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Influence of vitamin D on biomechanical microstructure and properties of patients with hip fracture

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I work for the FEIOMM to attend the 35th Congress of the ASBMR (Baltimore, 2013).

Summary

Introduction: To assess serum levels of 25-hydroxyvitamin D-25 (OH) D-hormones with influence on bone metabolism (parathormone -PTH- and insulin-like growth factor (IGF)-I), bone remodeling markers (BRM) (carboxy-terminal telopeptide of collagen type I- β -CTX- and amino-peptide pro-peptide of procollagen type I -PINP), bone mineral density (BMD), microstructure and biomechanics of the femoral neck, in patients with osteoporotic hip fracture (OH) versus arthritic patients (OA).

Material and methods: A cross-sectional observational study of 29 OH and 14 OA, age ≥ 50 years. We quantified hormonal serum levels and BRM (immunoassay), hip BMD (DXA), microstructure (micro-CT) and biomechanics (uniaxial compression tests, IGFA system). Analysis (SPSS 20.0.)

Results: OH patients had lower levels of 25(OH)D ($p=0.02$) and hip BMD ($p<0.05$), and higher PTH ($p=0.029$) and β -CTX ($p=0.04$). Levels of 25(OH)D correlated positively with IGF-I ($p=0.04$) and negatively with β -CTX ($p=0.003$). The PTH values were correlated negatively with hip BMD ($p=0.0005$) and positively with trabecular thickness (TbTh) ($p=0.006$). Patients with 25(OH)D <20 ng/mL presented higher levels of β -CTX ($p=0.006$), lower IGF-I ($p=0.007$) and TbTh ($p=0.04$).

Conclusions: Vitamin D levels are low in the elderly population, especially in patients with osteoporotic hip fracture. These patients also presented raised levels of PTH and BRM and descended from BMD. Patients whose 25(OH)D levels are below 20 ng/mL present higher bone remodeling, with lower levels of IGF-I and alterations of the bone structure (TbTh) that may be linked to a greater risk of fractures.

Key words: *vitamin D, osteoporosis, hip fracture, bone mineral density, microstructure and biomechanics.*

Introduction

Hip fracture is one of the main and most dreaded complications of osteoporotic disease. Among the risk factors that favor this type of fracture include a greater tendency to fall and a decrease in bone strength. Bone mineral density (BMD), the rate of bone remodeling, geometry, microstructure and bone tissue mineralization are fundamental properties associated with bone resistance¹.

Vitamin D is known to be essential, among other factors, in the maintenance of musculoskeletal health. Proper levels of 25-hydroxyvitamin D (25(OH)D) are needed to maintain the homeostasis of calcium metabolism and an insufficiency of these leads to a lower intestinal absorption of calcium, decreased levels of serum calcium, increased secretion of PTH, excessive rate of bone remodeling and, therefore, a lower amount and bone quality². Furthermore, levels below 30 ng/mL have been found to be associated with significant defects of bone mineralization and increase in the osteoid substance³. All of these disorders produce a decrease in bone strength and, thus, a greater risk of fracture. As for the muscle, several studies have shown the positive relationship between 25(OH)D levels and muscle strength, especially in the lower extremities in the elderly^{4,5}. Other research indicates muscular weakness and pain, as characteristic symptoms of Vitamin D-deficient syndromes⁶, as well as increased muscle strength and balance and reduced risk of falls following the administration of adequate vitamin D supplements⁷⁻¹⁰. Two meta-analyses evaluating controlled, double-blind and randomized studies conclude the beneficial effect of vitamin D supplementation, with a 19% reduction in falls, an 18% risk of hip fracture and 20% of the risk of any type of non-vertebral fracture^{11,12}. These same studies pointed out that anti-fall and antifracture efficiency of 25(OH) D is achieved when their levels are above 24 and 30 ng/mL, respectively.

In the adult population, a high frequency of inadequate levels of vitamin D has been reported, below a variable threshold that ranges between 20-40 ng/mL of 25(OH)D, according to the different authors, in several studies carried out in multiple communities in Europe and the United States¹³⁻¹⁵. In Spain, this has also been observed in the elderly population with different characteristics in different regions, with values below 15 ng/mL being described in 68% of 77-year-olds living at home, compared to 100% of those institutionalized in Andalusia¹⁶, a somewhat lower frequency in Cantabria¹⁷, and even lower levels (4.6 ng/mL as mean values) in patients with hip fracture in Madrid¹⁸.

These data reveal, on the one hand, the importance of vitamin D on muscle health and skeletal integrity and, on the other hand, the frequency of insufficient levels of the hormone among the adult population in general. However, the impact of serum vitamin D levels on the microstructural and biomechanical characteristics of bone tissue in patients with hip fracture has not been reported to

date. Therefore, the main objective of our study is to ascertain if vitamin D levels influence the microarchitecture and biomechanical properties of the bone tissue. Secondly, we also analyze the association of these levels with biochemical markers of bone remodeling and with hormones that influence bone formation and resorption (parathormone -PTH-, and insulin-like growth factor -IGF-I-, respectively) parameters, all of which contribute to bone quality and, therefore, the risk of osteoporotic fracture.

Material and methods

1. Patients

A cross-sectional observational study of 43 patients aged 50-93 years who underwent hip arthroplasty. The first group consisted of 29 (6 male and 23 female) patients with nontraumatic hip fracture (OP), considered as idiopathic (involution) osteoporosis. The second, as a comparative group, consisted of 14 patients (6 men and 8 women) with osteoarthritis (OA) and values of bone mass, at the hip level, T-score >(-2.5), with no personal history of fracture Osteoporosis or disease with influence on bone metabolism. Normal renal function was required.

All patients were recruited from the Traumatology and Orthopedics Service during the period from June 2014 to June 2015. Blood samples for biochemical determinations were collected in pairs with cases and controls in order to avoid seasonal bias in results. The protocol was reviewed and approved by the Center's Ethics Committee and all patients gave their informed consent.

The following data were collected: age, weight, body mass index (BMI), toxic habits (alcohol intake and smoking), semi-quantitative calcium intake (1 glass of milk=200 mg/day, 1 milk derivative=200 mg/day, 1 serving of cheese=200 mg/day), first-degree family history with osteoporotic fracture, treatment with vitamin D supplements and/or antiresorptive drugs. Bone mass was evaluated in the contralateral hip in the period between 15-30 days after surgery.

Bone samples were collected from the extracted femoral head for microstructural analysis and study of bone biomechanical properties after compression test.

2. Biochemical parameters

To carry out the biochemical determinations, blood samples were taken from the patients, fasting, in the first 48 hours after surgery. The following parameters, related to bone metabolism, were analyzed: calcium corrected for protein levels, phosphorus, reabsorption bone remodeling markers (carboxyl-terminal telopeptide of type I or β -CTX collagen) and formation (amino terminal propeptide of procollagen type I or PINP, parathormone (PTH), 25-hydroxyvitamin D (25 (OH) D) and insulin-like growth factor (IGF-I).

Serum levels of corrected calcium and phosphorus were assessed using the DAX-96 autoanalyzer. The levels of β -CTX and PINP were analy-

zed by immunoassay (electro-chemiluminescence), with the autoanalyzer COBAS e 601 (Roche, Spain), with coefficients of variation (CV) being interassayed <7.6% and <4.2%, respectively. Serum PTH was measured by immunoassay (electro-chemiluminescence), with autoantibody ADVIA Centaur (Siemens, Germany), with the CV interassay <5.8%. Serum 25(OH)D was analyzed by direct competitive immunoassay (electro-chemiluminescence), with the autoanalyzer LIAISON (DiaSorin, Italy), with the interassay CV <5.5%. Finally, serum IGF-I was quantified by immunoassay (electro-chemiluminescence), with the autoanalyzer IMMULITE (Siemens, Germany), with CV interassay being <3.9%.

3. Bone mass evaluation

Bone mineral density of hip and contralateral femoral neck was quantified using dual X-ray densitometry (DXA), with a Hologic-Densitometer (Hologic Inc.). The CV in vivo was 1% (total BMD).

4. Microstructural and biomechanical study of bone tissue

The analysis of the microstructure and the biomechanical properties has been made from the bone samples taken at the time of the surgical intervention for the prosthesis collation. From each femoral head, a trabecular bone cylinder from the primary compression region has been extracted with the longitudinal axis of the cylinder aligned with the main trabecular direction (MTD) (Figure 1).

Microstructural analysis of the biopsies has been carried out using computerized microtomography (micro-CT) using Bruker SkyScan 1172. The scanning of the sample was carried out at a resolution of 11 μ m, taking 2 images for each step of sample rotation (0.40°/pass, 180° total rotation). The images obtained have been reconstructed using the modified Feldkamp algorithm and later used for the quantitative and qualitative analysis of the trabecular bone microstructure.

The quantitative variables were: bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), degree of anisotropy (DA), which is a measurement of the symmetry of the object, or the presence/absence of structures aligned in a given direction, model structure index (MSI), indicating the relative prevalence of trabeculae in the form of plates or in the form of tube-cylinder and connectivity (trabecular pattern factor, Tb.Pf), which is an index of inverse connectivity, so that the higher the value the less connected the trabeculae are.

To assess biomechanical properties, uniaxial compression tests were carried out using the IGFA (image-guided failure analysis), applying a maximum force of 200 N, without reaching the elastic resistance limit of the sample. The variables that were quantified to describe the mechanical behavior of the bone tissue were the maximum stress (σ), or maximum internal resistance of the object to a force acting on it. The maximum deformation (ϵ), which represents the changes in the dimen-

sions of the object subjected to the action of force, from which microfractures occur and Young's modulus, or elastic modulus, which represents the slope of the elastic region.

5. Statistical analysis

The IBM SPSS statistical package version 20.0 (USA) was used. For the statistical analysis of the results of quantitative variables, we carried out comparisons of Student's t-means for independent samples. We did univariate linear analysis (ANCOVA) to take into account possible confounding factors such as age and BMI. Pearson's correlation test was used to assess the association between variables. To study the qualitative variables, we have analyzed contingency tables, χ^2 . In all cases, a level of $p < 0.05$ was required to consider significant differences.

Results

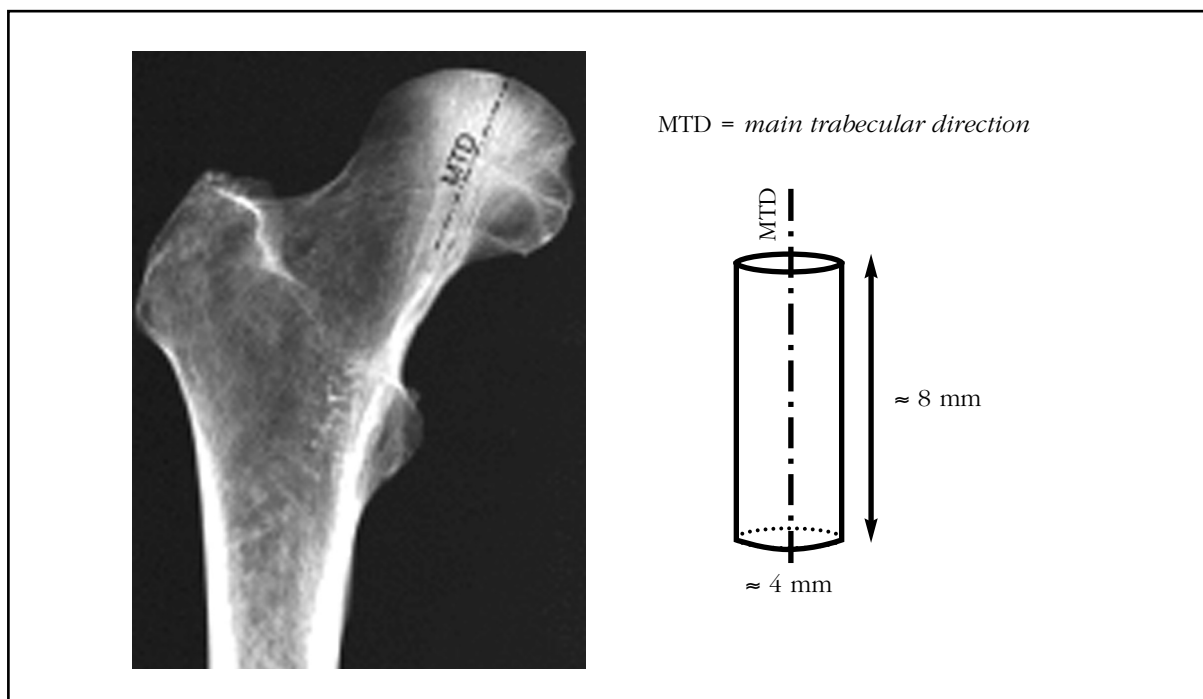
The characteristics of the patients studied in each of the groups are shown in table 1. As expected, patients with hip fracture had a significantly higher age, 81 ± 8 vs 68 ± 9 years, $p = 0.001$, a lower BMI 28.2 ± 5.8 vs 31.9 ± 4.4 , $p = 0.043$ and lower hip mass values ($p < 0.05$ for all locations). Since the group of patients with fracture and osteoarthritis were different in terms of age and BMI, the parameters analyzed were adjusted for these variables. In terms of habits, family history of first-degree osteoporosis, treatment with antiresorptive drugs and supplementation with vitamin D and calcium were similar in both groups.

1. Serum values of parameters related to bone metabolism

The biochemical parameters of the patients studied are shown in table 2. Serum levels of 25(OH)D were significantly lower in patients with fracture than in the group of patients with osteoarthritis (10.9 ± 6.9 vs 18.1 ± 10.7 ng/mL, $p = 0.02$). The values of PTH (60 ± 41.1 vs 38.2 ± 17.3 pg/mL, $p = 0.029$) and the bone resorption marker β -CTX (0.61 ± 0.26 vs 0.36 ± 0.19 ng/mL, $p = 0.04$) were significantly higher in the first group. Levels of corrected serum calcium, phosphorus, bone formation marker PINP and IGF-I were comparable in both groups. After adjustment for age and BMI, we verified that the differences that we found for 25(OH)D and β -CTX remained significant. The adjusted values for 25(OH)D were $10.7 \pm (95\% \text{ CI } 6.6-14.7)$ ng/mL for the hip fracture group and $19.6 \pm (95\% \text{ CI } 7-25.5)$ ng/mL, for that of arthrotic patients, ($p = 0.027$). The levels of β -CTX adjusted for the same variables were $0.63 \pm (95\% \text{ CI } 0.51-0.7)$ ng/mL, in the group of patients with hip fracture and $0.30 \pm (95\% \text{ CI } 0.1-0.5)$ ng/ml in the arthrosis group ($p = 0.012$).

Levels of 25(OH)D showed a significant and positive correlation with serum IGF-I ($r = 0.338$, $p = 0.044$) and negative with β -CTX levels ($r = -0.483$, $p = 0.003$). PTH levels were significantly negatively correlated with BMD-hip ($r = -0.617$, $p = 0.005$) and positively with trabeculae separation ($r = 0.530$, $p = 0.006$).

Figure 1. Localization of the trabecular bone cylinder extraction for the analysis of the microstructure and biomechanical properties



2. Bone tissue microstructure and biomechanics

Microstructural indices indicate lower bone quality in the group of patients with hip fracture than the arthrosic group. In figure 2, we show the result of scanned images of two patients, each belonging to a study group.

Although no parameter presented a statistically significant difference, we verified that the values of percentage of bone volume, as well as the thickness and number of trabeculae were lower in patients with fracture, whereas the separation between them was greater (Table 3).

The trabecular pattern index (inverse index of connectivity) presented higher values in the group of fractured patients. The structure index of the model, which implies a relative prevalence of trabeculae in the form of tube-cylinder compared to those of plate form, was higher in the group of patients with hip fracture, indicating a greater number of trabeculae in the form of a tube-cylinder, less resistant in these patients.

Biomechanics studies showed that Young's modulus values, maximal strain and maximum deformation, after applying compression tests, were lower in patients with hip fracture than in patients with osteoarthritis, although these differences were not significant (Table 3).

3. BMD and bone structure results in patients with insufficient levels of 25(OH)D (<20 ng/mL)

Serum levels of 25(OH)D in the studied population have shown very low values, in the range of 4.0-41.6 ng/mL when dividing patients into two groups, considering that these levels were less than or equal to 20 ng/mL or higher, we found the following results:

the two populations were similar in age, weight, BMI, bone mass values and biomechanical characteristics of bone tissue. However, those with levels below 20 ng/mL had higher serum β -CTX values (0.58 ± 0.25 vs 0.30 ± 0.15 ng/mL, $p=0.006$), lower IGF-I (49.8 ± 27.0 vs 83.5 ± 35.6 ng/mL, $p=0.007$) and the bone structure showed a smaller trabecular width (0.34 ± 0.17 vs 0.50 ± 0.1 mm, $p=0.04$) (Figure 3).

Discussion

This study allowed us to compare serum levels of vitamin D in patients with and without osteoporotic hip fracture and the association of these levels with markers of bone remodeling, hormones regulating bone metabolism, BMD, microstructural indexes and biomechanical properties of the femoral neck in these patients.

We found that patients with osteoporotic hip fracture had significantly lower serum 25(OH)D levels than non-fractured patients. In addition, we noted a high prevalence in elderly people with deficient levels (<20 ng / mL), especially in the OP group, in line with what was observed by other authors¹⁵⁻¹⁸, despite having approximately 3,000 hours of sunshine per year in our environment. As is known, insufficient levels of vitamin D are associated with an increased risk of falls and osteoporotic fractures^{11,12}. In our study, we also verified that patients with osteoporotic hip fracture also present higher serum levels of β -CTX and PTH, together with lower BMD values in all hip-measured locations, compared to patients without fracture. In addition, we demonstrated a significant negative correlation between 25(OH)D and β -CTX levels, as well as between PTH and BMD levels.

Table 1. Characteristics of the study population. Values expressed as mean \pm SD

	OA (n=14)	OP (n=29)	p
Age (years)	68 \pm 9	81 \pm 8	p=0.0001
Weight (kg)	77.8 \pm 17.9	68.7 \pm 13.5	n.s.
BMI (kg/m ²)	31.92 \pm 4.41	28.22 \pm 5.8	p=0.043
BMD neck (g/cm ²)	0.733 \pm 0.15	0.577 \pm 0.09	p=0.008
BMD hip (g/cm ²)	0.905 \pm 0.14	0.774 \pm 0.12	p=0.029
T-score neck	-1.3 \pm 1.1	-2.5 \pm 0.8	p=0.008
T-score hip	0.7 \pm 0.8	-1.5 \pm 0.9	p=0.034
Smoking	14.3%	11.5%	n.s.
Family history in 1st degree of osteoporosis	23.1%	30.8%	n.s.
Vitamin D treatment	23.1%	10.5%	n.s.
Intake of calcium (mg/day)	770.4 \pm 444.8	725.1 \pm 252.6	n.s.

n.s.: not significant.

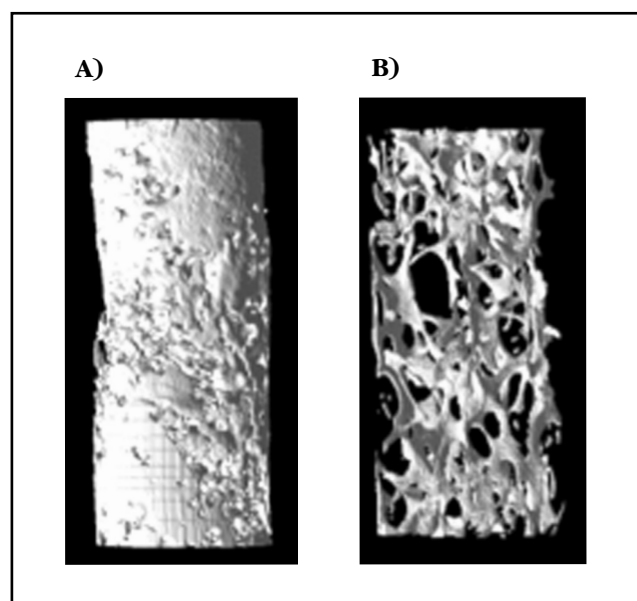
Table 2. Serum values of biochemical parameters of the study population. Values expressed as mean \pm SD

	OA (n=14)	OP (n=29)	p
25(OH)D (ng/mL)	18.1 \pm 10.7	10.9 \pm 6.9	p=0.02
PTH (pg/mL)	38.2 \pm 17.3	60 \pm 41.1	p=0.029
PINP (ng/mL)	55.8 \pm 37.6	57.6 \pm 38.0	n.s.
β-CTX (ng/mL)	0.36 \pm 0.19	0.61 \pm 0.26	p=0.04
Phosphorus (mg/dL)	3.1 \pm 0.5	3.1 \pm 0.8	n.s.
Corrected calcium (mg/dL)	9.5 \pm 0.6	9.4 \pm 0.4	n.s.
IGF-I (ng/mL)	66.3 \pm 36.6	51.7 \pm 29.0	n.s.

n.s.: not significant.

Taken together, these data once again explain that low levels of 25(OH)D are associated with high levels of PTH, that inducing a greater bone turnover would explain the higher values of bone remodeling markers. The imbalance in the levels of these parameters causes trabecular deterioration that could lead to increased risk of fracture. A high rate of bone remodeling increases the number of resorption cavities. These cavities act as "areas of stress accumulation", foci of weakness that may increase the risk of microfractures and macrofractures¹⁹. Excessive resorption may also lead to perforation of the trabeculae and permanent loss of connectivity. All these alterations described at the microstructural level of the trabecular bone make it less resistant to the load and, therefore, with an increased risk of fracture²⁰. In our study, all individuals with non-traumatic hip fracture and with osteoarthritis, except one in the latter group, had levels of 25(OH)D <40 ng/mL. Some authors report that men with radiologi-

Figure 2. Reconstruction of scanned images of patient biopsies with osteoarthritis (A) and hip fracture (B)



cal osteoarthritis of the hip have lower levels of 25(OH)D and a prevalence of deficiency higher than the control population²¹. It is generally known that up to this level of 40 ng/mL an inverse relationship between vitamin D and PTH¹⁶ is described. Although a low calcium intake may also be responsible for increasing PTH levels, in our case we found that calcium intake was low but similar in both study groups (<800 mg/day). Although this is a factor to be taken into account, we know that insufficient levels of vitamin D lead to elevated levels of PTH, despite adequate calcium intake, and conversely, sufficient levels of vitamin D maintain normal levels of PTH despite calcium intake <800 mg/day²².

Although patients with hip fracture presented lower BMD values than patients with osteoarthritis at all hip-measured locations, we did not find an association between these values and serum levels of 25(OH)D, as reported by other authors²³. However, we would like to point out that when all patients studied according to their 25(OH)D levels were <20 ng/mL or higher, the group of patients with lower levels also had lower levels of hip BMD, of 0.06 gHA/cm². These data may not be important at the individual level, but in terms of population level, taking into account each decrease of 1 standard deviation (SD), the risk of hip fracture increases by an average of 2.6 times^{24,25}. This would imply that, with the lowest bone mass found, the risk of fracture may be increased up to 1.5 times.

Many experts, based on broad population studies, have jointly pointed out that minimum levels of 25(OH)D of at least 20-30 are desirable to maintain overall health integrity, and bone in particular, Ng/mL (50-75 nM)²⁶⁻²⁹. The negative effect of insufficient levels of vitamin D on bone has been

assessed by surrogate factors such as PTH, BMD, and markers of bone remodeling. It has also been directly analyzed through its association with bone properties involved in bone strength and, therefore, the risk of fractures. In this sense, it has been verified by histo-morphometric studies of iliac crest that vitamin D insufficiency is associated with mineralization defects, showing higher levels of surface and volume of osteoid, and concluding that hormone levels higher than 30 Ng/mL to prevent pathological accumulation of osteoid³. One of the fundamental aspects of our study is the assessment of microarchitecture and biomechanical properties of the femoral neck of patients with and without osteoporotic hip fracture. Although the differences in the different parameters were not significant in any of the cases, as has been pointed out by other authors^{30,31}, we found that patients with hip fracture presented a percentage of bone volume (BV/TV), a number of trabeculae (Tb.N) and a width of these (Tb.th) 16%, 15% and 13% lower, respectively, than those of patients with osteoarthritis. In addition, the trabeculae were less connected and predominant than those presented as cylinder-tube in the fractured group, which is related to a lower bone resistance³². Along with this, biomechanical parameters also showed results between 10-14% lower in patients with hip fracture. We would like to highlight, as the most important point of this study, that it analyzes for the first time the serum levels of vitamin D, directly related to the microstructural properties of bone tissue in people with hip fracture. When comparing hormone levels among patients, we found that levels below 20 ng/mL were associated with a significant reduction in trabecular width, in addition to higher levels of β -CTX, indicating a greater activity of bone resorption, together with

Table 3. Microstructure and biomechanical bone properties of the study group. Values expressed as mean \pm SD

	OA (n=14)	OP (n=29)
BV/TV (%)	36.2 \pm 12.1	30.5 \pm 12.9
Tb.th (mm)	0.39 \pm 0.2	0.34 \pm 0.2
Tb.Sp (mm)	1.02 \pm 0.6	1.04 \pm 0.5
Tb.N (mm-1)	0.98 \pm 0.9	0.83 \pm 0.7
Tb.Pf (mm-1)	-0.08 \pm 4.2	2.81 \pm 5.0
SMI	0.44 \pm 1.3	1.07 \pm 0.8
AD	2.96 \pm 0.67	3.43 \pm 0.98
Módulo de Young (Mpa)	460 \pm 276	397 \pm 173
σ (Mpa)	9.1 \pm 4.9	8.2 \pm 3.6
ϵ	0.045 \pm 0.04	0.039 \pm 0.02

BV/TV: bone volume fraction; Tb.th: trabecular thickness; Tb.Sp: trabecular separation; Tb.N: number of trabeculae; Tb.Pf: trabecular connectivity; SMI: structural model index; AD: degree of anisotropy; σ : maximum voltage; ϵ : maximum deformation.

lower levels of IGF-I. Vitamin D status reportedly contributes to the determination of serum IGF-I levels³³. In turn, IGF-I has been shown to stimulate renal 1-hydroxylase activity, contributing to phospho-calcium metabolism, in addition to benefiting bone mass³⁴, associated with its reduction to a decrease in bone formation that contributes to bone loss in senile osteoporosis³⁵.

Our study has several limitations, especially in terms of sample size, which is relatively small. Furthermore, the dispersion of results of structural and mechanical parameters could be remedied, at least in part, with larger studies.

Overall, given the results obtained, we may conclude that the elderly population has a deficient vitamin D status, that these levels are even lower among patients with osteoporotic hip fracture and that serum concentrations below 20 ng/mL of the hormone can, directly or indirectly through PTH and IGF-I, condition an alteration in bone remodeling (β -CTX elevation) and BMD, with a consequent repercussion at the microstructural level (Tb.Th, among others) that lead to it being a low resistance bone where fractures easily occur.

Conclusions

These results indicate that patients with hip fracture have lower levels of 25(OH)D than patients with osteoarthritis, and that these induce raised PTH and increased bone resorption (with consequent increase in β -CTX levels) leading to decreased bone mass, decreased quality and increased risk of fracture. These alterations are more pronounced in patients with serum levels of 25(OH)D <20 ng/mL, in which there is also a decrease in IGF-I and trabecular width at the structural level of the femoral neck trabecular bone.

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Conflicts of interest: None of the authors have a conflict of interest.

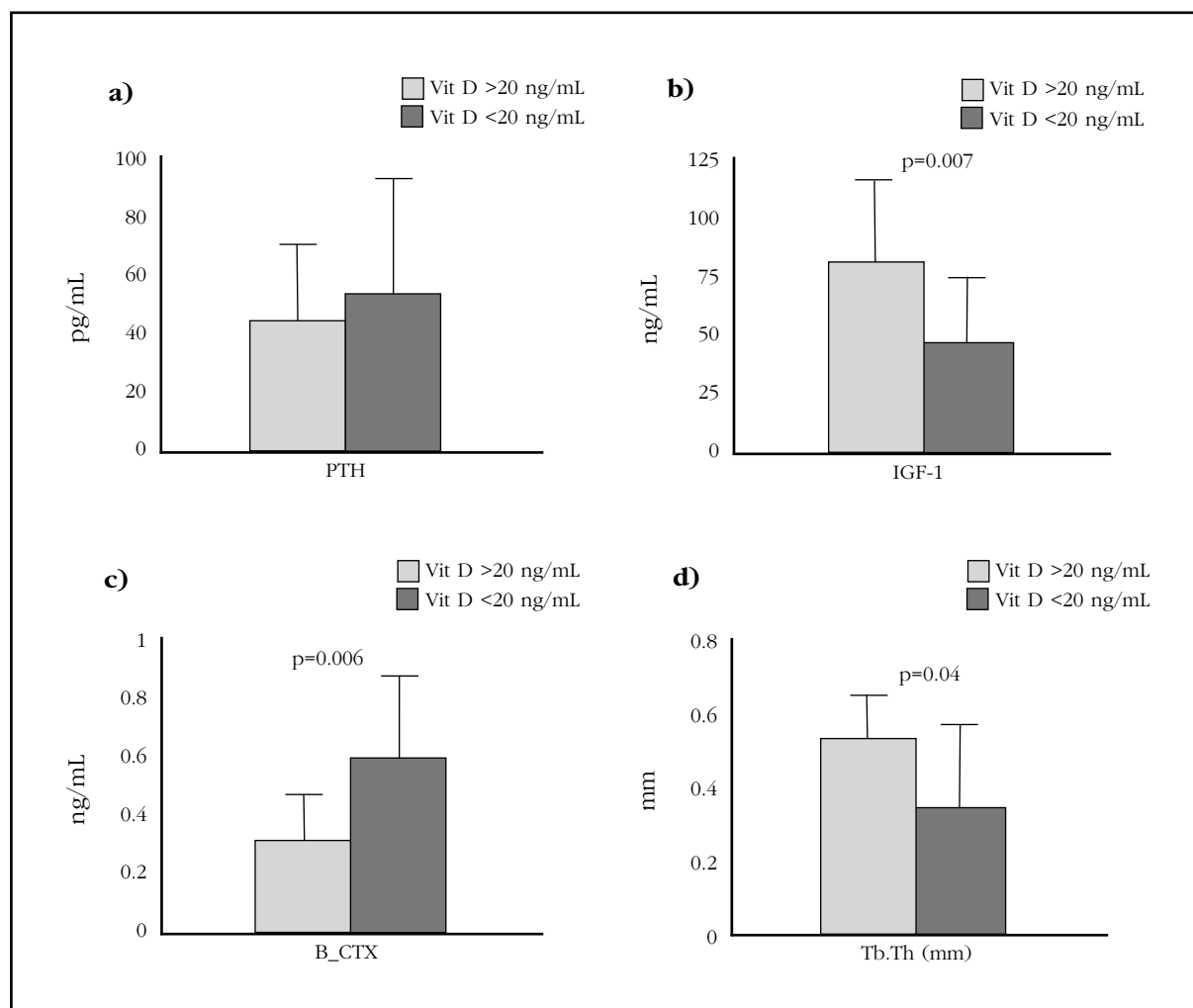
Ethics: The study was approved by the Institutional Ethics Committee (Macarena University Hospital, Seville, Spain) and informed consent was obtained from each participant.

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Figure 3. Serum levels of PTH (a), IGF-I (b), β -CTX (c) and Tb.Th -trabecular enlargement- (d) of patients with serum levels of 25(OH)D > 20 ng/mL (n=10) or \leq 20 ng/mL (n=33)



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