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Age related changes in the bone microstructure in patients with femoral neck fractures ☆

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ABSTRACT

Background: The risk of femoral neck fracture progressively increases with age. However, the reasons behind this consistent increase in the fracture risk can't be completely justified by the decrease in the bone mineral density. The objective of this study was to analyze the correlation between various bone structural features and age.

Study Design & Methods: A total of 29 consecutive patients who suffered an intracapsular hip fracture and underwent joint replacement surgery between May 2012 and March 2013 were included in this study. A 2 cm × 1 cm Ø cylindrical trabecular bone sample was collected from the femoral heads and preserved in formaldehyde. Bone mineral density (BMD), microarchitecture, organic content and crystallography were analyzed using a Dual-energy X-ray absorptiometry scan, micro-CT scan, and high resolution magic-angle-spinning-nuclear magnetic resonance (MAS-NMR), respectively. Statistical correlations were made using Spearmanś or Pearsonś correlation tests depending on the distribution of the continuous variables.

Results: The mean patient age was 79.83 ± 9.31 years. A moderate negative correlation was observed between age and the hydrogen content in bone (^{1}H), which is an indirect estimate to quantify the organic matrix ($r = -0.512$, $p = 0.005$). No correlations were observed between BMD, trabecular number, trabecular thickness, phosphorous content, apatite crystal size, and age ($r = 0.06$, $p = 0.755$; $r = -0.008$, $p = 0.967$; $r = -0.046$, $p = 0.812$; $r = -0.152$, $p = 0.430$, respectively). A weak positive correlation was observed between Charlsonś comorbidity index (CCI) and *c*-axis of the hydroxiapatite (HA) crystals ($r = -0.400$, $p = 0.035$).

Conclusion: The femoral head relative protein content progressively decreases with age. BMD was not correlated with other structural bone parameters and age. Patients with higher comorbidity scores had larger HA crystals. The present results suggest that the progressive increase in the hip fracture risk in elderly patients could be partially explained by the lower bone protein content in this age group.

Introduction

Osteoporosis is a silent disease characterized by a low bone mineral density (BMD), which is associated with a deterioration of bone microarchitecture. Accordingly, patients with osteoporosis have a higher fracture risk compared to the general population [1].

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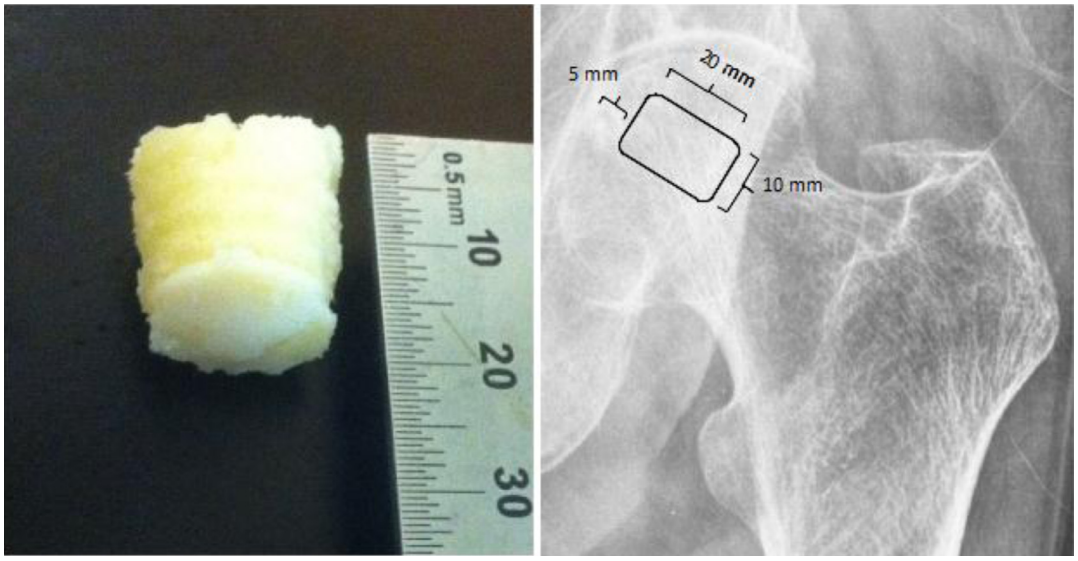

Fig. 1. Extraction of 1.0 cm Ø × 2.0 cm cylindrical bone samples from the cephalic region of the femoral head, 1 cm superior to the fovea.

The diagnosis of osteoporosis is usually established using a bone density scan (DEXA). The world health organization (WHO) defines osteoporosis as a - 2.5 standard deviation compared to the mean of a healthy young population [1,2]. BMD varies with age and gender, as well as with life habits such as physical activity [3,4] and nutrition [5]. Women peak their maximum bone density during adolescence, but it then gradually decreases with age. This progressive loss in the BMD is accelerated after menopause. On the other hand, males tend to experience a slower decrease in their BMD throughout their lives [6].

There is an indirect relationship between the BMD and the risk of suffering a fracture [7]. In the case of hip fractures, this risk increases by 2 folds for each standard deviation decrease in the BMD [8]. Within osteoporotic fractures, hip fractures are considered the most relevant clinically, due to their frequency and relatively high morbidity and mortality [9-14]. In addition, hip fractures represent a huge economic burden to countries around the world [1,15]. The hip fracture risk increases significantly after the seventh decade of age. However, the decrease in the BMD decelerates during the eight decade of life, particularly in females [16]. Moreover, some authors have reported that within patients with similar BMDs, the fracture risk is higher in older individuals [17].

For all the previously mentioned reasons, it would be reasonable to hypothesize that there could be other age-related-BMD-independent structural factors which could play a role in bone fragility. Accordingly, the objective of this study is to analyze the micro and nanostructural features of bone in patients who suffered a femoral neck fracture and to correlate them with age.

Patients and methods

The study was approved by the Scientific and Ethic Board of our Institution, and the Andalusian Public Health System Bio-bank.

We conducted a cross-sectional study on patients who suffered an intracapsular hip fracture and underwent a total or partial hip replacement during the period 1/5/2012–31/3/2013. The clinical data was withdrawn from our Orthopedic Surgery Department and National Public Health System databases. The following parameters were retrieved from patients´files and computerized records: age, gender, fracture side, body mass index (BMI), pre-surgical comorbidities (e.g., liver disease, ischemic heart disease, chronic renal failure), information on drug intake, history of smoking and alcohol consumption. The general health status was estimated using the age-factored Charlson comorbidity score [18].

Patients with pathological fractures (i.e., secondary to osteomalacia, Pagetś disease, primary bone tumors or bone metastasis), or diagnosed with avascular necrosis of the femoral head were excluded from the analysis.

Sample preparation

The femoral heads were collected during hip replacement procedures. A hollow awl was used to extract 1.0 cm Ø × 2.0 cm cylindrical bone samples from the cephalic region of the femoral head, 1 cm superior to the fovea (Fig. 1). The samples were then deposited in a 10% formalin solution and stored at 4 °C.

An additional 2.0 mm^3 fragment of bone was then extracted from the caudal end of each sample using a 1.0 Ø mm cylindrical burr adapted to a handpiece drill (Stryker, Hamilton, ON). Each fragment was then subjected to three cycles of cleansing with deionized distilled water at 25° for 60 min followed by three cycles of dehydration and defatting with 100% alcohol for 60 min [19]. Finally, a ceramic pestle was used to pulverize the samples, and the resulting dry powder was stored in separate tubes at 4 °C.

Micro-computed tomography (μ-CT)

The bone samples were scanned using a μ-CT (SkyScan1172; SkyScan; Kontich, Belgium) with a resolution of 10.88 μm, a voltage of 80 kV, current of 120 uA, a rotation step of 0.5° and an aluminum filter of 0.5 mm. The region of interest (ROI) included trabecular bone between 5.0 and 15.0 mm deep to the articular surface, to avoid potential areas with subchondral sclerosis (Fig. 2). The following parameters were then calculated: bone volume (BV), trabecular thickness (Tb.T), trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular pattern factor (Tb.Pf).

Bone densitometry

The bone density was analyzed using a bone densitometry device (GE Lunar PIXImus) with an image area of 100 × 80 mm, focal spot size of 0.25 × 0.25 mm, energy of 80 kV and a current of 400 uA. The region of interest was set at 10.0 mm deep to the articular surface. The following data were then calculated: bone mineral density (BMD), bone mineral content (BMC), and fat percentage.

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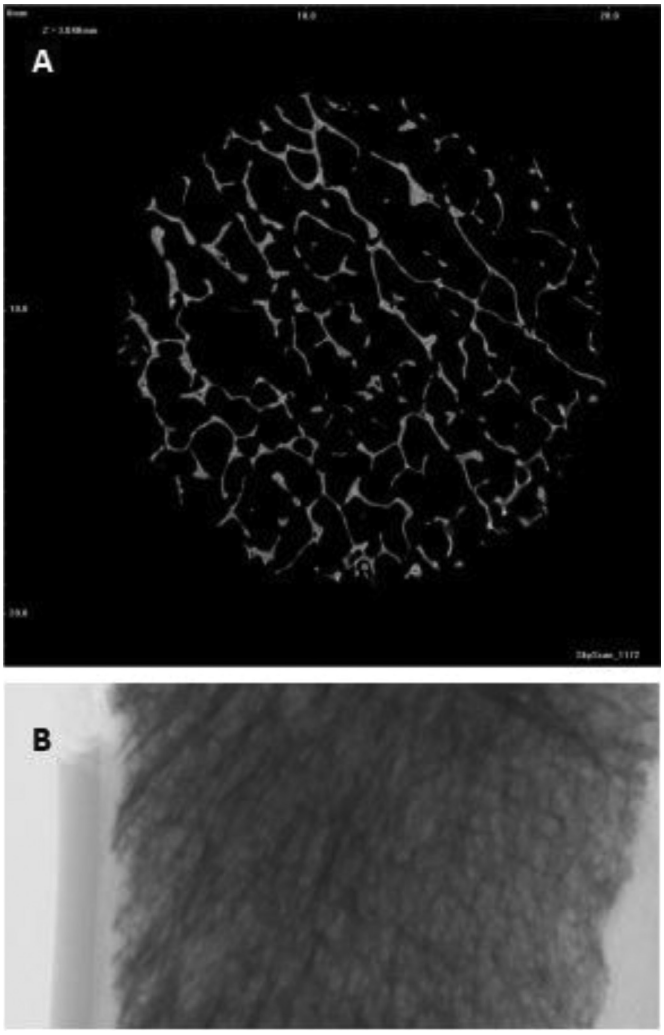

Fig. 2. A. Micro-computed tomography axial cut of a femoral head in a patient who suffered a femoral neck fracture. B. The selected region of interest on a coronal view.

X-ray powder diffraction

The crystallographic features of the hydroxyapatite (HA) crystals were analyzed using x-ray powder diffraction (XRD) (D8-Discover/GADDS, Bruker, Karlsruhe, Germany). The XRD patterns were recorded using a diffractometer with CuKα radiation (setting: 40 Kv, 40 mA, Theca1 15°, Theca2 15° scanning angle, frame width 23°, mode STEP and 1800 scan step time) [20]. DIFFRAC-plus EVA software (AXS, Bruker, Karlsruhe, Germany) was used to analyze the data obtained from each XRD spectrum [21].

The mean HA crystal size along the *a*-axis and *c*-axis for each bone sample were calculated using the (002) and (310) Bragg peaks of the XRD spectrum and Scherrer's formula (Eq. (1)). D is the average of domain lengths, K is the shape factor, λ is the x-ray wavelength, β is the line broadening at half the maximum intensity (FWHM), and θ is the Bragg angle.

$$D = k\lambda\beta\cos \quad (1)$$

Solid-state magic angle spinning (MAS) nuclear magnetic resonance (NMR)

The chemical composition of the extracted dry power was analyzed using a high-resolution MAS-NMR, which is a well-established technique for obtaining NMR spectra from solid materials. Measurements were carried out on a Bruker (Billerica,MA) MSL-600 NMR spectrometer, equipped with a 14 Tesla field strength magnet, yielding proton and phosphorus frequencies of 600.00 and 242.94, respectively. A Bruker solid-state magic angle-spinning (MAS) probe with B1 fields of 70–80 kHz (40 kHz for CP) was used to obtain solid-state 31P spectra with a single 90º pulse and with proton decoupling at a sample rotation speed of 2.5 kHz. All the measurements were performed consecutively, to avoid potential tuning changes on the spectrometer. Measurements were divided by the weight of the power, resulting in four different variables: ^{31}P relative concentration, ^{31}P with proton decoupling relative concentration, ^{1}H/^{31}P cross-polarization relative concentration, and ^{1}H relative concentration. The latter is an indirect measurement of the protein content of the bone matrix, since water and fat ^{1}H spins were eliminated during the preparation of the powdered samples. The parameters used for the NMR measurements can be found in Table 1.

Table 1
MAS Spectrometer parameters.

	Parameters						
Nuclei	90ºPD	NEX	PLA	SR	NP	RG	RD
1H	2.5	8	0	32k	32k	2k	1s
31P	10	100	−3	32k	32k	32k	20ms
1H/31P CP	12	300	0	2k	2k	32k	2s

Abbreviation: 90ºPD, 90º pulse duration; NEX, number of averages; PLA, power level attenuation; SR, spatial resolution; NP, number of points; RG, receiver gain; RD, recycle delay.

Statistical analysis

Data were analyzed using SPSS 22.0 software (SPSS Inc, Chicago, IL, USA), G*power 3.0.10 (Universität Kiel, Germany), and Origin Pro 8.0 (OriginLab Corporation, Northampton, USA). Mean values were expressed with their corresponding standard deviations (SD). The distribution of the variables was analyzed using the Shapiro–Wilk test and Q-Q plots. Correlations between normally distributed variables were determined using Pearson's test, whereas correlations involving at least one not-normally distributed variable were carried out using Spearman's rho. Results were considered significant when two-tailed *P* values were < 0.05. Power analysis were performed using two-tailed post-hoc *t*-test with an α- error probability of 0.05.

Results

A total of 29 femoral heads were collected and analyzed during the study period. The average patient age was 79.83 $\pm$ 9.31 years. The male-female ratio was 0.34, and the mean BMI was 25.7 kg/m^2. There were 8 (27.6%) smokers, and 2 (6.9%) patients who had a history of alcohol abuse. A total of 21 (72.4%) patients were able to mobilize without any aid before the injury. The mean age-factored Charlson comorbidity score of the participants was 5 (SD1.98) [18] (Table 2). The structural features of the femoral heads are illustrated in Table 3.

A moderate negative correlation was observed between age and hydrogen content (^{1}H), ($r = -0.512$, $p = 0.005$). No correlations were observed between the BMD, trabecular number, trabecular thickness, phosphorous content, crystal size and age ($r = 0.06$, $p = 0.755$; $r = -0.008$, $p = 0.967$; $r = -0.046$, $p = 0.812$; $r = -0.152$, $p = 0.430$, respectively). (Tables 4,5, and Fig. 3). A weak positive correlation was observed between the CCI and *c*-axis of the hydroxyapatite crystals ($r = -0.400$, $p = 0.035$) (Table 6).

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Table 2
Demographic features of the study group.

Characteristics	Intracapsular femoral fracture (n = 29)
Age, y	79.6 SD 10.1
Sex	
Men	8 (27.6)
Women	21 (72.4)
Smoking status	
Yes	8 (27.6)
No	21 (72.4)
Body mass index [a]	25.7 SD3.7
Operated Side	
Right	13 (44.8)
Left	16 (55.2)
Alcohol abuse	
Yes	2 (6.9)
No	27 (93.1)
CCI	5.27 (SD1.98)

Data are presented as No. (%) or mean ± SD.
Abbreviations: CCI, age factored Charlson comorbidity score. [a]Measured as weight in kilograms divided by the square of height in meters. *Statistically significant results.

Table 3
Structural characteristics of the femoral heads (Micro-CT, bone densitometry, MRI, X-ray diffraction).

Characteristics	Intracapsular femoral fracture (n = 29)
Percent bone volume (%)	20.37 (SD 6.58)
Trabecular separation (mm)	0.58 (SD 0.276)
Trabecular thickness (mm)	0.18 (SD 0.021)
Trabecular number (mm^{-1})	1.5 (SD 0.791)
TPF (mm^{-1})	−0.49 (SD 20.59)
BMD (g/cm^2)	0.28 (SD 0.048)
Trabecular area (cm^2)	4.62 (SD 1.179)
Bone area (cm^2)	5.05 (SD 1.24)
C size long *c*-axis† (nm)	17.9 (SD 2.2)
C size long *a*-axis† (nm)	18.3 (SD 2.5)
^{1}H concentration	0.956 (SD 0.075)
Relative 31P single pulse	6.90 (SD 1.90)
Relative 31P proton decoupled	7.18 (SD 1.99)
Relative cross polarization	1.25 (SD 0.33)

Abbreviations: TPF, trabecular pattern factor; BMD, bone mineral density; BMC, bone mineral content.
Data are presented as mean ± SD.
*Statistically significant results.
†Hydroxyapatite crystals.

Table 4
Correlation between Bone mineral density and other structural features on Micro-CT, bone densitometry, MRI and X-ray diffraction.

Characteristics	Correlation coefficient R	P values
Percent bone volume (%)	0.138	0.468
Trabecular separation (mm)	−0.083	0.662
Trabecular thickness (mm)	−0.049	0.799
Trabecular number (mm^{-1})	0.034	0.859
TPF (mm^{-1})	0.174	0.368
C size long *c*-axis† (nm)	−0.025	0.893
C size long *a*-axis† (nm)	−0.114	0.540
Trabecular area (cm^2)	0.495	0.005*
Bone area (cm^2)	0.525	0.002*
^{1}H concentration	−0.244	0.203
Relative 31P single pulse	−0.109	0.566
Relative 31P proton decoupled	−0.041	0.832
Relative cross polarization	0.010	0.960

Data are presented as mean ± SD.
* Statistically significant results.
† Of hydroxyapatite crystals measured in nanometers Spearman's rho.

Table 5
Correlation between age and the structural features on Micro-CT, bone densitometry, MRI and X-ray diffraction.

Characteristics	Correlation coefficient R	P values
Percent bone volume (%)	0.031	0.869
Trabecular separation (mm)	−0.144	0.447
Trabecular thickness (mm)	−0.083	0.664
Trabecular number (mm^{-1})	0.016	0.935
TPF (mm^{-1})	0.087	0.655
C size long *c*-axis† (nm)	0.015	0.936
C size long *a*-axis† (nm)	0.091	0.624
BMD	0.033	0.861
Trabecular area (cm^2)	0.074	0.692
Bone area (cm^2)	0.056	0.763
^{1}H concentration	−0.512	0.012*
Relative 31P single pulse	−0.222	0.238
Relative 31P proton decoupled	−0.065	0.738
Relative cross polarization	−0.333	0.072

Data are presented as mean ± SD.
* Statistically significant results.
† Of hydroxyapatite crystals measured in nanometers.

Discussion

We observed a negative correlation between age and the organic bone content in patients with intracapsular hip fractures. These results are in concordance with previous reports that observed changes in the collagen fibers with age, which could eventually cause a decrease in bone toughness [22,23]. Some authors suggest that the non-enzymatic glycation of collagen fibers could be an important contributing factor to the deterioration of bone tissue with age [24]. Moreover, some studies have measured the ratio between native and isomerized C-telopeptides of type I collagen in urine to estimate an index for bone matrix age [22].

The organic matrix, which is mainly composed of type I collagen, constitutes about 40% of bone tissue. Collagen fibers bind to each other in a triple helix structure, providing flexibility, elasticity and tension strength. Moreover, collagen fibers are longitudinally attached to hydroxyapatite crystals, providing additional rigidity and resistance against compression forces [25]. The degree of mineralization of bone tissue is correlated with the modulus of elasticity, and maximal strength [26]. Accordingly, variations in the composition of the collagen fibers could potentially alter bone tissue strength [16–18]. There are other non-collagenous proteins which could also play a significant role in the microstrutural organization of bone [22]. However, their contribution to bone strength is still largely undefined [22].

In this study, we analyzed the properties of the mineral and the organic matrix using phosphorus and proton solid-state MAS-NMR spectroscopy. This spectroscopic technique has been widely used to study ex vivo animal bone specimens [27]. However, the use of the corresponding solid state phosphorus and proton MRI methods in clinical practice has been very limited. In an in vivo study, analyzing the distal radius of 7 healthy humans, Wu et al, reported that solid-state 31P MRI achieved an adequate visualization of the bone mineral component [28]. In our study, we observed a moderate negative correlation between ^{1}H and age (Fig. 3). Our samples were completely dehydrated before the analysis; therefore, the remaining ^{1}H signal is an accurate indirect measurement of the organic composition of bone. Moreover, we also observed that there was no correlation between ^{31}P, which reflects the non-organic portion the bone matrix, and age. We can perceive from these results that the organic/non-organic matrix ratio may decrease with age. Our results are supported by recent research which reported a hyperminerilzation of osteocytic lacunae in patients diagnosed with osteoporosis [29]. Other reports have shown an age-related increase in the mineralization of osteocyte lacunae [30]. Moreover, a recent study has shown that a combination of high porosity and high mineralization, can lead to an increase in bone fragility [31].

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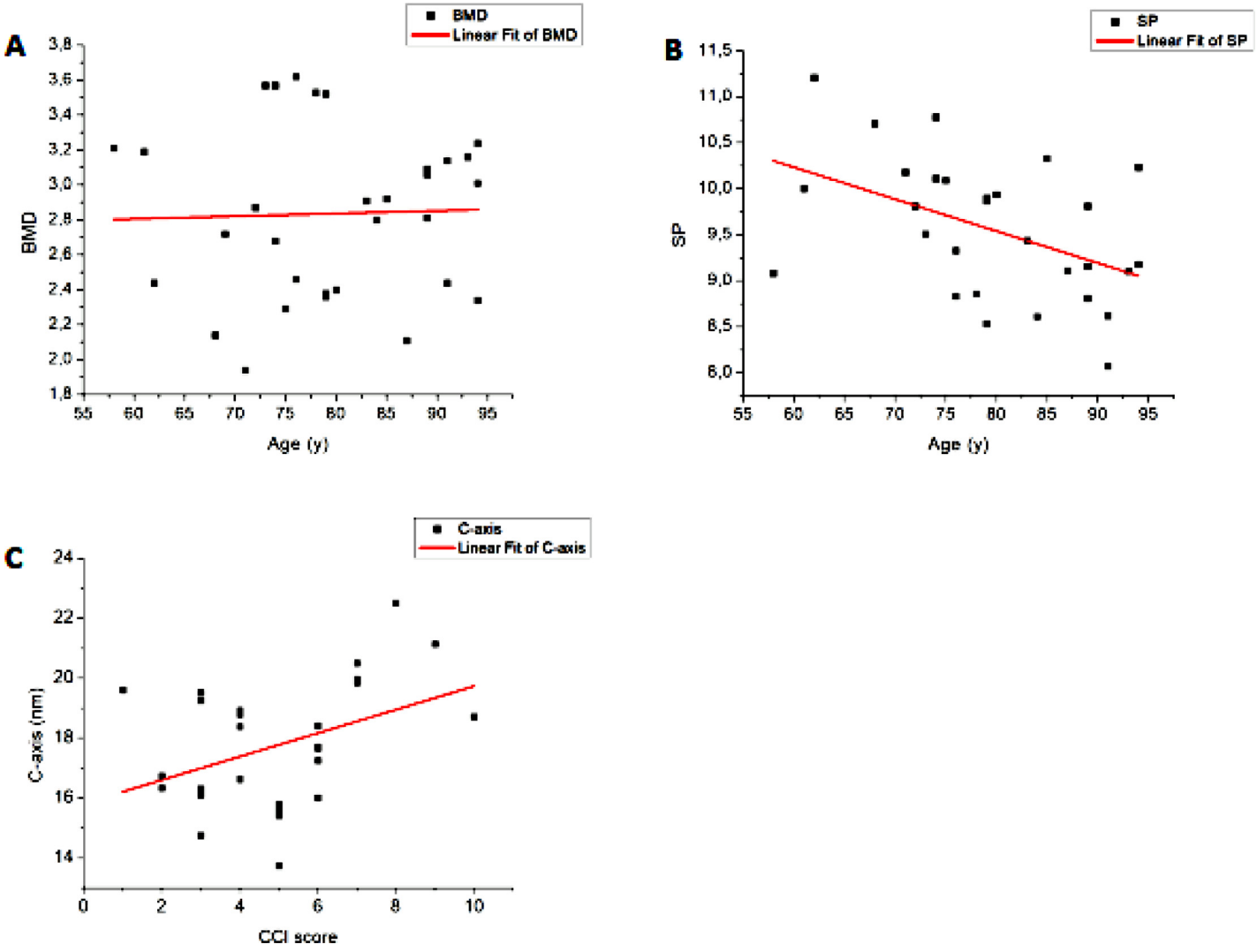

Fig. 3. A. Correlation between age, bone mineral density (BMD). B. Correlation between age and protein content from Single Pulse Hydrogen (SP). C. Correlation between the hydroxyapatite crystal size (*c*-axis) and patient comorbidity.

This study did not find a correlation between the BMD and age in patients who sustained an intracapsular femoral head fracture (Fig. 3). However, previous research has reported that the BMD usually decreases with age [1,6,32]. These studies refer to the general population, whereas our work only analyzed elderly patients who sustained a hip fracture. This could partially explain differences with previous reports. Moreover, some authors have reported that the femoral strength could vary more with age than with the BMD, and that age could be a more important risk factor for fractures than BMD [16,17]. In addition, other studies have shown that the predictive value of the DEXA scan for osteoporotic fractures decreases with age; therefore, it's a more useful tool in relatively younger elderly patients [7,33]. All these observations make us think that other age-related structural factors could influence bone strength, which could be independent form the BMD. Accordingly, the importance of other factors such as the bone microstructure, history of previous fractures, and risk of fall could play a more important role in the fracture risk of elderly patients [34].

On the other hand, we did not observe a correlation between age and certain structural features of bone, such as trabecular number, thickness or trabecular separation. Some authors have reported that the trabecular bone also experiences structural changes with age, which subsequently lead to a higher fragility [35]. Accordingly, the aging process leads to a loss of the trabecular number, thickness and connectivity [35]. A recent cross-sectional study reported that the bone volume/ trabecular volume could decrease by 22% and 18% between ages 60 and 90 years [36]. However, this was a cadaveric study on patients who represented the general population, and the bone samples were withdrawn from the base of femoral head and the base of femoral neck [36]. Moreover, the differences between this report and our results could be explained by the smaller sampler size in our study, and by the fact that our work does not represent the general population as all the processed samples were taken from individuals who sustained a femoral neck fracture.

Interestingly, we did not find a correlation between the hydroxyapatite (HA) crystal size and age. However, we observed a significant positive correlation between HA *c*-axis length and patient comorbidity using the CCI. The mineral component of bone tissue is formed mainly of calcium hydroxyapatite (Ca10(PO4)6(OH)2), which represents approximately 65% of its mass [37]. The HA crystals are arranged parallel to each other, and their *c*-axis is situated parallel to the collagen fibers, organized in a stepped pattern in the gap areas of the collagen fibrils [26]. Some authors have reported an association between the HA crystal size and bone strength [26,37,38]. HA crystals are arranged between collagen fib-

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Table 6
Correlation between CCI and the structural features on Micro-CT, bone densitometry, MRI and X-ray diffraction.

Characteristics	Correlation coefficient *R*	*P* values
Percent bone volume (%)	−0.269	0.166
Trabecular separation (mm)	0.131	0.505
Trabecular thickness (mm)	0.064	0.746
Trabecular number (mm^{-1})	−0.224	0.252
TPF (mm^{-1})	0.105	0.595
C size long *c*-axis† (nm)	0.400	0.035*
C size long *a*-axis† (nm)	0.159	0.418
BMD	−0.037	0.852
Trabecular area (cm^2)	0.073	0.713
Bone area (cm^2)	0.034	0.862
^{1}H concentration	−0.230	0.240
Relative 31P single pulse	−0.366	0.055
Relative 31P proton decoupled	−0.296	0.127
Relative cross polarization	−0.260	0.182

Abbreviations: CCI , Charlson comorbidity score (31), TPF, trabecular pattern factor; BMD, bone mineral density; BMC, bone mineral content; ^{1}H, single pulse Hydrogen.
Data are presented as mean ± SD.
* Statistically significant results.
† Of hydroxyapatite crystals measured in nanometers.

rils, and an excessive crystal size could damage the collagen fibers [26]. Moreover, longer crystals may affect the mobility of collagen fibrils, causing a decrease in bone ductility [26]. Accordingly, large HA crystals make bone tissue more brittle, whereas small crystals make it more ductile [26]. However, the HA crystal size which provides the optimal mechanical properties in human bone is still unknown [26]. In our study, individuals with higher degrees of comorbidity had an increased *c*-axis length of the HA crystals; therefore, these patients could potentially have weaker collagen fibers, and could potentially be exposed to a higher fracture risk. In addition, we did not find a correlation between HA crystal size, age and BMD. These results are in concordance with previous reports where the relationship between BMD and crystal size was not stablished [38]. However, some authors have reported a relation between age and crystal size [39]. Hanschin et al. observed that up to age of 25 years human bone experiences an increase in crystal length and crystal perfection and a decrease in thickness. Between 25 and 50 years there were few changes, but after age of 50 years, the mean crystal length slightly decreased [40]. HA crystal size in bone depends on multiple factors, such as the properties of collagen fibers, the distribution of other matrix proteins which are involved in the growth of the mineral crystals, diet, age, cell viability, pharmacological agents and mineral turnover rates [38]. Moreover, certain proteins such as osteopontin act as HA crystals growth inhibitors [38]. Accordingly, patients with high comorbidity levels could be potentially exposed to changes in the HA crystal size through any of these mechanisms. However, the exact pathways behind these changes remain unknown.

To the best of our knowledge this is the first study that shows a correlation between the organic matrix in bone tissue and age, using a MAS-NMR technique. In addition, this is the first study to report a positive correlation between patient comorbidity and increased *c*-axis length of the HA crystals. Moreover, we performed a comprehensive structural analysis of bone tissue which ranged from the crystal size to the macro-architecture of the trabeculae. However, this study has several limitations. Our sample size estimation was based on previous similar studies [24,28]. However, the sample size of the present study remained relatively small, and our post-hoc power analysis was limited to 70% with a 0.05 alpha error. In addition, we only included a relatively limited age range, confined only to elderly individuals; therefore, the magnitude of the collagen concentration change within younger individuals could not be defined. However, hip fractures occur typically in elderly individuals. Accordingly, the limited age range of this study was due to the absence of patients under 60 years of age with femoral neck fractures within our study cohort.

Conclusion

Femoral head relative protein content in elderly patients decreases significantly with age. Patients with higher degrees of comorbidity had an increased *c*-axis length of the HA crystals. The progressively increased fracture risk in elderly patients could be partially explained by the lower protein content of the organic matrix in these patients. More studies are needed to better understand how the microstructure of bone influences the development of a fracture. In the future, the development of new tools that measure other microstructural aspects of bone, such as high-resolution NMR, can provide important information to effectively prevent a fracture.

Declaration of Competing Interest

All authors declare no conflict of interest in relation to the content of this study.

References

[1] Hernlund E, Svedbom A, Ivergård M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden: a report prepared in collaboration with the international osteoporosis foundation (IOF) and the European federation of pharmaceutical industry associations (EFPIA). Arch Osteoporos 2013;8(1–2):136 Available from. doi:10.1007/s11657-013-0136-1.
[2] Watts NB, Bilezikian JP, Camacho PM, Greenspan SL, Harris ST, Hodgson SF, et al. American association of clinical endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of postmenopausal osteoporosis: executive summary of recommendations. Endocr Pract 2010;16(6):1016–19.
[3] Bielemann RM, Martinez-Mesa J, Gigante DP. Physical activity during life course and bone mass: a systematic review of methods and findings from cohort studies with young adults. BMC Musculoskelet Disord 2013;14(1):77.
[4] Neville CE, Murray LJ, Boreham CAG, Gallagher AM, Twisk J, Robson PJ, et al. Relationship between physical activity and bone mineral status in young adults: the Northern Ireland young hearts project. Bone 2002;30(5):792–8.
[5] Cashman KD. Diet, nutrition, and bone health. J Nutr 2007;137(11):2507S–2512S.
[6] Khosla S. Pathogenesis of age-related bone loss in humans. J Gerontol A 2013;68(10):1226–35 1.
[7] Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. J Bone Miner Res 2005;20(7):1185–94 7.
[8] Blake GM, Fogelman I. An update on dual-energy X-Ray absorptiometry. Semin Nucl Med 2010;40(1):62–73.
[9] Vun JSH, Ahmadi M, Pantelic M, Pountos I, Giannoudis PV. Dementia and fragility fractures: issues and solutions. Injury 2017;48S:S10–16.
[10] Smith M, Giannoudis PV. Fragility fractures: a complex interaction of the health care system- the patient and the bone: can we do better? Injury 2017;48S:S1–3.
[11] Aldebeyan S, Nooh A, Aoude A, Weber MH, Harvey EJ. Hypoalbuminaemia-a marker of malnutrition and predictor of postoperative complications and mortality after hip fractures. Injury 2017;48:436–40. doi:10.1016/j.injury.2016.12.016.
[12] Mori CM, Vicenti G, Carrozzo M, Picca G, Bizzoca D, Leone A, et al. The fake unlocked femoral nail: a configuration to avoid in stable pertrochanteric femur fractures. Injury 2018;3(49 Suppl):S32–6.
[13] Mustafa Diab M, Wu HH, Eliezer E, Haonga B, Morshed S, Shearer DW. The impact of antegrade intramedullary nailing start site using the SIGN nail in proximal femoral fractures: a prospective cohort study. Injury 2018;49(2):323–7.
[14] Rogmark C, Kristensen MT, Viberg B, Rönnquist SS, Overgaard S, Palm H. Hip fractures in the non-elderly-who, why and whither? Injury 2018;49(8):1445–50.
[15] Johnell O, Kanis J. Epidemiology of osteoporotic fractures. Osteoporos Int 2005;16(2):S3–7.
[16] Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, et al. Assessment of fracture risk. Osteoporos Int 2005;16(6):581–9.
[17] Rezaei A, Dragomir-Daescu D. Femoral strength changes faster with age than BMD in both women and men: a biomechanical study. J Bone Miner Res 2015;30(12):2200–6.
[18] Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40(5):373–83.

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[19] Marinozzi F, Bini F, Marinozzi A, Zuppante F, De Paolis A, Pecci R, et al. Technique for bone volume measurement from human femur head samples by classification of micro-CT image histograms. Ann Ist Super Sanita 2013;49(3):300–5.
[20] Hanlie H, Liyun T, Tao J. The crystal characteristics of enamel and dentin by XRD method. J Wuhan Univ Technol 2006;21(1):9–12 Available from. doi:10.1007/BF02861458.
[21] Xue J, Zhang L, Zou L. High-resolution X-ray microdiffraction analysis of natural teeth. J Synchrotron Radiat 2008;15(Pt 3):335–8 Available from. doi:10.1107/S0909049508003397.
[22] Sroga GE, Vashishth D. Effects of bone matrix proteins on fracture and fragility in osteoporosis. Curr Osteoporos Rep 2012;10(2):141–50.
[23] Leeming DJ, Henriksen K, Byrjalsen I, Qvist P, Madsen SH, Garnero P, et al. Is bone quality associated with collagen age? Osteoporos Int 2009;20(9):1461–70.
[24] Wang X, Shen X, Li X, Agrawal CM. Age-related changes in the collagen network and toughness of bone. Bone 2002;31(1):1–7.
[25] Stock SR. The mineral–collagen interface in bone. Calcif Tissue Int 2015;97(3):262–80.
[26] Bala Y, Farlay D, Boivin G. Bone mineralization: from tissue to crystal in normal and pathological contexts. Osteoporos Int 2013;24(8):2153–66.
[27] Wu Y, Ackerman JL, et al. Phosphate ions in bone: identification of a calcium-organic phosphate complex by 31P solid state NMR spectroscopy at early stages of mineralization. Calcif Tissue Int 2003;72:610–26. doi:10.1007/s00223-002-1068-8.
[28] Wu Y, Reese TG, Cao H, Hrovat MI, Toddes SP, Lemdiasov RA, et al. Bone mineral imaged in vivo by 31P solid state MRI of human wrists. J Magn Reson Imaging 2011;34(3):623–33.
[29] Increased proportion of hypermineralized osteocyte lacunae in osteoporotic and osteoarthritic human trabecular bone: implications for bone remodeling. Bone 2012;50(3):688–94.
[30] Busse B, Djonic D, Milovanovic P, Hahn M, Püschel K, Ritchie RO, et al. Decrease in the osteocyte lacunar density accompanied by hypermineralized lacunar occlusion reveals failure and delay of remodeling in aged human bone. Aging Cell 2010;9(6):1065–75.
[31] Guerado E, Cruz E, Cano JR, Crespo PV, Alaminos M, del Carmen Sánchez-Quevedo M, et al. Bone mineral density aspects in the femoral neck of hip fracture patients. Injury 2016;47:S21–4.
[32] Chun KJ. Bone densitometry. Semin Nucl Med. 2011;41(3):220–8.
[33] Blake GM, Fogelman I. The clinical role of dual energy X-ray absorptiometry. Eur J Radiol 2009;71(3):406–14.
[34] Kanis JA, Oden A, Johnell O, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int 2007;18(8):1033–46.
[35] Seeman E. Age- and Menopause-Related bone loss compromise cortical and trabecular microstructure. J Gerontol Ser A. 2013;68(10):1218–25.
[36] Chen H, Zhou X, Fujita H, Onozuka M, Kubo K-Y. Age-Related changes in trabecular and cortical bone microstructure. Int J Endocrinol 2013. [Internet]Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3614119/ .
[37] Su X, Sun K, Cui F, Landis W. Organization of apatite crystals in human woven bone. Bone 2003;32(2):150–62.
[38] Bone mineral crystal size. Osteoporos Int 2003;14(0):16–21.
[39] Boskey AL, Coleman R. Aging and bone. J Dent Res. 2010;89(12):1333–48.
[40] Hanschin RG, Stern WB. X-ray diffraction studies on the lattice perfection of human bone apatite (Crista iliaca). Bone 1995;16(4 Suppl):355S–363S.

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