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Age-related histological changes in calcified cartilage and subchondral bone in femoral heads from healthy humans

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ABSTRACT

Objective: Age is the most important risk factor for osteoarthritis (OA). It is suggested that changes in subchondral bone and calcified cartilage may occur in early OA. Therefore, the aim was to investigate age-related changes in the femoral head composition. We hypothesise that the thickness of the subchondral bone plate decreases with age, while the thickness of the calcified cartilage increases with age as seen in early-stage OA.
Methods: Femoral heads from 29 women (20–74 years) and 32 men (23–78 years), who had died suddenly and unexpectedly, were obtained at autopsy. Individuals with bone or joint diseases or macroscopic abnormal cartilage were excluded. Using design-based stereology, femoral head volume as well as thickness and volume of the calcified cartilage and subchondral bone plate were estimated and correlated to sex and age.
Results: The thickness and volume of the subchondral bone plate were not correlated with age. Calcified cartilage thickness and volume correlated positively with age in women, while the femoral head volume was correlated positively with age in men.
Conclusion: In human femoral heads obtained from a cross-sectional population without macroscopic OA changes, the thickness of the subchondral bone plate did not change with age, which differs from the thinning seen in early OA. Surprisingly, the age-related changes of the volume and thickness of the calcified cartilage and of the volume of the femoral head were different for women and men. This indicate that cartilage and bone metabolism is sex-specific, which may influence ageing of the hip joint.

1. Introduction

Osteoarthritis (OA) is the most frequent joint disease in western countries with socioeconomic expenses exceeding billions of dollars every year [1]. Age is a significant risk factor for OA [2]. Wear and tear are considered as the major cause of OA, and treatment strategies have therefore focused on reinforcing and rebuilding the damaged cartilage [3-5]. However, age-related changes in the mineralised joint tissue may occur prior to cartilage damage [6].

Studies suggest that the subchondral bone may also be involved in the pathogenesis of OA as it increases in thickness [7,8] causing bone sclerosis in late-stage OA [7,9]. However, in early OA stages, thinning of the subchondral bone has been observed in human [10] and animal studies [11,12]. These early subchondral bone changes suggest that the juxta-articular bone turnover may also play an important role in the pathogenesis of OA [13].

The calcified cartilage has been described to thicken in relation to local OA severity not only in animal studies [14,15], but also in human studies [16,17]. It has been hypothesised that alterations of the calcified cartilage may compromise its mechanical function as a connective and cushioning tissue, leading to degeneration of the overlying articular cartilage [18-20]. In aged humans, multiple tidemarks have been described [21], indicating advancement of the calcified cartilage, but whether this is due to age or early OA transformations is currently unknown.

The majority of human studies use μCT to investigate the bone

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compartments in OA joints. However, this technique lacks the ability to identify calcified cartilage, while histology enables differentiation of mineralised bone and calcified cartilage. In the present study, stereology was applied to estimate total tissue volumes and surface areas of the femoral head and different subvolumes herein from randomly sampled two-dimensional histological sections of entire femoral heads.

We hypothesised that changes in the mineralised tissues may occur in individuals without cartilage degeneration and that this could be a sign of premature OA. The aim of the study was to quantify the age-related changes in the calcified cartilage and the subchondral bone of complete femoral heads from healthy individuals using design-based stereology.

2. Methods

2.1. Study population

Femoral heads from 29 women aged 20–74 years and 32 men aged 23–78 years, who had suddenly or accidentally died, were obtained at autopsy from the Institute of Forensic Medicine, Aarhus University. Twenty-four of the femoral heads have been used in a control group in a previous study investigating differences in bone turnover and femoral head volume between healthy individuals and OA patients [13]. Subjects were excluded from the present study if they were diagnosed with OA, secondary OA, or other joint diseases, bone metabolic diseases, malignant diseases, or diabetes mellitus. At macroscopic inspection, femoral heads that were fractured due to high-energy pelvic trauma or had visible cartilage deterioration were also excluded from the study. The study was approved by The Regional Scientific Ethical Committee. J.nr: 10776, and the Danish Data Protection Agency (J.nr: 2003-41-3447).

2.2. Processing of tissue

Processing of the femoral heads, which provides the basis for the estimates of absolute volume and surface area, has previously been described in detail [17,22]. In brief, the femoral heads were fixed in 70% ethanol and using Systematic Uniform Random Sampling (SURS) and Vertical Uniform Random (VUR) sections [23] the femoral head was randomly rotated around a vertical axis through the top of the caput. A diamond saw was used to cut five to eight 7-mm-thick parallel slices. The bone slabs were halved, and alternating left and right halves were embedded in methylmethacrylate, the first being randomly chosen. A Jung model K microtome (R. Jung GmbH Heidelberg, Germany) equipped with a tungsten microtome knife was used to cut two 7-μm-thick sections from each bone slab. The sections were stained with either May-Grünwald toluidine blue or Masson-Goldner trichrome. Shrinkage is low in plastic embedded sections, and therefore no correction for tissue shrinkage was performed [24].

2.3. Definition of tissue structures

The caput was macroscopically and microscopically defined by the shape and the extent of the articular cartilage. Protrusions expanding from the natural curvature of the femoral head at the boundary between caput and collum were defined as osteophytes and excluded from the study. The calcified cartilage was identified deep to the articular cartilage. Articular cartilage is separated from calcified cartilage by a thin winding line called the tidemark. The underlying subchondral bone is separated from the calcified cartilage by the cement line. The subchondral bone plate was defined as the bone plate separating the marrow from the cartilage not including subchondral trabecular bone [25]. A 5-mm-thick subarticular bone region was defined including both the subchondral bone plate and cancellous bone. The 5 mm subarticular bone region ranged from the cement line and 5 mm profoundly into the femoral head (Fig. 1).

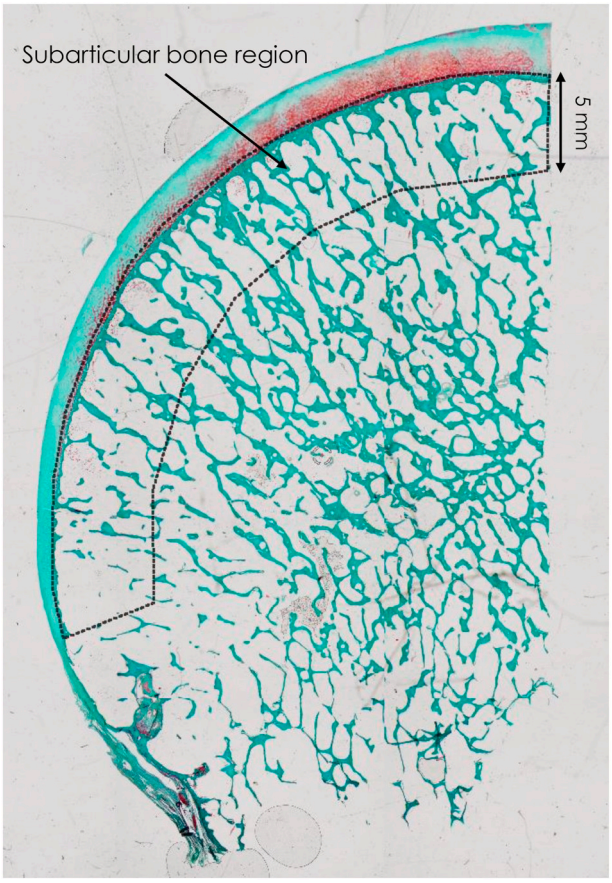

Fig. 1. A 7-μm-thick vertical uniform random section from a healthy female subjects' femoral head, stained with Goldner-trichrome. The black dotted box denotes the 5 mm deep subarticular bone region.

2.4. Stereology setup for volume and surface area

We were able to apply stereological methods because entire femoral heads were available, thus absolute 3-dimensional histological parameters of tissue volumes and surface areas could be estimated. All estimates are named according to the standard histomorphometric nomenclature [26]. The procedure for estimating volume and surface area have previously been described in detail [22]. In brief, data were collected using a light microscope (Nikon Eclipse 80i, Tokyo, Japan) connected to a computer running newCAST interactive stereology software (v. 3.4.1.0, Visiopharm, Hørsholm, Denmark). The surface area of the femoral head (FeHe.S), articular cartilage (ArCr.S), cement line (CeLi.S) and tidemark (Tm.S) were estimated according to the principles of SURS [27] and VUR sections [23], applying a line probe with an area per length (a(l)) of 0.99 mm. The natural curvature of the outer boundary of the articular cartilage was defined as the surface of the femoral head.

The volume of the femoral head (FeHe.V), articular cartilage (ArCr.V), calcified cartilage (CalCr.V), bone volume (BV), subarticular bone region (SubArB.V), subarticular marrow (SubArMa.V) and subchondral bone plate (SuBPl.V) were estimated according to the principles of Cavalieri [28]. Multiple point grids with different area per point were customized for each variable and regions of interest (ROI) were set to 100% for each bone slab. Bone volume fraction was estimated as the fraction of total bone volume of the total femoral head volume (BV/FeHe.V) and for the 5 mm subchondral region (SubAr.V/TV (5 mm)).

The precision of the volume estimates was calculated using the coefficient of error (CE) [28]. By applying a formula for estimating CE

from a previous methodological study [22], the CE values for volume estimates were found to be acceptable, varying from 0.03–0.06 between the different volume parameters. CE values for surface areas could not be calculated, because the intersectional size variation was too large.

2.5. Stereology-based sampling for corrected orthogonal thickness measurements

The method for measuring thicknesses has previously been described in detail [17]. In brief, a cycloid grid was superimposed onto each tissue section, from where sampling points were generated at the intersection between the lines of the cycloid grid and the surface of the femoral head. From each of these sampling points and perpendicular to the surface of the femoral head, the thicknesses of the articular cartilage (ArCr.Th), the calcified cartilage (CalCr.Th), and the subchondral bone (SubB.Th) were measured in triplets and the average thickness for each sampling points was used. Average thicknesses of articular cartilage, calcified cartilage and subchondral bone plate for complete femoral heads were calculated from the measurements as well as the subdivided average thicknesses according to the OARSI-grade [29].

Because of the stereological design and systematic uniform random sampling, all bone slabs were cut parallel to the vertical axis. To avoid overestimation of the thickness measurements due to the cutting angle, a predetermined formula was applied for correcting the measurements as previously described [30].

2.6. OARSI-grading and staging

For each of the systematic uniformly random sampled points at the femoral head surface, an OARSI-grade was given according to the OARSI scoring system of histological OA severity [17,29]. The OARSI-stage was later determined for each femoral head by counting the points with an OARSI-grade ≥ 1 and dividing it by the total number of points. Because the points were selected systematic uniformly random, the staging of the femoral head according to specific OARSI-grades of cartilage damage could be estimated [17,29].

2.7. Statistics

Sigmaplot 12.0 (Systat Software, inc. San Jose, California), was used for analyzing data. Normality was checked with the Shapiro-Wilk test. If normality was confirmed, the Students *t*-test was used for comparing groups. Otherwise, the Mann-Whitney *U* test was applied. Linear regressions were performed using the least-squares method on linear relationships as previously described [31]. Data for women and men were analyzed separately and plotted against subject age. For each regression line, *p*-values were calculated giving an estimate of the statistical significance of the relationship seen. The regression coefficients of the linear regression are denoted β_1 for the slope and β_0 for the *y*-axis intercept. *P*-values $< .05$ were considered statistically significant.

3. Results

3.1. Articular cartilage

Although, the OARSI-stage increased significantly with age in men and women and the average OARSI-grade increased significantly with age for men (Table 1), the articular cartilage degradation was minor as > 90% of the articular cartilage had an OARSI grade of 0 or 1. Similarly, the articular cartilage volume, surface area, and thickness were independent of age for both women and men (Table 2). The cartilage alterations were comparable for women and men as the regression coefficients for OARSI-stage, and grade did not differ between the two sexes.

Table 1
Mean value of sampling points and mean values and linear regression slopes of average OARSI-grade and OARSI stage.

	Mean value ± SD		β_1 (*p*-value)	
	Women	Men	Women	Men
Sampling points	45.1 ± 7.1	60.9 ± 9.1§		
Avg. OARSI-grade	0.4 ± 0.3	0.3 ± 0.2	0.08 (0.058)	0.009 (**0.007**)
OARSI-stage (%)	19.4 ± 15.6	16.9 ± 11.1	0.004 (**0.019**)	0.005 (< **0.001**)
OARSI grade 0	80.6 ± 15.6	83.1 ± 11.1		
OARSI grade 1	9.9 ± 7.9	8.1 ± 6.3		
OARSI grade 2	5.6 ± 6.5	3.7 ± 4.0		
OARSI grade ≥3	3.9 ± 5.6	5.1 ± 5.3		

Data are shown as mean ± SD and slope for the linear regression between the parameter and age with the corresponding P-value, significant P-values in bold. A *t*-test determined p-value < .05 between men and women is marked by §.

3.2. Subchondral bone plate and subarticular bone region

Volume and thickness of the subchondral bone plate did not correlate with age for either women or men (Table 2). The slope and *y*-axis intercept of the regression lines did also not differ between women and men. When subdividing thickness measurements according to the OARSI-grade of the overlying cartilage, we did not find any correlation with age in any group or a significant difference between the sexes (Table 2).

In the 5 mm subarticular bone region, the total bone volume decreased significantly with age in women, while the marrow volume increased significantly with age in men (Table 2). Thus, both men and women showed a statistically significant reduction in the subarticular bone volume fraction in the 5 mm region (SubAr.V/TV (5 mm)) (Table 2).

3.3. Calcified cartilage

Both calcified cartilage volume and thickness correlated significantly with age in women, but not in men (Fig. 2A–B), and consequently, the regression coefficients for the linear regression differed significantly between the sexes (Table 2). Furthermore, the calcified cartilage volume fraction of the femoral head increased significantly with age for women, but not for men (Fig. 2C) and again the slopes and *y*-axis intercepts differed significantly between the sexes (Table 2). We found no significant increase in the tidemark or cement line surface area (Fig. 2D–E). However, men had a significantly larger tidemark surface area than women, as the slope of the fit-lines did not differ between women and men, while the *y*-axis intercept was significantly larger for men.

By subdividing the calcified cartilage thickness measurements according to the OARSI-grade of the overlying articular cartilage, the regions with no cartilage alterations, *i.e.* scoring an OARSI-grade of 0, showed a significant positive linear relationship between calcified cartilage thickness and age in women, while it was independent of age in men. The age-related changes in calcified cartilage thickness in regions with an overlying OARSI-grade of 0 differed significantly between the sexes (Table 2).

3.4. Femoral head

Women had smaller femoral heads compared to men which is reflected by fewer sampling points for thickness measurements (Table 1). The volume of the femoral head increased significantly with age in men, but not in women (Fig. 3A). The regression coefficients of the linear regression, however, did not differ significantly between the two sexes (Table 2).

The femoral head bone volume showed no significant relationship

Table 2
Correlation of different tissue volume and thickness with age for women and men.

	Women (n = 29)				Men (n = 32)				β_1	β_0
	β_1	β_0	r	p	β_1	β_0	r	p	P	p
OARSI grade	0.08	−0.015	0.36	0.058	0.009	−0.10	0.57	< **0.001**	0.66	0.47
Femoral head										
Tissue volume (TV)	13.05	25,215	0.06	0.78	191.47	32,582	0.35	**0.048**	0.064	0.13
Bone volume (BV)	−22.06	6844	0.33	0.081	−8.33	10,007	0.08	0.67	0.48	**0.003**
Marrow volume (Ma.V)	20.67	10,441	0.14	0.45	141.79	14,006	0.38	**0.031**	0.063	0.28
BV/FeHe.V	−0.001	0.27	−0.49	**0.008**	−0.001	0.29	0.58	< **0.001**	0.45	0.22
Surface area	−2.36	3622	0.07	0.74	6.00	4361	0.16	0.38	0.22	**0.039**
Articular cartilage										
Volume (ArCr.V)	10.09	4200	0.14	0.47	19.62	56,812	0.23	0.20	0.53	0.06
ArCr.V/FeHe.V	0.000	0.17	0.14	0.47	−0.000	0.17	−0.12	0.50	0.14	0.72
Surface area (ArCr.S)	11.08	3350	0.22	0.25	14.31	4528	0.26	0.14	0.74	**0.022**
Thickness (ArCr.Th)	1.60	1224	0.13	0.49	0.50	1427	0.04	0.83	0.64	0.10
OARSI grade 0	0.44	1272	0.04	0.86	−0.20	1449	−0.01	0.94	0.80	0.18
OARSI grade 1	7.24	976.5	0.27	0.19	−3.74	1740	−0.10	0.60	0.13	0.05
OARSI grade 2	10.15	1901	−0.29	0.20	1.21	1347	0.03	0.89	0.21	0.26
OARSI grade ≥3	3.81	902.4	0.14	0.64	−20.70	2524	−0.47	**0.028**	**0.011**	**0.003**
Calcified cartilage										
Volume (CalCr.V)	5.10	39.07	0.53	**0.003**	1.89	179.0	0.32	0.07	**0.003**	**0.011**
Surface area (CalCr.S)										
Cement line (CeLi.S)	30.12	3810	0.32	0.086	23.72	5292	0.26	0.16	0.70	0.086
Tidemark (Tm.S)	10.19	2918	0.22	0.26	9.82	3842	0.23	0.20	0.96	**0.023**
Thickness (CalCr.Th)	1.53	27.74	0.59	< **0.001**	0.18	67.34	0.13	0.7	< **0.001**	**0.004**
OARSI grade 0	1.13	43.48	0.46	**0.013**	0.12	68.05	0.092	0.62	< **0.001**	**0.049**
OARSI grade 1	2.75	3.37	0.32	0.12	0.47	58.95	0.14	0.46	**0.001**	0.10
OARSI grade 2	3.10	6.37	0.46	**0.035**	0.71	75.98	0.08	0.72	0.23	0.51
OARSI grade ≥3	2.83	−1.58	0.47	0.086	0.51	60.27	0.26	0.25	< **0.001**	**0.015**
Subchondral bone										
Volume (SubBPl.V)	−2.65	710.7	0.16	0.41	−0.57	795.3	0.04	0.82	0.42	0.52
Thickness (SubBPl.th)	−0.92	259.4	0.21	0.28	−0.60	257.9	−0.18	0.33	0.61	0.96
OARSI grade 0	−1.13	268.8	−0.25	0.19	−0.84	264.5	−0.23	0.20	0.65	0.90
OARSI grade 1	−1.9	268.6	−0.20	0.33	−1.13	298.6	−0.16	0.41	0.91	0.67
OARSI grade 2	−0.63	211.6	−0.10	0.67	−2.20	336.0	−0.24	0.25	0.41	0.23
OARSI grade ≥3	−0.9	238.1	−0.03	0.93	−0.81	276.5	−0.11	0.62	0.71	0.66
Subarticular (5 mm)										
Bone volume (SubArB.V)	−16.58	3531	0.51	**0.004**	−13.57	4775	−0.29	0.11	0.72	**0.006**
Marrow volume (SubArMa.V)	9.84	7875	0.15	0.15	56.41	8392	0.47	**0.007**	**0.023**	0.61
SubArBV/TV (5 mm)	−0.001	0.31	−0.56	**0.002**	−0.002	0.35	0.57	**0.001**	0.65	0.11

Regression coefficients (β_0, β_1) and Pearson's correlations coefficient (r) for the linear relationship $y = \beta_0 + \beta_1 x$, where x is age and y the different parameter measured for women and men. We checked for differences between men and women by comparing slopes (β_1) and intercepts (β_0), which is shown with corresponding p-values. Significant P-values in bold.

with age in either sex (Table 2). However, men had a significantly larger y-axis intercept indicating that men have a greater bone volume than women. On the other hand, femoral bone volume fraction (BV/FeHe.V) decreased significantly with age for both women and men (Fig. 3B), and the age-related changes in (BV/FeHe.V) were similar in women and men (Table 2). Neither height, nor body mass index (BMI) correlated significantly with age.

4. Discussion

The study showed that the subchondral bone plate volume and thickness did not change with age in healthy subjects. Surprisingly, several parameters showed sex-specific correlation with age. Thus, women showed calcified cartilage thickening with age, while men showed enlargement of the femoral head with age.

4.1. Articular cartilage

OA has been understood as a progressive articular cartilage degradation and thinning. In the present study we wanted to investigate healthy individuals without OA and therefore excluded individuals with known joint diseases or macroscopic visual cartilage degeneration. However, we did find limited microscopic fibrillation of the articular surface and few fissures in the cartilage increasing the average OARSI-grade and OARSI-stage with age. These minor alterations of the articular cartilage may reflect age-dependent changes as we found no other signs of early OA. This is consistent with previous findings describing alterations on cellular and molecular levels, making the articular cartilage less resilient to mechanical stress with age [32]. Nonetheless, the average OARSI-grade was < 0.5 for both women and men, thus reflecting only minor superficial damage, which permitted investigation of age-depended changes without loss of substance. We did not find any correlation between subject articular cartilage volume or thickness and subject age. An MRI study found articular cartilage thinning in knees with age especially in women older than 40 years [33]. However, in contrast to the present study, adjustments were done only for radiological osteoarthritis and not macroscopical or microscopical changes making it difficult to exclude early osteoarthritic changes. Furthermore, for women, it is well-known that the incidence of OA accelerates around the menopause [2]. Changes in the hormone balance during the menopause may be linked to articular cartilage thinning found at this age, but further research is needed to elucidate this. According to the present study, age itself does not seem to induce cartilage loss.

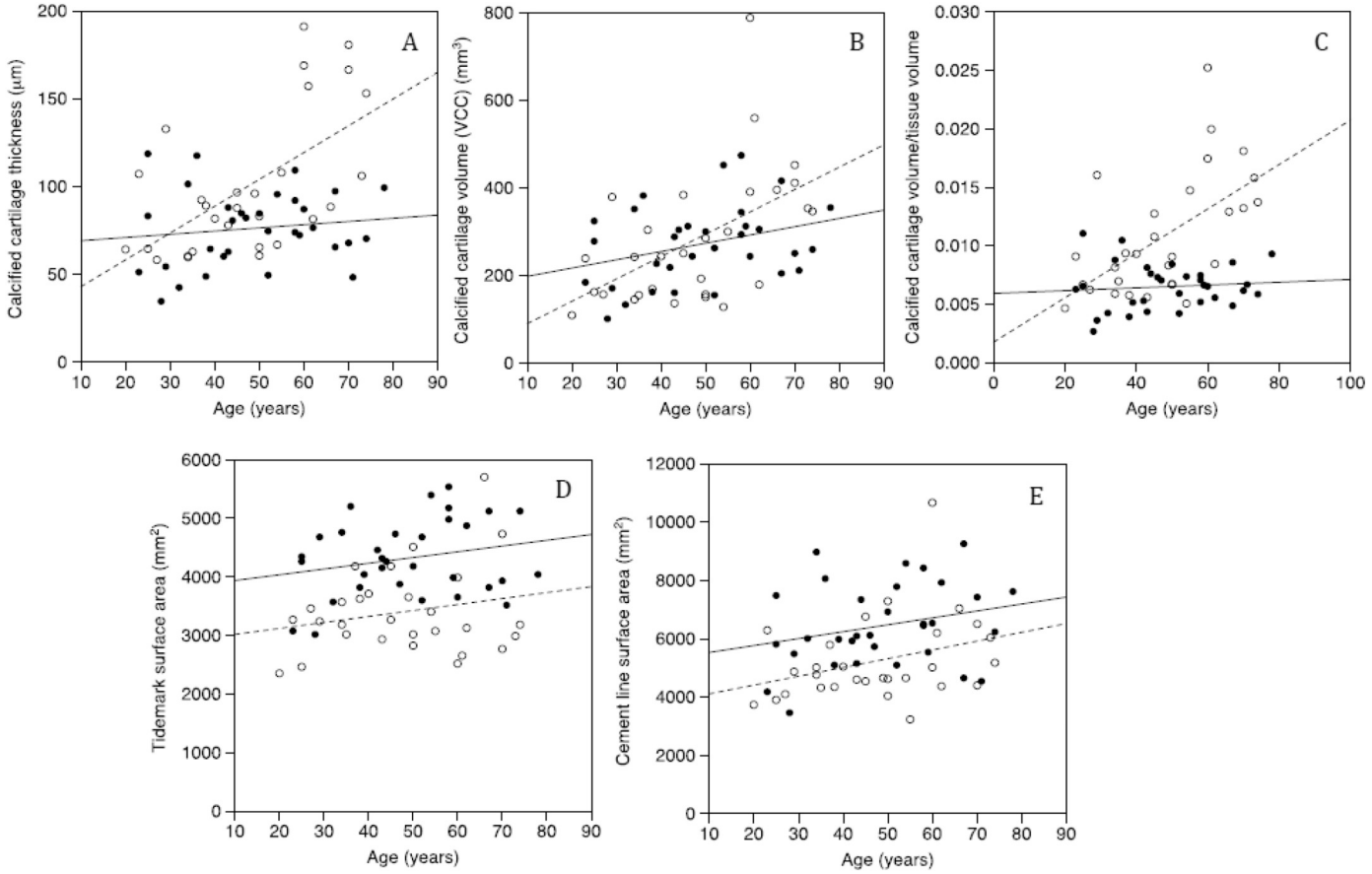

Fig. 2. Age correlation to calcified cartilage thickness, calcified cartilage volume, the total volume fraction of the calcified cartilage in the femoral head, tidemark surface area and cement line surface area.

4.2. Subchondral bone plate

A well-described characteristic of OA is the increased thickness of the subchondral bone plate as demonstrated in radiological studies [8]. The present study did not find any indications of changes in the subchondral bone plate as neither the volume nor the thickness of the subchondral bone plate correlated with age. This emphasizes that the age-related changes differ from OA alterations. Moreover, a previous study using the same methods found that thickening of the subchondral bone plate first occurred in regions with severe OA lesions, as indicated by the local OARSI grade [13,17]. Animal studies have also demonstrated that joints undergo an initial subchondral bone plate thinning in early OA, with bone sclerosis in late-stage OA in which the joint surface is completely denuded [7,9,11,12]. Human studies have found similar observations in late-stage OA [17]. However it is still unknown how minor cartilage erosions in early OA affects the subchondral bone plate, but a recent study has found increased bone remodelling in early OA [13]. The present study demonstrated that the thickness of the subchondral bone plate was similar in areas with overlying minor articular cartilage alterations (OARSI 1–2) and in areas with overlying normal

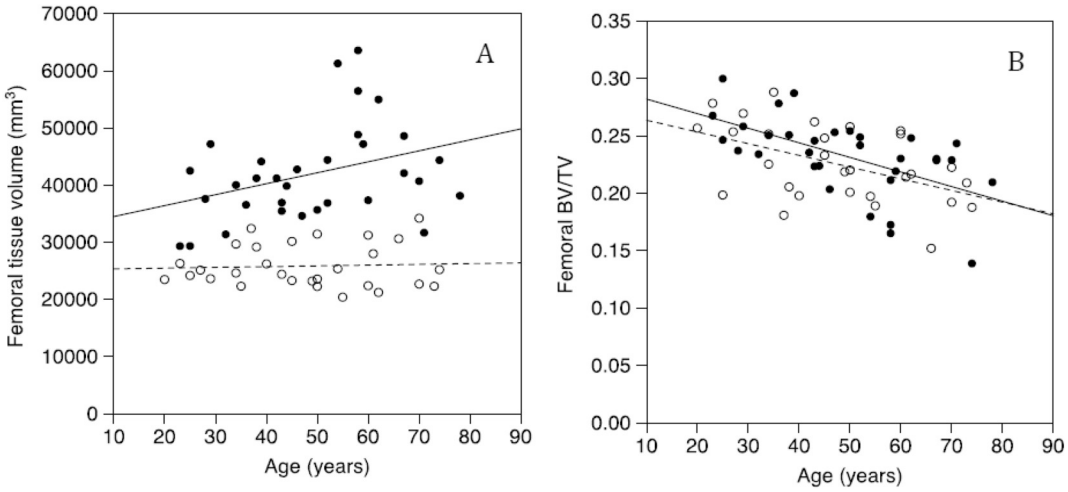

Fig. 3. Age correlation to femoral tissue volume and total volume fraction of the bone in the femoral head.

cartilage (OARSI 0). This is in contrast to animal studies, which have found early subchondral bone plate thinning in OA with minor cartilage fibrillation (OARSI 1) [9,34]. Furthermore, we found no correlation between age and subchondral bone plate thickness in regions underlying undamaged articular cartilage (OARSI 0).

Age itself does therefore not seem to cause preliminary OA changes with subchondral bone plate thinning prior to articular cartilage alterations. Moreover, age itself does not seem to cause thickening of the subchondral bone plate.

4.3. Calcified cartilage

Animal studies have shown calcified cartilage thickening in OA joints [14,15]. Therefore, it has been suggested that growth of the calcified cartilage contribute to the pathogenesis of OA resulting in later endochondral ossification and femoral head enlargement [29]. In the present study, the calcified cartilage volume and thickness correlated with age in women but only showed a positive trend in men. However, in regions with an overlying intact articular cartilage (OARSI 0) calcified cartilage thickness correlated with age in women only. Age itself may, therefore, have an impact on the thickening of the calcified cartilage and explain the tidemark advancement found in other studies [14,21]. Furthermore, the finding of calcified cartilage advancement indicates that calcified cartilage is a dynamic tissue, which can be stimulated to expand possibly by vascular ingrowth [35,36]. Vessels located in the deep part of the calcified cartilage communicate with the marrow cavity wherefrom growth factors may induce bone formation [36]. This endochondral ossification may explain how joint enlargement occurs with age and OA [6,37].

These sex-related different findings were not expected. However, divergence in the ageing of calcified tissue between women and men is well-known as bone turnover changes significantly in women after the menopause [38-40]. Thus, sex hormones may also differentially influence the calcified cartilage and the femoral head growth causing the composition of joint tissues to differ between the sexes as the fraction of calcified cartilage to tissue volume of the femoral head increases significantly in women only. This may be due to a higher conversion of calcified cartilage to bone in men causing the femoral head to expand faster and the calcified cartilage to become thinner [37].

The tidemark and cement line are seen to wind into the surrounding structures in OA, because of bone turnover and calcified cartilage growth results in bone expansion [6] and tidemark advancement [41]. This may be due to differences in the growth response or exposure to load and stress facilitating OA in the femoral head. In the present study, we did not find any statistically significant age-related changes in the surface area of either the cement line or tidemark, but we saw an increasing trend. The calcified cartilage may act as a reactivated growth zone, but further studies are needed to confirm this hypothesis. The present study suggests sex-specific ageing of the calcified cartilage questioning whether the pathogenesis of OA may influence women and men similarly.

4.4. Fractional bone volume

Changes in subchondral bone in OA joints has been described in both the subchondral bone plate and in the underlying trabecular bone [42].

The ratio between the bone volume and the total tissue volume (bone + marrow) known as the bone volume fraction (BV/FeHe.V) can be altered by two scenarios: Either through a change in bone volume without a proportional equal change in marrow volume or through a change in marrow volume without an equivalent change in bone volume. In the present study, the bone volume fraction declined with age in both women and men for the subchondral region as well as for the femoral head in general. This is a well-known age-dependent phenomenon [43]. However, the net results reflect two different underlying causes.

In men, the marrow cavity volume increased significantly with age, while the total bone volume was unchanged. The reduced bone volume fraction implicates that the growth of the femoral head caused the marrow cavity to expand proportionally more than the bone volume. Another study validates this phenomenon as it found the femoral head diameter increased with age especially in men [37]. However, in that study, they did not control for OA as in the present study, and therefore femoral enlargement might also be due to increasing incidence of OA with age causing the results to be significant for both men and women. Femoral head growth seems therefore not limited to the adolescence. Bone growth with age has also been observed in other joints for men. The average cross-sectional area of the vertebral bodies has been shown to increase by 25–30% from the age of 20 to 90 years [44]. This phenomenon has also been described in OA studies where OA joints were found to be significantly larger than healthy joints [6,30]. Because the joint is covered by cartilage, we suggest that endochondral growth may cause both osteophyte formation as well as femoral head expansion in OA joints. Importantly, the growth found in the present study only reflected femoral head expansion. We found very few, and minor osteophytes, which could only be acknowledged microscopically, and all were excluded from the final analyses. Furthermore, the larger sizes of the femoral heads could not be explained by height or BMI of the included subjects. However, the sample size is small.

In women, the size of the femoral head did not change with age indicating that the loss of subchondral bone volume fraction was caused by a thinning or loss of subchondral trabecular bone. Women have an accelerated bone loss around the menopause [38-40], after which the incidence of OA are found to increase rapidly [45]. Bone turnover has been hypothesised to be an essential factor in the pathogenesis of OA and a previous study from our group has shown an increased bone turnover in OA individuals [13]. Furthermore, animal studies have indicated that a pharmaceutical inhibition of bone turnover in OA may decrease the progression rate of the disease making this a possible target point for future treatment [46]. However this has not been confirmed in human studies [47].

Our results confirm that structural alterations of the femoral head in general and of the subchondral region occur with age, although the influence of age differs in women and men. How these alterations relate to OA needs further investigation.

4.5. Strengths and limitations

The histological material investigated in the present study is one of the most extensive collections of complete non-osteoarthritis human femoral heads, providing a unique opportunity to study the impact of age on the hip joint. Our analyses were performed on random samples representing entire femoral heads strengthening the validity and precisions of the results compared to biopsy studies, which are limited to focal knowledge.

Because complete femoral heads were available, stereological methods could be applied to obtain a global understanding of the histological structures. Using design-based stereology, three-dimensional estimates of absolute surface areas and volumes could be obtained from uniform randomly sampled two-dimensional sections. In contrast, conventional histomorphometry considers two-dimensional tissue sections without the possibility of relating the parameters to absolute numbers in three dimensions.

The histological analysis of the femoral heads was a major advantage making it possible to discriminate between the different tissue compartments. Especially the calcified cartilage could be investigated in contrast to imaging techniques like CT and MRI, which are unable to distinguish this structure from the underlying bone [48,49].

We applied stereology for estimating volumes and a stereology-based method for measuring thicknesses. It is a strength of the study that corresponding data were obtained with two independent methods.

Furthermore, we found no difference concerning minor articular cartilage alterations in men and women making the sexes comparable.

The present study was conducted as a cross-sectional study limiting our knowledge of the individualized ageing. However, as our investigations are destructive by nature, it is impossible to conduct a longitudinal research study. Consequently, our data only reflect the ageing of the femoral head based on our study group and therefore other methods need to be developed to attain a deeper understanding of the ageing process.

Although being a large population included in a histological study of complete femoral heads, an even larger population would have strengthend the results reflecting the true ageing of the different tissues in hip joints.

5. Conclusion

In the studied cross-sectional population without macroscopic OA degradation, the thickness of the subchondral bone plate did not change with age. Therefore, it is not likely that age *per se* is essential for development of OA-related early thinning or late thickening of the subchondral bone plate.

Surprisingly, the effect of age on calcified cartilage and on the dimension of the femoral head were different in women and men. Therefore, ageing might also impact the development of OA differently in women and men. Furthermore, future studies on the medical targeting of OA could consider the sex-specific effect of the calcified joint tissues.

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Author contributions

All authors made a substantial contribution to the conception and design, drafting of the article and participated in critical revision of the article for important intellectual content for the final approval. Louise Brøndt Hartlev acquired the femoral heads collaborating with Lene Warner Thorup Boel. Jesper Skovhus Thomsen contributed to the setup and execution of the statistical analysis. Andreas Wiggers Nielsen conducted the data collection, statistical analysis, and interpretation in the guidance of Rasmus Klose-Jensen, Kresten Krarup Keller and Ellen-Margrethe Hauge.

Declaration of competing interest

Ellen-Margrethe Hauge reports personal fees from MSD, personal fees from Pfizer, personal fees from UCB, personal fees from Sobi, grants from Roche, grants from Novartis, outside the submitted work. Kresten Krarup Keller reports personal fee from Pfizer outside the submitted work. For all other authors, there are no conflicts of interest to declare.

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