section of the femoral neck into three zones to separate cortical bone, endocortical bone area and cancellous bone [38]. The absolute cortical bone area was negatively associated with age within the whole tissue area of the cross section, whereas cancellous bone showed positive age-association. This finding is consistent with previous histological studies [39–41]. Interestingly, no age-associated change was detected in the endocortical bone area, either before or after adjustment for height. Although cortical thinning with age might be associated with the
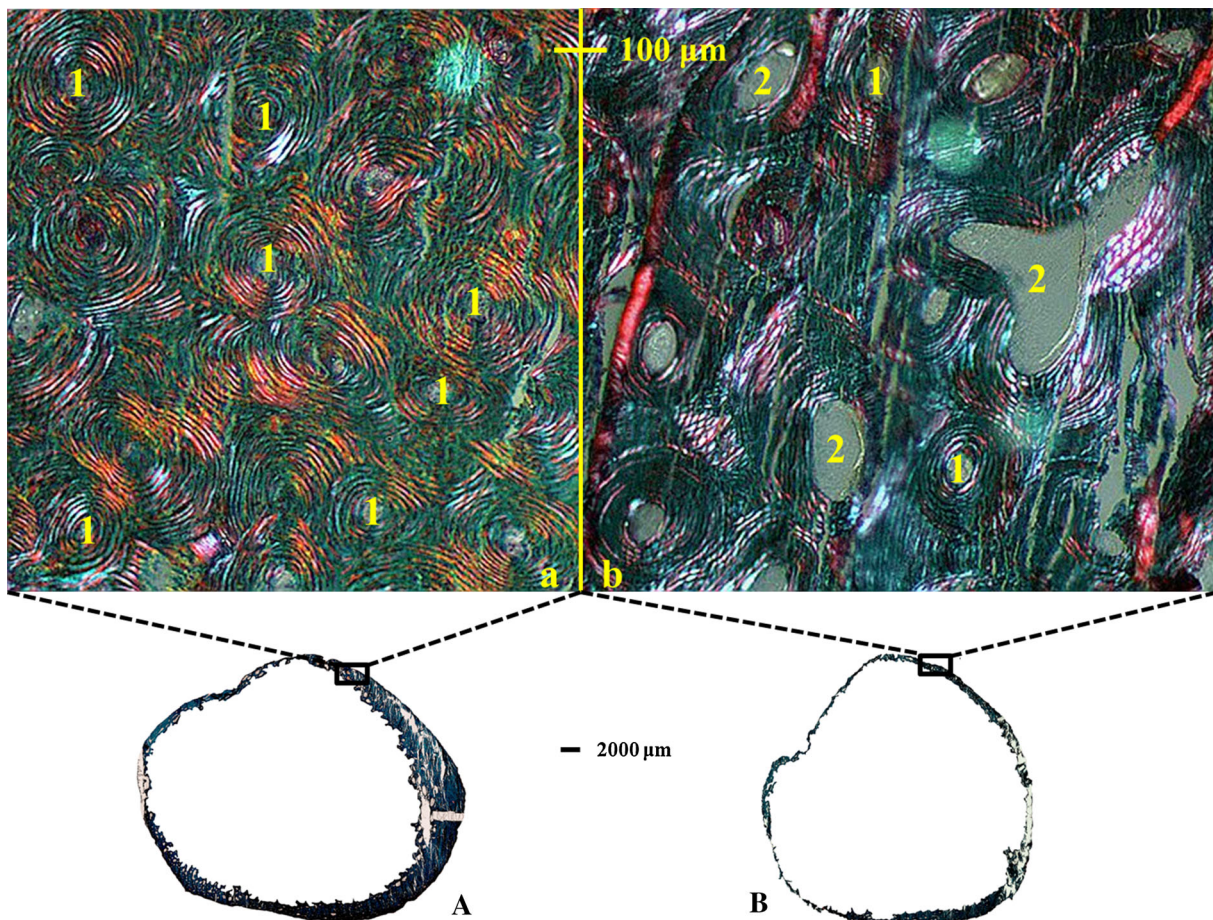
Fig. 5 The age-associated loss in osteonal system is exemplified by two cross-sectional samples A (29 years) and B (82 years) (×5). The magnified images show the osteonal structures under polarised light (×100). Osteons undergoing normal remodelling (1) were more frequent in young sample (**a**), than in older sample (**b**). Osteons with excessive deep resorption and adjacent merging (2) were found more in older sample (**b**) than in younger sample (**a**)

conversion of compact to cancellous bone arising from disordered Haversian remodelling [42, 43], losses of cortical and cancellous bones on different time axes have been found [34, 44]. Furthermore, both endocortical perimeter and periosteal perimeters were positively associated with age, although the significances of these trends were lost after adjustment for height. It has been suggested that the generalised bone loss (e.g. endocortical resorption) could be compensated for by the periosteal apposition to some extent. Thus, the hip strength and its resistance to fracture could be maintained [45, 46]. Some studies have suggested that the periosteum of the femoral neck is disrupted by the attachment of the capsule of the hip joint, and that periosteal bone apposition does not occur at the femoral neck [47, 48]. Other studies, however, have shown that bone formation occurs on the outer periosteal surface of the femoral neck [49, 50]. We still cannot draw the conclusion that the femoral neck expands with ageing.

In this cohort, the inferior cortex was the thickest and the superior cortex was the thinnest. Similar findings have been shown before [51, 52]. The superior cortex has been shown to get thinner three times faster with age than the inferior cortex [53]. The structural changes of cortical bone at the femoral neck are both age-associated and anatomical region-dependent.

Femoral osteonal morphology depends on the remodelling balance achieved within individual remodelling units. Structural changes of osteonal and Haversian canal area may thus result from different resorption depths as well as osteonal combination [54, 55]. We observed negative age-associated changes in osteonal and Haversian canal area. In contrast, the osteonal diameter showed no significant age-association. Although one study found similar changes at the iliac crest [24], some other studies have suggested that osteonal dimensions could never be systematically affected by age and sex [56]. The lack of agreement in osteonal

Table 3 High-magnification cortical bone parameters (×100) in two age groups (≤50 years, >50 years)

Parameters	Age ≤ 50 (N = 12)		Age > 50 (N = 8)			
	Median	SE	Median	SE	Diff.	P value
On.Ar/Sa.Ct.Ar (%)[a]	9.43	0.72	6.21	0.67	3.22	**0.014**
On.Pm (μm)[b]	574	11.36	549	22.02	24.5	0.939
Min.On.Dm (μm)[c]	134	2.73	133	3.52	1.3	0.165
Max.On.Dm (μm)[d]	189	3.89	188	4.28	1	0.280
W.Wi (μm)[e]	62	1.44	60	1.87	2.3	0.190
N.On (#)[f]	286	14.06	202	17.91	84.5	0.105
H.Ar (mm²)[g]	0.41	0.03	0.3	0.02	0.11	**0.014**
H.Pm (μm)[h]	149	3.57	144	4.36	5.1	0.758
Po.Ar (mm²)[i]	2.51	0.22	1.92	0.16	0.59	**0.007**
N.Po (#)[j]	116	6.61	126	10.65	−10	0.699
Porosity (%)[k]	4.17	0.37	3.18	0.26	0.99	**0.007**
O.Pm/Ec.Pm (%)[l]	1.73	0.37	1.92	0.17	−0.19	0.589
E.Pm/Ec.Pm (%)[m]	0.99	0.28	0.84	0.18	0.15	0.643

Median values with standard error [SE] are shown

Bold values indicate statistical significance ($P < 0.05$)

[a] Osteonal area/cortical area (%)

[b] Mean osteonal perimeter (μm)

[c] Min osteonal diameter (μm)

[d] Max osteonal diameter (μm)

[e] Mean wall width (μm)

[f] Osteon number (#)

[g] Haversian canal area (mm²)

[h] Mean Haversian canal perimeter (μm)

[i] Pore area (mm²)

[j] Pore number (#)

[k] Porosity (%)

[l] Osteoid perimeter/endocortical perimeter (%)

[m] Erosion perimeter/endocortical perimeter (%)

P values were obtained with Mann–Whitney U test

changes with ageing may reflect the differences in methodology, sample size as well as various definitions of parameters and cortical boundaries. In our study, the osteons with excessively enlarged Haversian canals (>82 μm) due to the adjacent merging were excluded, because previous studies have suggested that the enlargement and clustering of the osteonal system may predict the formation of cortical pores [57, 58]. As a result, the age-associated loss in osteonal system suggested by the present study was reasonably explained. Moreover, we also showed that the changes of osteons undergoing normal remodelling (without excessive deep resorption [59]) were negatively associated with age. In addition, the excessive resorption is more likely to lead to osteon merging and the formation of "giant" canals [60], the prevalence of which has a negative

influence on the ability of the cortical shell to withstand stresses associated with a fall [61]. Therefore, the age-associated loss in normal osteons may potentially increase the age-associated fragility at the femoral neck.

The pore area and porosity were negatively associated with age. This seems contradictory to the previous studies but the definitions of the cortical bone boundaries have varied [62–64]. Based on our histological criteria, the "absolute cortex" consisted of pure cortical bone tissue only, and was defined mainly based on the size of composite canals and their distance to an additional "guide" boundary [32]. As a result, many endocortical canals were excluded, while some previous studies might have included them. On the other hand, age-associated cortical expansion may lead to super-osteon formation, which is supported by the fact

Table 4 The mean values of high-magnification cortical bone parameters ($\times 100$) in femoral neck cross-section octants ($N = 20$)

Parameters	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Area 7	Area 8	H (df)	P
On.Ar/Sa.Ct.Ar (%)	9.55	9.79	8	7.56	6.99	6.52	7.96	10.01	23.65 (7)	**0.001**
On.Pm (µm)	552.2	564.9	548.8	542.4	551.5	539.8	554.3	558.1	2.95 (7)	0.890
Min.On.Dm (µm)	135.8	136.8	133.8	133.7	131.3	130.1	134.6	139.2	10.43 (7)	0.166
Max.On.Dm (µm)	188	194.7	190.2	188.4	195.4	189.9	194.2	192.2	2.64 (7)	0.916
W.Wi (µm)	61.5	63	60.3	60.9	60.1	60.3	62.5	63.1	6.45 (7)	0.488
N.On (#)	34.4	34.9	29.2	28	26.9	25.4	28.3	35.6	24.14 (7)	**0.001**
H.Ar (mm^2)	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.05	17.73 (7)	**0.013**
H.Pm(µm)	144.5	145.1	152.3	141.8	156.5	145.5	145.7	145.8	7.08 (7)	0.421
Po.Ar (mm^2)	0.2	0.31	0.41	0.33	0.38	0.38	0.26	0.24	7.54 (7)	0.375
N.Po (#)	12.9	16.7	17.5	14.8	15.8	15.3	13.6	14.1	6.09 (7)	0.529
Porosity (%)	2.71	4.17	5.44	4.4	5.1	5.08	3.43	3.13	25.6 (7)	**0.001**
O.Pm/Ec.Pm (%)	0.17	0.2	0.2	0.43	0.28	0.33	0.29	0.23	10.94 (7)	0.141
E.Pm/Ec.Pm (%)	0.12	0.2	0.16	0.11	0.17	0.1	0.1	0.08	1.93 (7)	0.963

Bold values indicate statistical significance ($P < 0.05$)

Femoral neck cross-section areas: *Area 1* infero-anterior, *Area 2* antero-inferior, *Area 3* antero-superior, *Area 4* supero-anterior, *Area 5* supero-posterior, *Area 6* postero-superior, *Area 7* postero-inferior, *Area 8* infero-posterior (Fig. 3)

*P* values were obtained with Kruskal–Wallis test

that the periosteal region has the highest density of clustered osteons [10]. Therefore, the formation of cortical pores predicted by the enlargement and clustering of the osteonal system [57, 58] may primarily occur in the periosteal cortex. These periosteal pores would predominantly compose the cortical porosity according to our criteria. This was confirmed by our finding that number of pores tends to be positively associated with age, although the differences were not significant. However, this positive age-associated change is restricted to the periosteal cortex, which is not enough to be responsible for the increase in porosity, because the pore area in the endocortical bone has been found to be much greater than in the periosteal cortex [63].

Therefore, the exclusion of partially trabecularised cortical bone with larger pores would lead to the decreased porosity in present study. Based on our criteria, the trabecularised cortical bone with larger pores had been predominantly included into the endocortical bone area. The transitional zone between cortical and cancellous bone has been very rarely defined as the endocortical bone area by previous studies, which may be explained by the fact that increased trabecularisation of the cortical bone complicates the identification of endocortical features [65, 66]. Thus, in our study, the positive age-associated changes in porosity and pore area might be the key characteristics of the endocortical bone. This was also consistent with our other finding that the endocortical bone area tends to be positively associated with age, although the changes were not significant.

The regional comparisons of the femoral neck cross section revealed some differences. Osteonal area and osteonal number were higher in the antero-inferior area and infero-posterior area compared to the postero-superior area. The Haversian canal area was higher in the infero-posterior area compared to the postero-superior area. As described previously, the inferior cortex was much thicker than the superior cortex. Therefore, the regional differences of osteonal structure could be explained with cortical thinning. Since the thinner cortex offers less volume in which remodelling may occur, the probability of including relatively intact osteons is correspondingly reduced as well. As a result, the values of osteonal structure-related parameters were higher in the inferior-adjacent regions. On the other hand, our result also suggested that the porosity was higher in the antero-superior area, supero-anterior area and supero-posterior area compared to the infero-anterior area. This is consistent with previous findings that, at the femoral neck, the superior cortex is more porous than the inferior cortex [64, 67]. In accordance with the results of Yeni and Norman [68], we did not find any differences in porosity between the inferior and posterior cortex at the femoral neck. However, our results showed a porosity increase in the anterior cortex projecting to the superior region at the normal femoral neck, which may help to explain the specific porosity difference between the anterior and inferior cortex in the findings by Malo et al. [31]. Moreover, the cortical loss was found primarily along the infero-anterior to supero-posterior axis in cases with femoral neck fracture [61, 69]. Our results suggested a significant cortical porosity difference along exactly the same axis; therefore, this may reveal the underlying morphometric changes that might predispose to fragility fracture of the femoral neck.

The positive association between the superior cortical width and erosion surface could be explained by the loss of
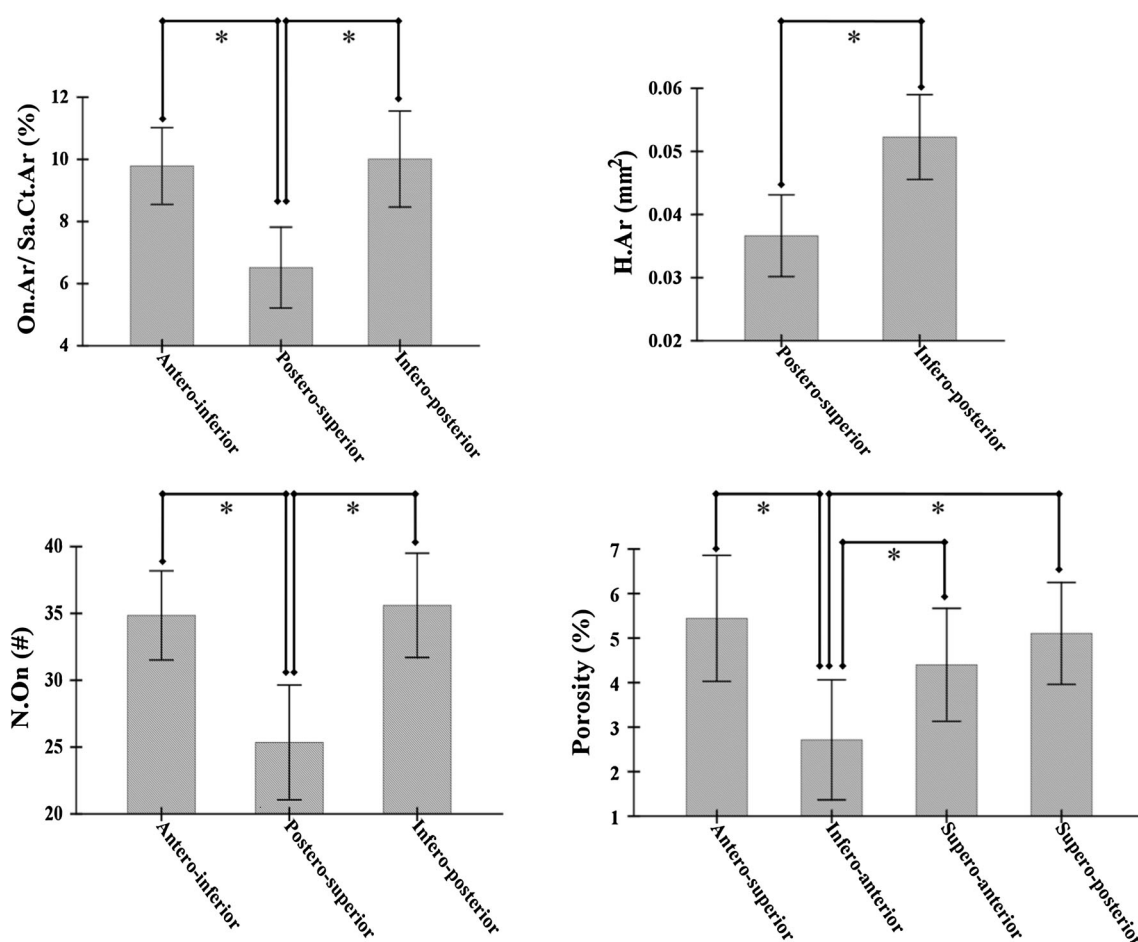
Fig. 6 Significant variation of high-magnification parameters between eight anatomical areas. Osteonal area and osteonal number were higher in the antero-inferior area and infero-posterior area compared to the postero-superior area. The Haversian canal area was higher in the infero-posterior area compared to the postero-superior area. Porosity was higher in the antero-superior area, supero-anterior area and supero-posterior area compared to the infero-anterior area. *$P \le 0.002$. N. *On* osteon number, *On.Ar/Sa.Ct.Ar* osteonal area/ cortical area, *H.Ar* Haversian canal area

cortical bone through the trabecularisation of the endo-cortical region [44]. As mentioned previously, cortical thinning shortens the volume in which remodelling may occur, which may reduce the probability of detecting a resorption space as well.

Our study has some limitations. The number of samples is relatively small, especially for studying age-related changes. Thus, the statistical power to show significant changes may have been inadequate for some parameters. The secular changes in cortical dimensions and architecture cannot be excluded, as this was a cross-sectional study. Moreover, this study only takes male subjects into account. Since many previous studies have examined the effect of ageing on cortical bone in the hip with a cross-sectional study design in women [35, 41, 44], and women have cortical thinning with ageing that is two times faster than in men [53], we do not know whether similar changes of histological property will be observed in female subjects.

In conclusion, we found thinning of the superior cortex of the femoral neck with age in healthy males. Investigations of the underlying structural changes that might predispose to fracture have been largely limited to computed tomography-based approaches, as well as to the skeletal sites that do not fracture, such as the iliac crest. Studies on femoral neck biopsies from healthy subjects are rare. Therefore, our study provides useful insight into the histological changes at femoral neck with age. These changes may explain the underlying mechanisms that give rise to an osteoporotic femoral fracture. Further studies to confirm these findings are needed. Moreover, since most previous studies have applied various definitions both for parameters and bone boundaries, our method could be used to obtain data that are more comparable between studies.

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