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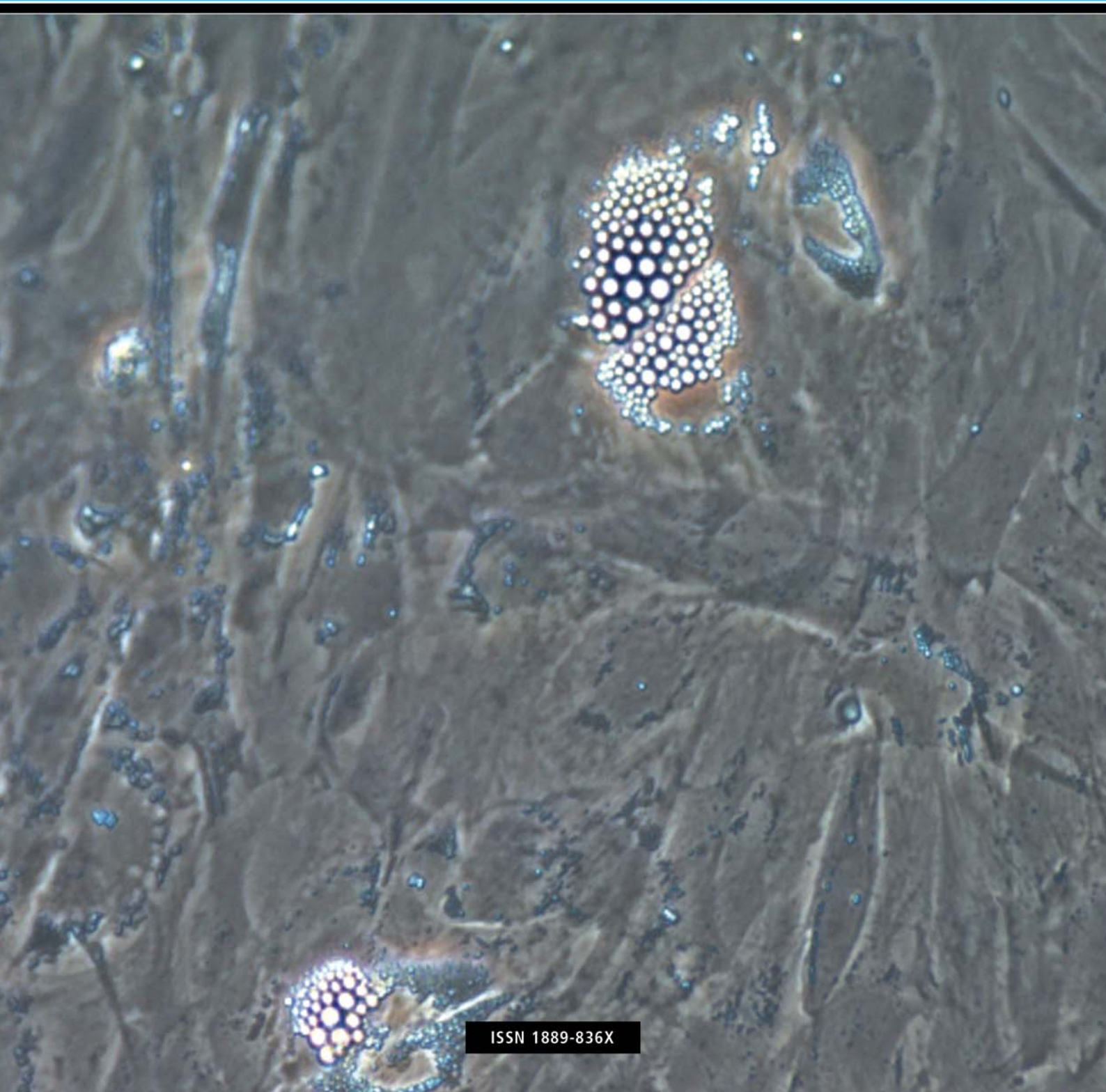

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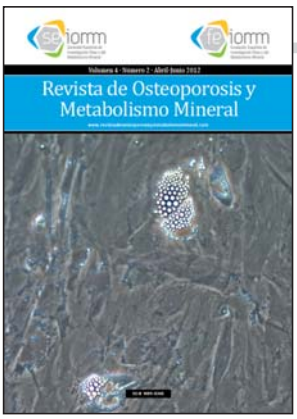

Our cover

Adipocytes in culture of mesenchymal stem cells isolated from human bone marrow cells induced to differentiate into osteoblasts in presence of acids omega-6 polyunsaturated fatty

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Osteoporosis during pregnancy and breastfeeding

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Both generalised and regional osteoporosis are diseases which, exceptionally, are associated with pregnancy and breastfeeding, although, undoubtedly, diseases that are underdiagnosed.

Compensatory physiological mechanisms allow, in the majority of cases, those requirements necessary for the formation and mineralisation of the foetal skeleton and the nutrition of the new born to be met, overcoming this period without major difficulties[1]. However, some mothers experience bone demineralisation which may become complicated with fractures[2], and a small group suffers regional demineralisation which temporarily disables them[3].

The fundamental problem in these situations is the diagnosis since, on the one hand, some of the associated symptoms may be attributed to "normal" problems related to gestation, such as pelvic pain, which are frequently seen in the typical clinical picture during the third quarter of pregnancy, while on the other, there is the impossibility of carrying out diagnostic procedures such as DXA during pregnancy.

In addition, ultrasounds do not help to establish clearly changes in bone mineral density associated with gestation[4]. And markers for bone remodelling do not help either, since during gestation they have significant biases in interpretation due to the haemodilution which occurs, the increase in glomerular filtrate and the placental production of alkaline phosphatase. It should also be added that the ranges of normality during gestation have not been established.

During pregnancy and breastfeeding, compensatory mechanisms occur which meet the needs of the foetus. Calcium is actively transferred through the placenta, especially in the third quarter, with the aim of ossifying the collagen matrix of the foetal skeleton. The blood levels of calcium in the mother are reduced by the haemodilution, and the greater requirements are compensated for essentially by an increase in intestinal absorption, related with an increase in the production of 1,25 $(OH)_2D$.

Initially it was considered that gestation resulted in a state of hyperthyroidism; however, with advances in technology, is has been possible to establish that levels of PTH are found to be slightly reduced in the first quarter and normal in the other two. Nevertheless, the level of the peptide related with PTH (PTHrP) is found to be raised during gestation. This is a prohormone which produces many peptides with various biological properties. This finding has opened up an exciting field in the study of these molecules, with various sources of production being identified, such as the placenta, myometrium, breast, decidua, amniotic membranes and foetal parathyroids[5].

In spite of the fact high levels of calcitonin are found in pregnancy, produced by hypertrophied thyroid C cells, and possibly in the breast and placenta, it does not appear to have a significant impact on calcium metabolism during pregnancy.

This is not the case with vitamin D, which increases its blood levels to twice that in non-gestation, possibly related to an increase in globulin transporters produced in gestation, although an increase in blood levels of its free parts has also been found, due more to an increase in production than a reduction in clearing. This increase is independent of the action of PTH and is, principally, due to an increase in the activity of 1α-hydroxylase in the maternal kidney. Estrogens, prolactins, placental lactogen and PTHrP also increase renal enzyme activity. The placenta and

the foetal kidneys may also be additional sources. Vitamin D crosses the placenta and in the foetus reaches levels of around 20% lower than the mother's.

A question has been raised about what the impact of these adaptive changes would be on the maternal bone. Histomorphometric studies in gestating animals suggest that there are no substantial modifications in the mineral content of bone, nor in its structure[6].

Some studies whose evaluation of bone mineral density was carried out immediately after birth, or a miscarriage at different times in the gestation period, show results which are variable and difficult to interpret due to the presence of factors which create confusion, such as changes in the composition, weight and volume of the skeleton[7]. Nevertheless, it appears that gestation does not significantly affect the density or resistance of the bone and, in fact, epidemiological studies in the postmenopause have established no relationship between giving birth and the period of breastfeeding and low bone mass and risk of fracture later in life[8,9].

During breastfeeding, 400 mg/day is lost due to giving milk. Differently from the way that this happens during gestation, where the main mechanism for the maintenance of calcium homeostasis is an increase in intestinal absorption, the increase in demand is mainly offset by an increase in bone resorption and, partly, by an increase in renal resorption. Both mechanisms are found to be mediated by high levels (some 1,000 times higher than those in gestation) of PTHrP, which is mainly secreted by the breast, and not by PTH, which remains low during breastfeeding. The intestinal absorption of calcium returns to pre-gestation levels after birth.

The increase in prolactin during breastfeeding is associated with a decrease in estrogens which are subject to the increase in PTHrP resulting in an observation of loss of bone mass of around 2-3% per month of breastfeeding. However, these losses are recovered rapidly after the end of breastfeeding, with gains of between 0.5 and 2.0% per month, which means that the recovery is total after a period of 2-6 months. It has been noted that supplementing calcium during breastfeeding does not prevent the bone loss which occurs during this period [10].

In view of the above, It can be stated that in a healthy woman pregnancy and breastfeeding induce changes in the bone, but that they do not, however, effect the health of the bone over the long term.

It is not known why in some women osteoporosis occurs during pregnancy. The rareness of this association means that the series of cases published is short, and therefore that no patterns of risk which may be associated with osteoporosis during gestation have been established, although primiparity, maternal history of fracture, the use of corticoids and low weight have all been indicated.

The diagnosis is usually carried out in the presence of severe back pain making it possible for vertebral fractures to be identified. It is accepted that there is no recurrence in later pregnancies, although cases followed up over the long term with a number of pregnancies in the same woman are very scarce.

Transitory osteoporosis during pregnancy happens suddenly in the third quarter, and progressively immobilises the mother. Radiological studies show a large loss of bone mass and oedema which may result in fracture[11]. It has been suggested that the origin of this may be found in neurological problems (compression of the obturator nerve), vascular compromise, oedema in the bone medulla, or nutritional deficiencies, but none of these clearly explains the clinical picture.

In addition, no definitive course of treatment has been established, with recommendations varying from bed rest to waiting for the end of gestation to start antiresorptive treatment or to perform orthopaedic surgery.

In summary, it is important to consider generalised or regional osteoporosis during pregnancy, although it is a rare association, by carrying out differential diagnosis of pain which appears suddenly and leads to immobilisation, especially in the third quarter of pregnancy, in order to make an early diagnosis and avoid complications such as fracture.

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Pregnancy-associated osteoporosis. Presentation of 5 cases and long term monitoring

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Summary

Background: Pregnancy-associated osteoporosis (PAO) is a clearly described and relatively frequent entity, although there are few studies which have carried out long term monitoring of the disease.
Material and methods: 5 women affected by osteoporosis who were monitored over the long term, between 4 and 16 years. In all the patients a questionnaire on lifestyle and risk factors was completed and a physical examination carried out, as well as densitometry after the pregnancy and subsequently every year during the follow up period. At the end of this period, a lateral X-ray of the dorsal and lumbar spine was performed.
Results: In 3 cases there was a spontaneous fracture as the first manifestation of PAO, while in 2 cases a very low densitometric value was observed, without fractures, in the immediate postpartum period. All the patients received calcium and vitamin D supplements, and in 3 cases a biphosphonate (risedronate) was indicated. No new fractures were observed in any of the cases over the follow up period. The bone mineral density (BMD) increased in the lumbar spine in all the patients, but in one a decreased measurement was observed in the hip, both in the femoral neck and the total hip. None of the patients became pregnant again in the period of the study.
Conclusions: While none of the patients with PAO studied over the long term suffered a new fracture, and in all an increase in the bone mineral density in the lumbar spine was observed, which makes us think that there is a recovery of DMO over time, in one case there was a considerable decrease in bone mineral density in the proximal femur, for which reason we believe that it is advisable to carry out long term monitoring of these patients.

Key words: *osteoporosis, pregnancy, fracture, densitometry, monitoring.*

Introduction

Pregnancy-associated osteoporosis (PAO) was described for the first time more than 50 years ago[1]. It usually appears in women of between 25 and 30 years of age in their first pregnancy and may develop with lumbar pain, loss of vertebral height and compression fractures[2-5], although in most cases the disease usually passes unrecognised, since it symptoms may be attributed to back pain similar to that produced by the exaggeration of lumbar lordosis in pregnant women. Although it appears not to be a frequent disease, its exact prevalence is unknown[6], and is possibly underestimated for the aforementioned reason, combined with the avoidance of X-rays of the spine and, obviously, of densitometry in pregnant patients.

Some authors have described four types of osteoporosis associated with pregnancy and breastfeeding[7]: a) idiopathic osteoporosis in pregnancy, b) transitory osteoporosis in the hip during pregnancy, c) post-pregnancy or breastfeeding-associated lumbar osteoporosis, and d) drug-induced osteoporosis.

Case-based descriptions of PAO usually consist of few cases, and with a follow up normally over a limited time period[6,8-11]. Although it is accepted that fractures do not tend to occur in subsequent pregnancies and that this type of osteoporosis is usually self-limiting[12,13], there are few descriptions which include densitometric and clinical follow up over the long term, especially in our country.

Therefore, we present in this study a series of 5 cases of as many patients diagnosed with PAO and followed up over several years.

Patients, materials and methods

In carrying out this work, between 1989 and 2010 5 patients who were diagnosed with pregnancy-associated osteoporosis in the Bone Metabolism Unit of the Insular University Hospital were studied. The diagnosis of the disease was made by means of a radiological study and densitometry carried out due to the presence of back pain on the final three months of pregnancy, or post-partum, either by the detection of a fragility fracture and/or by obtaining a densitometry T-score of ≤ –2,5. All the patients were Caucasian. Their informed consent was requested and the study was approved by the ethics committee of the Insular University Hospital of Gran Canaria.

A complete clinical history of all patients was taken, along with a detailed physical examination, in order to note risk factors for osteoporosis[14]. The current intake of calcium was estimated using the Cummings method[15].

Determination of bone mineral density by dual energy X-ray absorptiometry (DXA)

A measurement of bone mineral density (BMD) was made both in the lumbar spine (L2-L4) and in the proximal extremity of the femur using at the first stage a Hologic® QDR-1000 densitometer and subsequently Hologic® QDR-4500 Discovery. In order to calculate the T-score the following formula was applied:

T-score = (observed value – value of peak BMD)/typical deviation from BMD.

The peak BMD values were those previously published as normal in the Canarian population[16]. A variation coefficient of 0.75 ± 0.16% was obtained. All the determinations of BMD were carried out by the same technician (JS).

Diagnosis of fractures

The vertebral fractures were diagnosed by means of a lateral X-ray of the dorso-lumbar spine, from D3 to L5, applying Genant's diagnostic criteria[17]. The presence of non-vertebral fractures was garnered from reports provided by the patients and subsequently confirmed by hospital records. Fragility fractures were considered to be those produced by a minor trauma or by a fall to the ground from a maximum height of the person themselves. Excluded were fractures resulting from traffic accidents or from falls from a greater height, as well as cranial, face, metacarpal and phalangeal fractures.

Results

Table 1 shows the baseline characteristics of the patients studied. All the women had a current intake of calcium lower than 800 mg a day and were sedentary, except one who had high levels of physical activity. None of the patients smoked or drank alcohol during their pregnancy, but one of them returned to smoking after pregnancy and four started to drink alcohol, albeit moderately. All the patients returned to their normal weight after pregnancy, with the exception of one woman who remained overweight.

Table 2 lists the year of diagnosis of PAO, the year of the last review and period of time that each patient was followed. The period of follow up was variable, varying between 4 and 16 years. In three cases the follow up was longer than 12 years.

Table 3 includes gynaecological data. The age of the patients during the pregnancy which resulted in PAO was higher than 30 years in all cases, except for one patient, who was 29 year of age. In three cases the PAO happened during the 2nd pregnancy, and in the two remaining patients, during their first gestation. All the patients, with the exception of one, breastfed their children, although lactation lasted only a few months, less than, or equal to, 6 months in all cases. Subsequent to the pregnancy which resulted in the PAO and after the follow up, none of the patients became pregnant again, or suffered a miscarriage. Over the follow up period two of the patients experienced the menopause (those with the longest period).

Table 4 show the clinical data relating to the patients. None had comorbidity: no cases of AHT, diabetes mellitus, hypercholesterolemia or urolithiasis, and in only one case was there a congenital mitral prolapse. In three cases the PAO pro-

Table 1. Baseline characteristics of the patients studied

	Patient 1 RMM	Patient 2 FGB	Patient 3 PMH	Patient 4 MSE	Patient 5 TMP
Age (years)	31	31	37	38	35
Size (cm)	175	160	165	155	170
Weight (kg)	64	61	58	52	78
BMI (kg/m^2)	20.9	23.8	21.3	21.6	26.9
Current intake of Ca (mg/24h)	750	650	650	750	750
Consume tobacco	No	Ex-smoker	Yes	No	No
Consume alcohol	Occasional	Occasional	Yes	Yes	No
Physical activity	Sedentary	Sedentary	Sedentary	Sedentary	High

BMI: body mass index; Ca: calcium

Table 2. Year of diagnosis of PAO and follow up time in each case studied

	Patient 1 RMM	Patient 2 FGB	Patient 3 PMH	Patient 4 MSE	Patient 5 TMP
Diagnosis year	1993	1991	1997	2004	2007
Year last reviewed	2009	2007	2010	2010	2011
Year follow-up	16 years	16 years	13 years	6 years	4 years

gressed with fracture at the time of diagnosis: in two patients the fractures were costal and in one, vertebral, without previous trauma in either case. After the pregnancy, drug treatment with risedronate was indicated in three patients, and calcium and vitamin D supplements in all. None of the patients had a further fracture during the years of follow up. In three cases, new diseases appeared: hypophyseal adenoma, hypothyroidism and bilial lithiasis.

In Table 5 can be seen the changes produced in BMD, both in the lumbar spine and in the proximal extremity of the femur. The change is highly variable, since in one case the recuperation of BMD reached 137% in L2-L4 after 16 years of follow up, while in another patient a significant decrease was found in BMD in total hip, with a loss of 17.6% after 4 years of follow up. In all the women there was an increase in BMD in the lumbar spine, while in four cases the BMD in the total hip increased. In the femoral neck there was a decrease in BMD in two cases.

Discussion

PAO is a syndrome known since 1955, since the initial description of Nordin and Roper[1], which was followed that same year by another article from Bret[18]. In the first publications only isolated cases were presented, until 1985 when Smith et al. put together a list of eight women who had been followed up for 10 years[5]. In this British series the patients mainly began with a vertebral fracture, which was not reproduced in successive pregnancies. A bone biopsy was carried out on these patients and in only one case were signs of increased bone resorption observed, the other seven cases being normal. Also normal were the analysis data, including vitamin D and calcitonin. The authors concluded that in these patients there could have been a transitory failure in the calciotropic hormones which prepare the maternal skeleton for birth.

Almost 60 years after Nordin and Roper's description, we still do not know what is the physiopathological mechanism by which PAO is produced. Many possible causes have been suggested, such as an insufficient ingestion of calcium and vitamin D[19], a change in the regulation of parathyroid hormone (PTH) or of the peptide related to PTH (PTH-RP)[7] or even the coexistence of a previous disease which could cause the alteration of bone mineral homeostasis, such as the coexisting treatment with corticoids or with heparin, a weak form of osteogenesis imperfecta or anorexia nervosa[3].

In our series of 5 cases, the average age of the patients during the pregnancy producing the disease was 34.4 years. In a larger series concerning

Table 3. Gynaecological data of each patient studied

	Patient 1 RMM	Patient 2 FGB	Patient 3 PMH	Patient 4 MSE	Patient 5 TMP
Age at menarche (years)	14	12	14	12	13
Age at pregnancy* (years)	31	29	36	32	35
Total number of pregnancies	2	2	2	1	1
Pregnancy in which PAO occurred	2º	2º	2º	1º	1º
Previous miscarriages	1	No	No	No	No
Later miscarriages	No	No	No	No	No
Subsequent pregnancies	No	No	No	No	No
Breatsfeeding*	2 months	6 months	1 month	No	6 months
Current menopause	Yes	Yes	No	No	No
Ca supplement during pregnancy*	Yes	Yes	Yes	Yes	Yes
Increase in dietary Ca*	Yes	No	Yes	Yes	No

* what time of diagnosis of the PAO; Ca: calcium

PAO, the average age of the patients was 27 years[3], but this was published in 1995 after 24 years of follow up, which means that most of the patients had had their pregnancy in the 1970s. It is well known that nowadays, for sociocultural reasons, women usually become pregnant later in life, which means that these data are not compatible. The age of occurrence of PAO in other series varied widely, between 25 and 40 years of age[1-10,20-23].

Our patients did not have a high risk profile for osteoporosis. At the start of the pregnancy which resulted in the disease, only one was a smoker, after which she stopped smoking. None had family history of fragility fractures (particularly maternal), none had taken corticoids, and while their dietary intake of calcium was somewhat low, with a median of 750 mg a day, in all cases they had received calcium and mineral supplements during pregnancy.

One of the most controversial matters in relation to this syndrome is the understanding of whether the skeleton recuperates in the long term, if there is a high risk of new fractures in future pregnancies and if it is necessary or not to indicate treatment, and with which drug. The initial publications of Smith et al.[3-5] suggested a favourable outcome the patients. In fact, in a series of 24 patients followed over 24 years, only one patient continued to have new fragility fracture, but suffered from a weak form of osteogenesis imperfecta. 10 patients had 14 pregnancies after that in which the PAO happened, which progressed completely normally without new fractures. However, other cases have been published in which the development was not so favourable. Thus, in a series of 11 patients, followed up over between 1 and 19 years, 3 patients had new fractures after the pregnancy[24]. In our series, none of the patients had any new fractures. In spite of this, none became pregnant again.

The BMD was determined in all our patients after pregnancy and in no cases were earlier densitometries available. In all cases we found low values of BMD in the lumbar spine, in four of the five cases below a T-score of -2.5. In one case the T-score value was -2.2, which, combined with the presence of costal fractures, justified the diagnosis of PAO. The changes in BMD in our patients over time was disparate. As can be seen in Table 5, in the lumbar spine a gain was seen in all cases, with an increase in BMD which varied between 1.7 and 137%. However, in the BMD in the femoral neck, in some patients a marked improvement was observed, whilst in others a fall of up to 17.6% was observed, despite the fact of four years having passed since the pregnancy and the patients having received treatment with risedronate, calcium and vitamin D. This wide variation in the development of BMD has also been described in other series[2-4,7-9,12,22] and suggests a significant heterogeneity in the clinical characteristics of those patients who have suffered from PAO[25].

In conclusion, a long term follow up has been carried out of a series of 5 women who suffered from PAO. None of them became pregnant again, nor did they suffer new fractures. Although the BMD improved in all cases in the lumbar spine (L2-L4), in two cases a decrease was found in different locations of the proximal femur. While PAO appears to be a transitory disease, which progres-

Table 4. Clinical data of the patients at the time of diagnosis of pregnancy-associated osteoporosis (PAO) and at the end of follow up

	Patient 1 RMM	Patient 2 FGB	Patient 3 PMH	Patient 4 MSE	Patient 5 TMP
AHT	No	No	No	No	No
Diabetes	No	No	No	No	No
Urolithiasis	No	No	No	No	No
Other diseases present PAO	No	No	No	Mitral valve prolapse	No
Presence of fracture*	Costal	Costal	No	No	Vertebral
Later fractures	No	No	No	No	No
Treatment for OP after pregnancy	Risedronate Ca + Vit D	Ca + Vit D	Risedronate Ca + Vit D	Ca + Vit D	Risedronate Ca + Vit D
Other new diseases	Adenoma of hypophysis	No	Hypothyroidism	Litiasis biliary	No

* in the moment of the diagnosis of the PAO

Table 5. T-score values from the baseline densitometry and changes in BMD at the end of the follow up

	Patient 1 RMM	Patient 2 FGB	Patient 3 PMH	Patient 4 MSE	Patient 5 TMP
T-score L2-L4	-2.6	-2.2	-2.7	-3.4	-4.4
L2-L4 (%)*	137	4.6	1.7	16.9	29
T-score femoral neck	-0.9	-0.2	-1.0	-2.2	-2.1
Femoral neck (%)*	34.5	-2.5	18.9	1.9	-5.5
T-score Total hip	-1.2	-0.2	-0.5	-0.9	-2.2
Total hip (%)*	5.9	8.7	32	0.3	-17.6

* percentage change in BMD at the end of the follow-up period

ses favourably, given the absence of new fractures, it is advisable to carry out a follow up of these patients, since there are cases in which BMD is not normalised and could result in an increased risk of fracture.

Conflict of interest: The authors declare that they have no conflicts of interest of any kind.

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Seasonal variation in vitamin D levels in patients attending in Basic Healthcare Center

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Summary
Background: Previous reports have shown a high prevalence of vitamin D deficiency among different populations in our country. However fewer longitudinal studies about seasonal changes in serum vitamin D have been published. The aim of the present study was to determinate seasonal variation in serum vitamin D in patients attending in Basic Healthcare Center.
Patients and method: Prospective longitudinal cohort study of 82 patients attending in Basic Healthcare Center. In all cases, serum levels of calcium, 25OHD and PTH were determined during January, February and March (Period 1) and September and Octuber (Period 2).
Results: Serum calcium levels did not differ between Period 1 and Period 2. During Period 1, 50.6% presented 25OHD levels < 15 ng/ml and 3.65% presented 25OHD levels > 30 ng/ml. During Period 2, 25OHD levels increased (31.88 vs 15.75 ng/ml, p < 0,001). Prevalence of patients with 25OHD levels < 15 ng/ml decreased (2.7 vs 50.6%, p < 0,001) and prevalence of patients with 25OHD levels > 30 ng/ml increased (50.68 vs 3.65%, p< 0,001). Negative correlation between 25OHD and PTH concentrations during both periods was observed.
Conclusions: These results show vitamin D deficiency during winter months in the majority of patients attending in Basic Healthcare Center. The prevalence of patients with vitamin D deficiency decreased after summer, however only half of the patients reached optimal vitamin D levels. Based on our results, to guarantee optimum vitamin D levels in the general population, the promotion of sanitary policies is recommended.

Key words: *vitamin D deficiency, secondary hyperparathyroidism, sunlight.*

Introduction

At the end of the 1930s the chemical structure of vitamin D[1] was described. Human beings obtain vitamin D naturally through the diet and by exposure to the sun. Ultraviolet B solar radiation penetrates the skin and converts 7-dehydrocholesterol into previtamin D_3, which is rapidly transformed into vitamin D_3. The vitamin D_3 in the skin, and that originating from the diet, are metabolised in the liver into 25-hydroxy-vitamin D–25(OH)D– whose determination constitutes the best index for the evaluation of vitamin D reserves in the organism. The 25(OH)D is metabolised in the kidneys by the action of the enzyme 25-hydroxy-vitamin D-1 α–hydroxylase into its active form, 1,25-dihydroxy-vitamin D–1,25(OH)2D-[2].

In recent decades there has been a notable increase in the understanding of the role of vitamin D in the pathogeny of some diseases, and it has been confirmed that its deficit is extraordinarily common in many sections of the population, which has led some to suggest an almost "epidemic" character to this deficiency[3]. In addition, the confirmation of the presence of vitamin D receptors in numerous tissues and cells of the organism suggests that vitamin D may play a role in the pathogeny of various cardiovascular and autoimmune diseases[4].

The estimated prevalence of vitamin D deficiency in the general population of the US is 9%[5]. In Europe, where, differently from the US, only a few foods are enriched with vitamin D, the general population would have a higher risk of suffering this deficiency[5], in the region of around 40%[6], with a lower prevalence in the Scandinavian countries, while the estimated prevalence in other continents varies between 30% and 94%[7].

In those studies published in our country, the prevalence varies between 30% and 70% for independent people of an advanced age, and is even higher (70-100%) in people who are institutionalised or with multiple pathologies[8]. In other sectors of the population, such as postmenopausal women[9], patients with bone fractures[10], and individuals with risk of osteoporosis[11], the prevalence varies between 39 and 70%. Lastly, studies in the young healthy population show different degrees of vitamin D deficit with a prevalence which varies between 27 and 56%[12].

Exposure to sun plays a fundamental role in vitamin D concentrations. Various studies show significant differences in the values of 25(OH)D according to the season of the year in which the determination of the concentration of vitamin D was carried out[3].

In our country, most of the works concerning vitamin D deficiency previously published are transversal, and study this deficiency in certain sectors of the population, such as institutionalised older people, postmenopausal women or patients with osteoarticular pathology. While, on the other hand, studies carried out in a healthy population, of prospective and longitudinal design, are scarce. The objective of this work was to understand the influence of exposure to sun, according to the season of the year, on blood concentrations of vitamin D in a cohort of subjects being treated at a primary healthcare clinic.

Material and method

Design and ambit

Observational, analytical, longitudinal and prospective study of a cohort of mobile adults aged between 18 and 80 years, treated at a primary healthcare clinic of the Orcasitas Health Centre pertaining to the Primary Care Area 11 of Madrid, over two periods: Period 1, in the months of January, February and March, and Period 2, in the months of September and October.

Sample and inclusion criteria

The minimum sample size, according to an α risk of 5%, an absolute precision of 10%, an expected proportion of 0.25 and 10% of losses, was 77 people for each group, who were selected by simple random sampling. Pregnant women, subjects with limited mobility, patients with chronic systemic diseases (nephropathies, hepatopathies, neoplastic disease and malabsorption syndromes) endocrinopathies (thyroid, parathyroid and suprarenal disease) and/or in treatment with drugs which affect calcium metabolism, such as vitamin D, biphosphonates, calcitonin, calcium supplements, multivitamins, glucocorticoids, theophylline, lithium, diuretics, statins, anti-convulsives, isoniacide or oral anticoagulants.

The participants were informed of the objectives of the study, gave their verbal consent in all cases and the approval of the Ethics and Research Committee of the Primary Healthcare Area 11 region was given. With the aim of avoiding the influence of variables other than seasonal exposure to sun, the subjects received instructions not to modify their dietary habits or their normal degree of exposure to sun during the period of the study.

Biochemical determinations

Venous blood samples were extracted, in fasting, for the biochemical study. The following determinations were carried out: haemogram, basic biochemistry, total calcium, phosphorus, calcium corrected according to blood albumin, albumin, magnesium, thyrotropin (TSH), intact parathyroid hormone (PTH) and 25(OH)D.

The figures for calcemia were corrected according to the albuminemia with the following formula: total calcemia + [0.8 (4 – total albuminemia)].

The concentrations of 25(OH)D were quantified by chemiluminescent immunoanalysis with the Isys (Vitro®) autoanalyser (reference values 14 – 75 ng/ml), with intra-trial and extra-trial coefficients of variation of 3% and 4.6% respectively. The concentrations of intact PTH were measured by chemiluminescent immunoanalysis, with the Immulite 2000 autoanalyser (Siemens®) (reference values 7 – 57 pg/ml) with intra-trial and extra-trial coefficients of variation of 4.1% and 5.9% respectively.

Table 1. The average concentrations of calcium, 25(OH)D and PTH during Periods 1 and 2

Variables	Period 1 (January-March)	Period 2 (September-October)	Significance
Corrected calcium (NV[a]: 8.4-10.2 mg/dl)	8.99 (CI 95%: 8.90-9.08)	8.97 (CI 95%: 8.87-9.07)	p = 0.653
25(OH)D (NV[a]: 14-75 ng/ml)	15.75 (CI 95%: 14.35-17.15)	31.88 (CI 95%: 28.76-35)	p < 0.001
% 25(OH)D <15 ng/ml	50.6	2.7	p < 0.001
% 25(OH)D >30 ng/ml	3.65	50.68	p < 0.001
PTH	32.68	27.95	p = 0.001
(VN[a]: 7-57 pg/ml)	(CI 95%: 29.95-35.40)	(CI 95%: 24.37-31.53)	

[a]Normal values

Definition of vitamin D deficit

The cut off point was established at 15 ng/ml in our study, according to earlier publications which showed an increase in the values of PTH in subjects with concentrations of 25(OH)D equal to or less than this value[7]. The optimum level of vitamin D was established at values of 25(OH)D higher than 30 ng/ml[8].

Statistical analysis

The quantitative continuous variables are expressed as the mean, with the confidence interval (CI) at 95%, and qualitative variables as relative frequencies or percentages.

To compare the qualitative variables between the two periods the Student's t test was used for paired data and the chi-squared test for qualitative variables.

To study the relationship between variables the Pearson correlation coefficient was used.

In all cases the test was considered to be statistically significant when $p < 0.05$. The statistical study was carried out using a social science statistics software package (SPSS 9.0.1).

Results

In Period 1 82 subjects were included, 52% women, with an average age of 54.93 years (CI 95%: 50.94 – 58.91). The average concentrations of corrected calcium, 25(OH)D and PTH are shown in Table 1. 50.6% of the subjects showed values of 25(OH)D lower than 15 ng/ml, and 2.4% values of PTH above the upper limit of the normal range.

In Period 2 73 subjects were included (9.88% losses compared with Period 1), 52.3% women with an average age of 53.89 years (CI 95%: 51.37 – 57.20). The values of corrected calcium were similar to those obtained in Period 1. A significant increase was seen in the values of 25(OH)D compared with Period 1, along with a decrease in the percentage of subjects with values lower than 15 ng/ml. The percentage of subjects with values of 25(OH)D higher than 30 ng/ml was increased significantly during Period 2 (50.68% vs 3.65%, $p<0.001$). The values of PTH in Period 2 fell significantly (Table 1). In both periods there was a significant inverse correlation between the values of PTH and those of 25(OH)D (Figures 1 and 2).

Discussion

The results of our study show that more than half of the population treated at a primary healthcare clinic had vitamin D deficiency during the winter months. While there are different classifications of the degrees of hypovitaminosis D[13], in our study we established vitamin D deficiency as being a concentration of 25(OH)D equal to or less than 15 ng/ml, the value above which a compensatory secretion of PTH is initiated[7]. Given this cut off point, the prevalence of vitamin D deficit of our population is higher than that described in our country in a population at risk of osteoporosis[11] and in women of a fertile age[14], and lower than that in a group of postmenopausal women from a rheumatology clinic[15]. However, the use of other criteria for vitamin D deficiency, the absence of a

Figure 1. Correlation between 25 (OH) D and PTH during Period 1

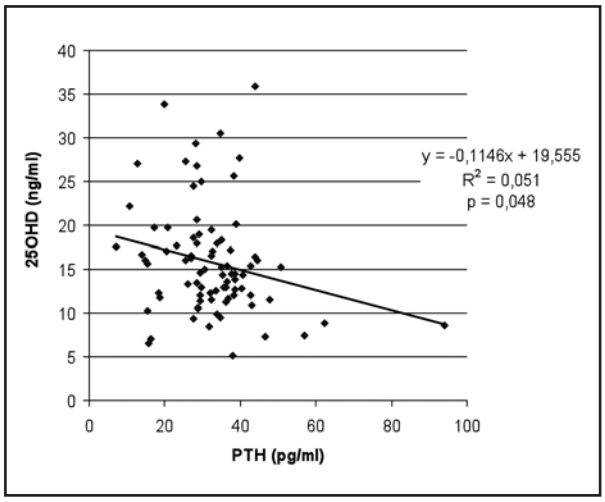
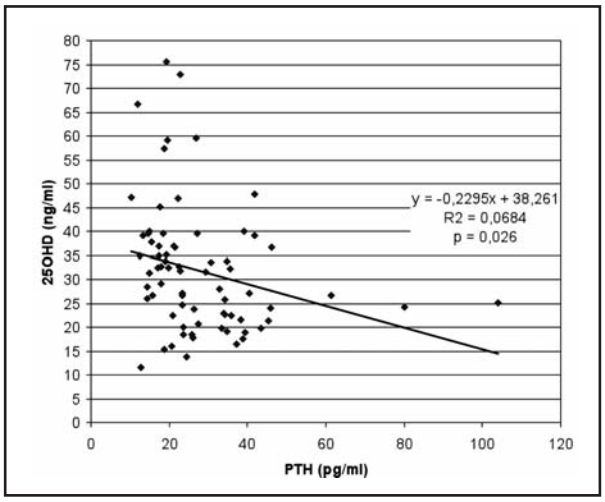


Figure 2. Correlation between 25 (OH) D and PTH during Period 2



precise definition of the season in which the samples were taken, and the presence of pathologies and circumstances which predispose a person to vitamin D deficiency in these earlier works, are circumstances which limit comparison with our results.

Although, obviously, the prevalence of vitamin D deficit recorded in our study cannot be extrapolated to the general population, it approximates to that outlined in McKenna's review[6], in which more than 40% of adults from western and central Europe had vitamin D deficiency during the winter months.

The exposure of the skin to sunlight represents the most important source of vitamin D (80-100% of requirements). Since the 1970s[16], the annual cyclical variation in blood concentrations of 25(OH)D, has been known, reaching maximum values in the final phases of the summer and minimum values in the winter. In parallel, bone mass falls during autumn and winter, while it improves or remains stable during the spring and summer[7,17,18].

The concentrations of 25(OH)D in the patients in our study determined during the months of September and October were double those obtained in the period January – March, with the prevalence vitamin D deficit dropping from 50% in the winter months to less than 3% at the end of the summer. Although various studies published in our country[8] show the seasonal influence on values of vitamin D, our work highlights the magnitude of the change of status in vitamin D which happens after the summer months, similar to that in other longitudinal studies carried out in different European countries[18-20] and in different states of North America[17,21,22].

The drop in concentrations of vitamin D and the subsequent reduction in blood levels of ionised calcium, detected by calcium sensors in the parathyroid glands, triggers an increase in the expression, synthesis and secretion of PTH[7]. Hence, PTH values constitute a highly sensitive indicator of vitamin D deficit, increases in this hormone being caused even in situations of moderate deficiency. In addition, some studies have shown[23] that the increase in concentrations of PTH bears a closer relationship to bone mineral density than that measured by the concentration of vitamin D, especially in older people, in whom secondary hyperparathyroidism is a determining factor in bone fragility. The negative correlation found between PTH and 25(OH)D in both periods of our study, the fall in concentrations of PTH and of the percentage of patients with blood PTH higher than the upper limit for normality recorded in the post-summer period confirm the improvement in vitamin D status in our population by the end of the summer months.

Exposure to sunlight is responsible for the maintenance of adequate levels of vitamin D in most of the world's population[24]. However, numerous circumstances may modify the cutaneous production of vitamin D. In addition to the seasonal changes already referred to, variations in latitude[25], daily hours of sunlight and the period of exposure to the sun[18], the area of the skin's surface exposed to the sun[25], the type of skin[26] and the person's race[22], the percentage of body fat[22], and even the use of different types of clothes for cultural or religious reasons in individuals in the same zone[27], are factors which may alter considerably the synthesis of vitamin D. On the other hand, the intake of foods, natural, or artificially enriched, with a high vitamin D content, contributes moderately to the status of vitamin D in most of the countries studied[7].

The results of our work show that after the summer months, the values of 25(OH)D of the population studied doubled, reducing the percen-

tage of patients with vitamin D deficit and secondary hyperparathyroidism, changes undoubtedly related to the increase in the cutaneous synthesis of vitamin D due to a greater exposure to sunlight. However, in our study the influences of aforementioned factors which may modify the production of vitamin D, were not analysed, and neither were the variations in dietary intake of vitamin D which might have happened during the period of observation, facts which constitute limitations to our work. Although before embarking on the study the subjects received instructions not to change their dietary habits or their degree of normal exposure to sunlight, the possibility cannot be entirely discounted that some variations in these variables may have contributed to the change in status of vitamin D observed at the end of the summer. In fact, an observational study[17] similar to ours did record spontaneous increases in the ingestion of vitamin D and calcium in the population studied, which contributed to the seasonal changes in bone mass and calciotropic hormones observed.

Even though the overall improvement in vitamin D status recorded after the summer months is notable, it must be highlighted that only half the subjects of our study reached levels of 25(OH)D higher than 30 ng/ml, a level which constitutes an optimum status for vitamin D8, ensuring bone health and prevent vitamin D deficiency during the winter months[25]. Thus, the results of our study confirm the difficulty of achieving an optimum level of vitamin D only by exposure to sun, in accordance with that highlighted by Quesada and Sosa[8].

The emerging understanding of the involvement of vitamin D deficit in a predisposition to many neoplastic, inflammatory, autoimmune and metabolic diseases[4], in addition to the unequivocally demonstrated skeletal changes, reinforce the need to achieve an optimum level of vitamin D in the population of our country, which, as in other European populations[6], has a high prevalence of vitamin D deficit. Only the active promotion of public health policies which strengthen the development of food enriched with vitamin D, and even preventative use of pharmacological supplements of vitamin D, will allow the eradication of a deficiency which is already reaching almost pandemic proportions.

In conclusion, more than half the patients treated in a primary care health centre had vitamin D deficiency during the winter months, a situation which improves notably by the end of the summer, even though only half of them reach optimum levels which guarantee an adequate vitamin D status. In the light of our results, and in accord with other authors, the promotion of health policies which optimise the nutritional status of vitamin D in the general population of our country would be recommended.

Conflicts of interest: the authors declare that they have no conflicts of interest.

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Prevalence of vertebral fractures in patients wit h Chronic Obstructive Pulmonary Disease admitted to a University Hospital

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Summary
Objective: Chronic obstructive pulmonary disease (COPD) is a widely distributed disease with high morbimortality, associated with important pathologies, among which is included osteoporosis. The objective of this study was to evaluate the prevalence of vertebral fractures in those patients with chronic obstructive pulmonary disease and to determine some factors which heighten the risk of fracture in these patients, especially the severity of the COPD and the use and dosage of inhaled corticoids.
Material and method: Retrospective, observational transversal study, in which were included patients admitted to the Rio Hortega University Hospital during the year 2006 diagnosed with COPD who had a lateral thoracic X-ray. A control group was included, without COPD, of similar age and sex, admitted to the internal medicine service over the same period. The vertebral fracture was determined using Morphoxpress®.
Results: 115 patients with COPD and 87 control patients were included, with a higher prevalence of vertebral fractures in being observed in patients with COPD, although without there being a statistically significant difference with respect to the control group. However, if we consider only the moderate-severe fractures (Gennant Type II and III), there is a greater prevalence, which is related to the severity of the disease, measured by the decrease in FEV1 (Forced expiratory volume in one second). We found no relationship between the prevalence of fractures, the different types of treatment and the morbidity determined by the number of admissions.
Conclusions: Our study shows the tendency of patients with COPD to have an increased prevalence of vertebral fractures which are associated with the severity of the COPD and the seriousness of the fractures themselves. We found no relationship between the different inhaled corticoids, individually or grouped, and the presence of fractures. Nor did we find a relationship between the number of vertebral fractures and the number of flare-ups, treatment with broncodilators, corticoids, home oxygen therapy, or the diagnosis of, or previous treatment for, osteoporosis.

Key words: *COPD, osteoporosis, vertebral fractures, Morphoxpress®.*

Introduction

Chronic obstructive pulmonary disease (COPD) is a widely distributed disease and with high morbimortality, principally associated with smoking, over and above the susceptibility of an individual to develop the disease. It is a chronic disease which brings with it significant comorbidities, among which is osteoporosis[1]. Both the age at which it develops, and the habit of smoking itself, and the systemic and inflammatory effects of the disease have an influence on the presence of a high risk of fracture, but it is, possibly, the use of corticoids in its treatment which is the most important factor in this association[2].

Accord to current guides, the treatment of COPD is based on the use of β2-adrenergics of short and long duration and anticholinergics, which improve the quality of life and tolerance of exercise, and reduce exacerbations. The inhaled corticoids are added to the former in patients with moderate to severe COPD. They have been shown to reduce the frequency of exacerbations and to improve quality of life, above all combined with β2-adrenergics, this being more effective than each of them separately. Oral corticoids are used in patients with severely exacerbated COPD, for a short period of time[3].

The presence of fractures in patients with COPD results in limitations to physical activity, sedentariness and the necessity for elderly nursing care. From the respiratory point of view, the thoracic vertebral fractures result in a reduction in lung volume which causes a restrictive ventilatory defect[2], with a reduction in forced vital capacity of up to 9%[4], an effect which is seen to be increased by the pain which accompanies it. However, although the role which oral corticoids play in osteoporosis and the risk of fracture in COPD has been widely demonstrated, it is not so clear to what extent the inhaled corticoids have similar effects on the bone.

The aim of this study was to evaluate the prevalence of vertebral fractures in those patients with chronic obstructive pulmonary disease, and to attempt to determine which factors cause the risk of fracture in these patients, especially the severity of the COPD and the use and dosage of inhaled corticoids.

Material and methods

A retrospective, observational transverse study was carried out in which were included patients admitted to the Río Hortega University Hospital during the year 2006, diagnosed with COPD, older than 40 years of age, and who in the 4 months previous to their admission had had a baseline lateral thoracic X-ray and arterial gasometry. None of the patients were excluded due to the poor quality of the thoracic X-ray. The control group was obtained from patients admitted in the same year who had had a lateral thoracic X-ray in the previous 4 months, and who had not been diagnosed with COPD, or previously been in treatment with inhaled or oral corticoids. In both groups, those whose thoracic X-ray was not sufficiently clear for the measurement of vertebral crushing and fractures, or who had degenerative disease or spinal deformities, were excluded.

The data evaluated in the patients and controls were: age, sex, smoking habit (non-smoker, ex-smoker, active smoker), previous diagnosis of osteoporosis and, in the case of the affirmative, the treatment followed, and the existence of earlier fractures. The patients with COPD were classified as a function of their FEV1 (forced expiratory volume in 1 second) as light (FEV1>80%), moderate (FEV1 60-80%) and severe (FEV1<60%), and the number of admissions due to the exacerbation of COPD over the 5 years up to 2006 was recorded. The treatments carried out at admission, especially bronchodilators and inhaled and oral corticoids were recorded, differentiating the different types of inhales corticoids (fluticasone, budesonide, beclamethasone), the daily dose of each of them and the number of courses of oral corticoids in the last year.

The presence of vertebral fractures was measured using the Morphoxpress® fracture detection programme, a software package which allows the detection of vertebral fractures through original digitised lateral X-rays. The analysis of the fractures is initiated manually, by marking up the upper and lower corners of the anterior and posterior walls of the vertebrae situated immediately above and below the vertebra to be evaluated.

Subsequently, the system analyses automatically the distances marked between these points and marks on the vertebra the evaluation of the corners of the anterior and posterior walls it has calculated. The marking of the points is carried out by the observer and by the system itself independently, subsequently comparing both measurements. The difference corresponds to the degree of crushing or fracture of the vertebra studied. The fractures found are classified according to the Genant classification as light or grade I (20-25%), moderate or grade II (25-30%) and severe or grade III (>40%).

Statistical analysis.

The database was established and its analysis carried out using the SPSS v15.0 statistical programme officially licensed o the University of Valladolid.

The continuous variables were described as mean ± ND (normal distribution), and the qualitative variables as frequencies and percentages. The Kolmogorov-Smirnov test was used to determine the normality of the distribution. To study the association between qualitative variables the Chi-squared test was used with the Fisher's exact test or verisimilitude ratio when the conditions required it. To study the differences between the means, parametric or non-parametric statistical tests were used as required by the conditions which applied (Student's t, Mann-Witney U, ANOVA with the Bonferroni post-hoc test, Kruskal-Wallis). The level of significance was considered to be at $p < 0.05$.

Results

115 patients with COPD and 87 control patients were included, with comparable average ages (73 ± 11 years vs 74 ± 10 years, p>0.05) and with an age range of between 40 and 90 years. Of the 115 patients with COPD included, 8 (7%) were women, all postmenopausal, and 107 (93%) men. The controls had a similar distribution by sex. The majority (70%) were ex-smokers and only 8 (16%) continued to smoke. Only 5 (4.3%) had been diagnosed with osteoporosis, and 4 (3.5% of the total) had received treatment with calcium, vitamin D or bisphosphonates. The presence of previous fractures were found in the personal histories of 11 patients.

46 (40%) of the patients did not have a previous spirometry recorded, for whom it was not possible to determine the severity of their COPD. Of the remainder, 49 (43%) had a severe form, 10 (17.5%) moderate and 10 (17.5%) light. This admission was their first in the last 5 years for 48 (42%) patients, while 39 (34%) had been admitted due to exacerbation on 3 or more occasions over the same period of time.

Most of the patients were receiving treatment with beta-blockers and inhaled corticoids, 84 (73%) with beta-blockers and 81 (70%) with inhaled corticoids. Among the corticoids analysed only 2 patients (2%) were in treatment with beclamethasone, while 18 (16%) were taking budesonide, and 61 (53%) fluticasone. There were 34 patients (30%) who did not have, or could not recall having had, treatments with inhaled corticoids. With respect to the dosage, 11 (73%) were taking more than 400 µg daily of budesonide and 42 (70%), more than 500 µg of fluticasone. In the previous year, 43 (37%) received at least a course of oral corticoids and 32 (28%) had previously had domiciliary oxygenotherapy.

There were 50 patients with COPD with at least one fracture, as opposed to 29 in the control group (43.5% vs 33%), with no statistically significant differences (p=0.14), the total number of fractures being 79 (39%). 64% of those patients with COPD had a single fracture as opposed to 70% in the control group. In analysing the fractures as a function of their severity, significant differences were found between the two groups. The patients with COPD had a greater number of moderate or severe fractures, while light fracture predominated in the control group. The results appear in Table 1.

The severity of the COPD was a determining factor in the frequency of fractures: there was 1 fracture in patients with light COPD (2%), 6 in patients with moderate COPD (12%) and 24 in those patients with severe COPD (49%), the intergroup differences being statistically significant, p<0.05 (Figure 1). No differences were observed in the number of fractures with the taking of inhaled corticoids, their dosage, number of exacerbations, domiciliary oxygenotherapy and previous diagnosis of osteoporosis (Table 2).

Discussion

Our study shows a high prevalence of vertebral fractures in patients with COPD, although without seeing statistically significant differences with the control group made up of patients admitted to the internal medicine department without COPD. However, if we only consider moderate-severe fractures (Gennant Type II and III), there is a higher prevalence which is related to the severity of the disease, measured by the decrease in FEV1. We did not find a relationship between the prevalence of fractures, the various types of treatment and the morbidity determined by the number of admissions. The prevalence of vertebral fractures is higher than that observed in other recently published series[5,6], although, if we exclusively consider moderate or severe COPD, it is similar[7]. Recently, in a population of Spanish COPD patients the risk of fracture has been determined using the FRAX® tool[8]. This study showed the risk of a major osteoporotic fracture at 10 years of 1.8% (CI 95% 0.9-3.6). Major osteoporotic fractures include vertebral fractures, and our data suggests a higher prevalence of this type of fracture, which probably means that in this population the FRAX® tool underestimates the risk of fracture, similar data to that observed in the Spanish osteoporotic population[9].

A greater prevalence of fractures in patients with COPD has been related to a variety of factors associated with the disease. Tobacco smoking is associated with a reduction in bone mineral density both in active smokers and in ex-smokers[10,11]. Given that women appear to be more susceptible to the negative effects of tobacco on the lungs[12], the increase in the number of women smokers and their susceptibility to osteoporosis after menopause gives them a greater propensity to bone problems. Secondly, as happens with other chronic diseases, patients with COPD have higher levels of inactivity and weight loss, and a deficit in calcium and vitamin D , accentuated by a lack of exposure to sun, factors with which are associated a higher risk of osteoporosis and fractures[13-15]. Lastly, it has been suggested that the systemic effects of the disease itself may play a part in the bone damage which occurs in patients with COPD[16].

These systemic effects would be related to pro-inflammatory cytokines and active inflammatory cells which would reach the bloodstream from the lungs and would intervene in bone turnover, either expressing or secreting the receptor activator of NF-κB ligand, either by producing cytokines (TNF-α and interleukins) which activate bone remodelling independently of the osteoblasts, or by the modification of the enzymes responsible intracellular metabolism of the corticoids, increasing their transformation into their active metabolites[17,18].

It has been estimated that the prevalence of osteoporosis in patients with COPD who have not taken corticoids is twice as high as in healthy subjects and asthmatic patients[19]. This makes COPD a possible independent risk factor for a reduction in bone mineral density[20], and for vertebral[21] and hip[22] fractures.

Table 1. Prevalence of fractures in COPD and control

	COPD	Control	Signification
Total fractures	50 (56.5%)	29 (66.7%)	NS
Minor fractures	17 (34%)	22 (33%)	NS
Moderate-severe fractures	27 (54%)	6 (21%)	p<0.05

Table 2. Prevalence of fractures according to treatment

	No Fracture	Fracture	Signification
No Oxygenotherapy	51 (61.4%)	32 (38.6%)	NS
Oxygenotherapy	14 (43.8%)	18 (56.3%)	NS
No 2-inhaled	20 (64.5%)	11 (35.5%)	NS
2-inhaled	45 (53.6%)	39 (46.4%)	NS
No budesonide inhaled	56 (57.7%)	41 (42.3%)	NS
Budesonide inhaled	9 (50%)	9 (50%)	NS
No Fluticasone inhaled	32 (59.3%)	22 (40.7%)	NS
Fluticasone inhaled	33 (54.1%)	28 (45.9%)	NS
No Oral Corticosteroids	43 (59.7%)	29 (40.3%)	NS
Oral Corticosteroids	22 (51.2%)	21 (48.8%)	NS

Although in our study we did not find a statistically significant relationship, a tendency was observed to a higher number of fractures in patients with COPD than in the controls. It is possible that the lack of statistical significance bears some relation to the high prevalence of fractures in the control group. However, this relationship becomes significant if the patients with COPD are grouped according to its severity. It was seen that the most severely affected patients had more fractures, a relationship which had been observed before[23], this association being much higher among the men[24]. In addition, it was observed in our study that the severity of the fractures also increased with the severity of the COPD, such that the moderate and severe fractures are more frequent among those patients with COPD than in the control group, thus confirming other results[25].

The factors which influence these results, leaving aside the systemic and inflammatory effects of the disease itself, which will be more intense the greater the severity of the COPD, could be related to the corticoid treatment. This supposes that those patients included with the most severe degree of the disease have required treatment with inhaled and oral corticoids more frequently in response to a higher number of exacerbations.

Our study tried to demonstrate a relationship between the taking of inhaled corticoids and the prevalence of vertebral fractures, independently of the severity of the disease and, above all, of the taking of oral corticoids. This relationship is a current cause of concern, given that the use of inhaled corticoids in COPD has become widespread since it has been demonstrated to improve the quality of life and reduce the frequency of exacerbations, and even more if it is associated with β-adrenergics and used as an underlying treatment. There are numerous studies on this subject in which it is not clear that the taking of inhaled corticoids significantly increases the risk of fracture, or increases the likelihood of osteoporosis. On the one hand, most studies do not hold that inhaled corticoids have an effect on osteoporosis, or on the risk of fractures. In 2003, Rich et al.[26] made a systematic review of studies carried out of this relationship to date. Firstly, they found that there was a significant relationship between the pharmacological dose of inhaled corticoids and a reduction in bone mineral density in the lumbar region and the femoral neck in studies lasting at least two years, and that this relationship was much stronger with high doses of inhaled corticoids and with greater periods of exposure. However, the same study concluded that, generally, there were no data which related the taking of inhaled corticoids and the risk of fracture, giving greater significance to other associated factors, such as the presence of previous fractures,

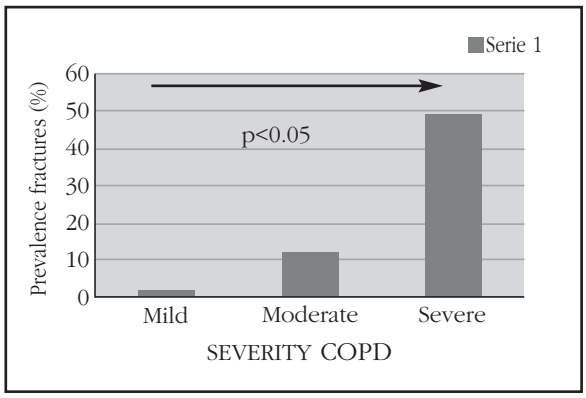
the earlier diagnosis of osteoporosis and advanced age. In terms of the classes of inhaled corticoids studied, the meta-analysis indicates that triamcinolone is the one which is most associated with bone mineral loss, followed by beclamethasone and budesonide, with no data in this study for fluticasone. Other studies have confirmed these data. Johannes et al.[27] found no relationship between the taking of inhaled corticoids and the presence on non-vertebral fractures, independently of the corticoid inhaled and the dose used. Nor did Nuti et al.[24] find any relationship between the taking of inhaled corticoids and the risk of vertebral or non-vertebral fractures. However, not all the data are uniform; other studies affirm that there is a relationship, and that it occurs at high doses inhaled corticoids. Hubbard et al.[28], describe an association between the taking of inhaled corticoids and the risk of fracture, independently of other factors, such as the taking of oral corticoids, the severity of the COPD, the use of bronchodilators and daily physical activity, but dependent on the dosage. And Pujade et al.[29] found small increases (not significant) in the risk of fracture associated with high doses of corticoids which were independent of the degree of COPD and the taking of oral corticoids. However, this relationship does not always appear to be dose dependent[30], or this is lost when the data are adjusted for other confusion factors such as the severity of the COPD and the class of bronchodilator[21]. In addition, the clinical significance of the effect of high doses of inhaled corticoids on the prevalence of vertebral fractures does not appear to be much higher than that of other risk factors such as the taking of antipsychotic or hypnotic medication, low body mass index, cerebrovascular disease and active tobacco smoking[31].

In our study we also observed no association between the taking of inhaled corticoids and the prevalence of vertebral fractures, a fact that remains when the corticosteroids are grouped by class or dose.

In terms of the other treatments which may have an influence on the higher presence of vertebral fractures in patients with COPD, what is notable is the absence of an association between the taking of oral corticoids and the incidence of fractures, a fact widely illustrated in the literature. This may be due principally to the short time period over which the data has been collected, which means a lack of knowledge about the quality of corticoids taken previously.

It should also be highlighted that no association was found between the taking of β-adrenergics and the incidence of fractures. It has been found that the overstimulation of the adrenergic system is related to a low level of bone mineral density and greater bone fragility[32], which is produced by the activation of the β-adrenergic receptors in the osteoblasts, which increase the production of the receptor activator of NF-κB ligand and encourage the activation of the osteoclasts[33], above all salbutamol. On the other hand, two

Figure 1. Prevalence (%) of fractures according to the severity of COPD.



recent studies have concluded that the risk of fracture associated with the β-adrenergics diminishes when adjusted for the use of oral corticoids and the severity of the disease, and that, in any case, this risk is higher for the oral β-adrenergics, and at low doses[34].

There are various limiting factors which may also influence the results. Firstly, the fact that the population selected has a wide spread of ages (of between 40 and 90 years, with an average of 77 years). Secondly, the absence of more complete information from the of data collected. There is little data related to the style and quality of life of the subjects, such as daily physical activity, nutrition and exposure to sun. The lack of more exhaustive data in relation to the quantity of inhaled or oral corticoids taken previously, and the loss of subjects due to missing data, mainly regarding the severity of the COPD and due to the lack of knowledge of any treatments at the time of their inclusion in the study, reduced the sample size. This would explain why no association was found between the prevalence of vertebral fractures and oral corticoids, or between the prevalence of vertebral fractures and domiciliary oxygenotherapy, which is usually associated with the severity of the COPD. The control group is not a healthy group, but is formed of patients admitted to the internal medicine service for other reasons, which may explain its high prevalence of vertebral fractures. Finally, the fact that the vertebral fractures were quantified using the Morphoxpress® system gives the study greater objectivity, but it does not rule out the fact that the diagnosis is still dependent on the skill of the observer.

In conclusion, our study shows the tendency of patients with COPD to have an increased prevalence of vertebral fractures, these being associated with the severity of the COPD and the seriousness of the vertebral fractures themselves. We found no relationship between the different inhaled corticoids, individually or grouped, and the presence of fractures. Nor did we find a relationship between the number of vertebral fractures and the number of exacerbations, treatment with bronchodila-

tors, corticoids, long term domiciliary oxygenotherapy or the earlier diagnosis and treatment of osteoporosis. Given the consequences of these fractures on the morbimortality of the COPD, a more aggressive approach on the part of the clinician to the diagnosis and treatment of osteoporosis in this population is necessary.

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Role of DNA methylation in the regulation of osteogenesis

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Summary
Recent studies suggest that epigenetic mechanisms, such as the methylation of DNA, play a critical role in cellular differentiation. Osteoclasts are cells with the capability of reabsorbing bone. Their differentiation is strictly regulated by the RANKL-OPG-RANK signalling pathway. Recently, our group has reported that the expression of RANKL and OPG by cells of the osteoblastic lineage is regulated by the methylation of the promoter regions of those genes. This review summarizes current knowledge about the influence of DNA methylation on the regulation of osteoclastogenesis.

Key words: *epigenetic, RANKL, OPG, methylation, differentiation.*

Introduction

Osteoclasts are cells specialising in bone resorption. These cells are formed through a complex process of differentiation (osteoclastogenesis), from haemopoietic precursors in the bone medulla[1]. The process of maturation is led by the induction of the expression of certain genes characteristic of the functional osteoclasts. The expression of these genes initiates a series of changes in the precursors which finally allow the mature osteoclast to erode the bone.

The activity of the osteoclasts is essentially to bring about bone remodelling, a process whose purpose is to periodically renew skeletal tissue, substituting new bone for old[2]. Changes in the normal activity of these cells have a significant impact on bone mass, which means that the molecular mechanisms which drive the differentiation of the osteoclast precursors need to be tightly controlled.

For some time now there have been various works which have indicated the possible role of epigenetic mechanisms as regulatory axes for the processes of cell differentiation. Although there is still little information available about the role these processes may play in the bone, various studies indicate the involvement of these marks in the regulation of the expression of genes critical to bone biology[3-5]. The aim of this review is to re-examine the role of the epigenetic mechanisms, most specifically DNA methylation, in the control of osteoclastogenesis.

DNA methylation

All the cells of the human body, with the exception of the belonging to the germ line, share the genome. However, there are different types of cells, with different functions and behaviours. This fact indicates that there should be mechanisms which strictly regulate the progress from totipotent cells to cells which are totally differentiated and functional. In addition, these mechanisms should ideally be capable of modulating the process of maturation in response to microenvironmental stimuli, promoting cell differentiation which is time and place dependent. Numerous studies suggest that the epigenetic mechanisms are capable of regulating various types of these processes of cell differentiation[6].

Epigenetic mechanisms are defined as inheritable changes in DNA, which do not affect the base sequence, are reversible and which are manifested as specific patterns of gene expression[7]. Differently from the genome, it is known that the epigenome (the combination of epigenetic marks) is dynamic, changing in response to the environment, not only cellular, but also of the individual. Up until now, three types of epigenetic mechanism have been described: DNA methylation, the post-translational modification of histones and the microRNAs[8].

The methylation of DNA is, by and far, the epigenetic mechanism most studied, perhaps due to its involvement in neoplastic processes. DNA methylation is carried out by some enzymes known as DNA methyl transferases (DNMTs). In mammals, the DNMTs catalyse the addition of the methyl group to position 5' of the cytosines which precede a guanine, dinucleotides known as CpGs[9]. In general, these CpGs appear methylated. There are certain regions rich in CpGs, known as CpG islands, which appear preferentially in regions close to the gene promoters or in the promoters themselves. Curiously, these regions are usually generally demethylated, and their pattern of methylation may change between different cell types[10].

Generally, it may be considered that DNA methylation has a repressor role in promoter regions with CpG islands, blocking gene expression[11]. Although for the moment the mechanisms by which methylation is able to modulate the expression of a gene is not known with certainty, there are data which suggest that the presence of the methyl groups make the bonding of the transcription factors to their target sequences difficult, thus making it hard to initiate the transcription[12]. On the other hand, the presence of these methyl groups is capable of promoting changes in the conformation of chromatin, making certain regions critical for the initiation of transcription less accessible (Figure 1)[13].

It is important to say that methylation does not only take a significant regulatory role in gene expression. In fact, as has already been mentioned, most of the CpGs appear to be methylated independently of whether their location is exonic, intronic or intergenic. It is thought that the methylation in these zones contributes significantly to the stability of the genome[14].

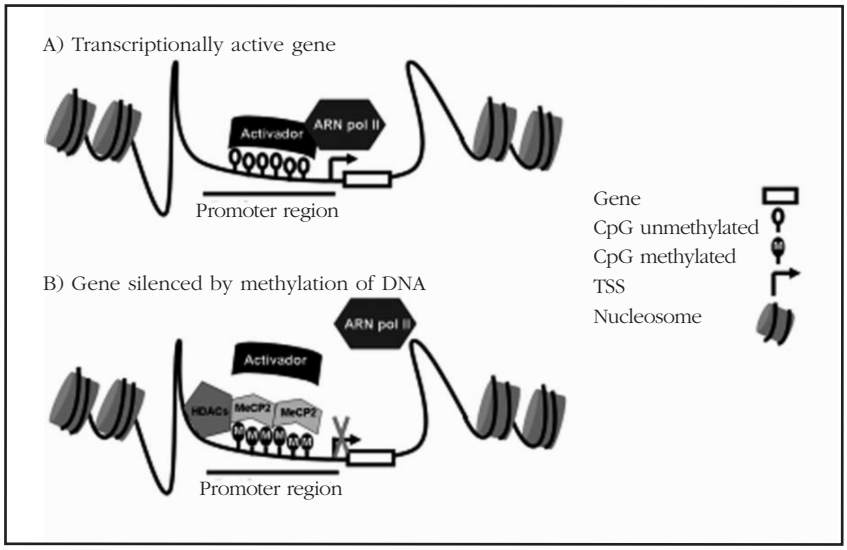
The role of the epigenetic mechanisms in the bone, and specifically that of DNA methylation, has started to be studied recently. However, as has already been said, there is a range of evidence which invites us to think that these mechanisms may be important in the biology of the bone[15]. For example, it is known that DNA methylation regulates the progress of the differentiation of the osteoblast precursors, as well as regulating the expression of various genes involved in bone homeostasis[16-18]. On the other hand, little is known about the role DNA methylation has in the regulation of osteoclastogenesis.

Epigenetic regulation of osteoclastogenesis: DNA methylation

The process of differentiation of an osteoclast from the haemopoietic monocyte-macrophage line cells generally takes place near the surface of the bone. Currently, it is known that there are two cytokines essential for correct osteoclast differentiation: macrophage colony stimulating factor (M-CSF) and the receptor activator of nuclear factor κB ligand (RANKL)[19,20]. RANKL is a protein produced by various types of cells. Although until recently it was considered that the osteoblasts were the principal source of RANKL in the environment of the bone, recently, it has been proposed that the osteocytes may also actively produce this protein[21]. The bonding of RANKL to the recep-

tor activator for nuclear factor κB (RANK), present in the osteoclast precursors, triggers a series of molecular mechanisms which promote the initiation and progress of the differentiation of the osteoclast precursors (Figure 2)[22]. Basically, after the interaction between RANKL and RANK , the latter is activated and the signal is transmitted to the interior of the cell by means of the signalling complex TRAF-IKK-NFκB, in the end achieving the activation of the transcription factor known as nuclear factor for activated T cells c1[23,24]. This, in cooperation with other factors induces the specific genes which determine the osteoclast phenotype. On the other hand, in addition to RANKL, the osteoblasts are also capable of producing OPG, a protein which can bond with RANKL and block its interaction with RANK[22,25]. In fact the relationship between the production of RANKL and OPG is considered to be an important determinant of the rate of osteoclastogenesis.

Figure 1. Silencing of gene expression by the methylation of the CpG dinucleotides in promoter regions. A) In the absence of methyl groups in the CpGs located in the promoter region, the activator factors for transcription are capable of bonding to their target sequences and of recruiting RNA polymerase II to start the transcription. B) in the presence of methyl groups, these are recognised by the MeCP2 proteins which block the bonding of the transcription factors with their target sequences, impeding the start of the transcription. In addition, the MeCP2 proteins are capable of recruiting histone deacetylase enzymes (HDAC) which are in charge of the remodelling of chromatin, inducing a less accessible conformation

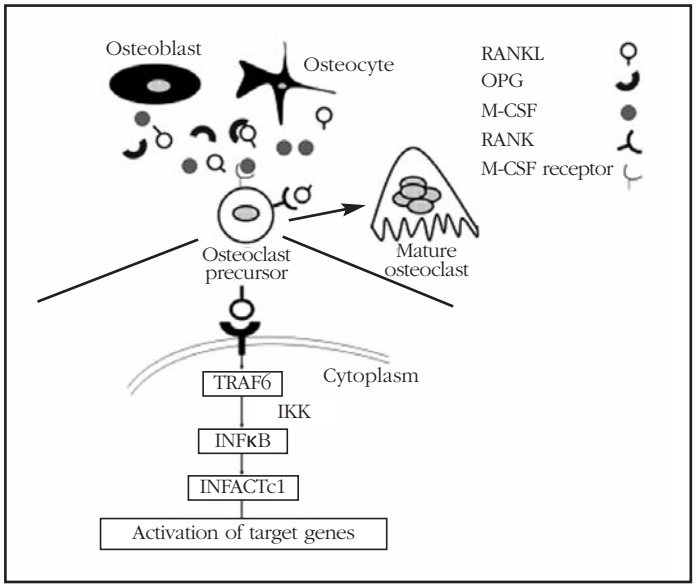


Given its key role in this process RANKL has become a valuable therapeutic target for the treatment of prevalent diseases of the skeleton in which bone mass is altered. In fact, various recent studies have demonstrated how the blocking of RANKL by neutralising antibodies results in an increase in bone mass in patients[26]. In spite of its importance, at present it is not known with certainty what molecular mechanisms regulate its expression. However, our group has recently proposed that the expression of RANKL and OPG is regulated by means of the methylation of the regions which promote these genes[27].

Through a bioinformatic analysis we identified two CpG islands in the RANKL gene, one situated on the isoform I TSS (CpG 1), and the other, a few bases before the start of the gene (CpG 2). We also identified another CpG island in the OPG gene. After the analysis of the expressions and the degree of methylation in various osteoblast cell lines we observed that the expression of RANKL is inversely associated with the degree of methylation of island 1, but not island 2. Similarly, the expression of OPG is inversely associated with the degree of methylation in the island discovered. The treatment of cells which had not previously produced either RANKL or OPG, which had raised levels of methylation, with a demethylisation agent provoked a 15% drop in the levels of methylation in the islands studied. In parallel with this drop in methylation the treatment induced the expression of both genes, confirming the relationship which exists between degree of methylation and expression. On the other hand, we observed that the expression of RANKL, as well as the RANKL/OPG quotient, were significantly higher in bone tissue from osteoporotic patients than in arthrosic patients. However, the islands selected for study appeared to be hypomethylated in the bone tissue, and we found no differences between the two groups of patients. Overall, our results suggest that there is an association between levels of expression of these genes and the methylation of their CpG islands, in such a way that low levels of methylation will allow the expression of RANKL, and the consequent induction of osteoclastogenesis[27].

It should be mentioned that, in addition to the direct role of the methylation of the CpG islands of RANKL and OPG in the activation of osteoclastogenesis, other epigenetic mechanisms involved in the process have been described. For example, it has been demonstrated that the demethylisation of histone residues in the NFATc1 gene playa a critical role in the progression of the differentiation of the osteoclast precursors[28]. On the other hand, there are a number of works which suggest the production of certain microRNAs are also involved in the regulation of osteoclast differentiation[29,30].

Figure 2. RANKL-RANK signalling pathway in the cell differentiation of osteoclast precursors. The osteoblasts and the osteocytes produce and secrete RANKL into their environment. The interaction of RANKL with its receptor RANKL triggers a whole series of molecular reactions in the cytoplasm of the osteoclast precursor. In the end, the NFATC1 transcription factor is activated. This factor, along with others, induces the expression of various target genes which direct the differentiation of the precursors towards being mature osteoclasts



Conclusions

The increase in life expectancy in today's society has resulted in an increase in the number of patients suffering from diseases related to aging. Among these, osteoporosis is one of the most representative. Most of its current treatments centre on the modulation of bone resorption, thus reducing the loss of bone mass. Therefore, understanding in greater depth the mechanisms of the RANKL-OPG-RANK signalling pathways, regulatory axis for osteoclast formation, is of vital importance. The identification and study of this type of mechanism will permit the identification of new therapeutic targets, as well as understanding better the pathogeny and physiopathology of osteoporosis.

On the other hand, various epidemiological studies suggest that the epigenetic mechanisms, and specifically DNA methylation, may also be involved in the relationship between the genome and the environment, a relationship which on many occasions is a determining factor in the development of diseases of the skeleton[31,32], Although at the moment a direct relationship has not been found between epigenetic profiles and bone deficiencies, it cannot be discounted that the identification of epigenetic markers specifically associated with diseases of the skeleton may be useful in the future for their diagnosis and prevention.

Overall, while the data are still scarce, the existing studies make it possible to venture the view that epigenetics will be an important topic in the field of bone research and in the development of new antiresorptive drugs in the coming years.

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Action of beer on the bone

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Summary
Although it has been shown that excess alcohol is a significant risk factor for osteoporosis, the moderate consumption of beer appears to have beneficial effects on the bone. This review comments on the scientific evidence regarding the possible beneficial effects of beer on bone metabolism, describes which of its elements may be responsible for these positive effects, and reports that both the polyphenols and the flavonoids, among them lignane, and above all silicon, all of which are components of beer, act positively on bone metabolism and bone mass.

Key words: *alcohol, polyphenols, flavonoids silicon, bone mass, osteoporosis.*

Introduction: diet and osteoporosis

Osteoporosis is a disease of bone metabolism very common in human beings. It was initially defined by Fuller Allbright as "too little bone". Nowadays, the accepted definition by consensus is "systemic skeletal disease characterised by a reduction in bone resistance, with the consequent increase in bone fragility and susceptibility to suffering fractures."[1]. The essential elements of this definition are low bone mass and changes in its microarchitecture, which distinguish osteoporosis from other bone diseases. Any bone fracture is related to the strength of the bone, bone mass, expressed as bone mineral density (BMD), being the main contributor to this strength.

The change in microarchitecture is characterised by the loss, thinning and lack of connection between the bone trabeculae, along with a series of factors, such as alterations in remodelled bone, the geometry of the bone itself, etc., which have been grouped under the concept of bone quality[2]. All this produces a deterioration in the structural integrity of the bone and causes skeletal fragility, which results in an increase in risk of fractures.

The etiopathology of osteoporosis is multifactorial, and, although the genetics and the hormonal factors influence enormously the degree of bone loss related with age, poor nutrition, with a low intake of calcium and vitamin D[3], tobacco and excess intake of alcohol, as well as the absence of physical exercise also significantly affect this bone loss[4].

Among a number of factors, foods rich in calcium and vitamin D[5] have been shown to have a significant positive effect both on the acquisition of a good peak bone mass, and in delaying this loss of mass which appears from a certain age. Other foods rich in minerals which the bone contains such as magnesium, potassium and fluorine, or in trace elements such as zinc, copper, boron and manganese[6], are associated with bone mass, and a deficiency of these elements with reduced bone mass, or with slow consolidation of fractures.

Alcohol and bone

We know that an excess intake of alcohol is considered to be a significant risk factor for osteoporosis. The physiopathological mechanism which relates alcohol with osteoporosis is complex[7], although it appears that it would be related through a depression in bone formation and an increase in urinary excretion of calcium. This is an osteoporosis with low remodelling, whose most significant clinical expression is that which happens in cirrhotic alcoholics, and which is closely related to the duration of the consumption of alcohol. It should not be forgotten that in alcoholics, with or without hepatopathy, other changes in mineral metabolism may also be associated, included among which are a reduction in secondary vitamin D due to a deficit in hepatic hydroxylation, and a reduction in the production of the proteins bonded to vitamin D. Magnesium deficiency is another parameter to be studied in these alcoholic patients, which is accompanied by hypoparathyroidism with hypocalcemia and resistance to PTH, all of which contribute to bone loss.

However, moderate consumption of alcohol could be beneficial to the bone, both in men and in menopausal women[8-10]. In the Framingham Osteoporosis Study, extracted from the cohort of the Framingham study, Tuckery et al.[11], evaluated the femoral BMD in a group of 1,182 men, 1,289 postmenopausal women and 248 premenopausal women, relating it to the variables included in, at least, two questionnaires carried out over a 5 year period, and in which were included data regarding the quantity of beer, wine and spirits which the subjects ingested, valuing a 350 ml glass as one unit of beer, a glass of 118 ml as a unit of wine, and a 42 ml glass as a unit of spirits. In this study the men were mainly drinkers of beer and the women drinkers of wine, and when compared with the non-drinkers, the BMD in the femoral neck was, in those subjects who drank 1 or 2 units a day, between 3.4 and 4.5% higher in the men, and 5-8.3% higher in the women, compared with those who were abstemious.

In another study carried out in 5,865 people over 65 years of age in the US within the Cardiovascular Health Study[12] the annual intake of beer, wine and other spirits was quantified, and the incidence of fractures evaluated, and in 1,567 subjects, the BMD was determined by DXA. Over the 12 years the study lasted, in comparison with the group who were abstemious, a relationship between the risk of hip fracture and alcohol intake was found. The hazard ratio for hip fracture was 0.78 (C.I. 95%, 0.61-1.00) among the consumers of up to 14 glasses a week, and 1.18 (C.I. 95%, 0.77-1.81) among those consuming 14 or more drinks per week. A relationship was also found between the BMD in the total femur and femoral neck, with a BMD approximately 5% higher (C.I. 95%, 1-9%) in those consuming up to 14 drinks a week compared with those who were abstemious. This relationship was similar in men and women.

On the other hand, a moderate intake of alcohol is associated with an acute reduction in bone resorption when a marker for bone resorption, such as CTX (carboxy-terminal telopeptide of type I collagen) is used[13].

It has been suggested that one of the mechanisms related to this beneficial effect is the presence of polyphenols in alcoholic drinks. It has been confirmed that moderate drinkers of wine have less cardiovascular disease than drinkers of other alcoholic drinks, highlighting the role of certain components of alcohol, the polyphenols, and especially resveratrol, as possible causes of this effect. The oestrogenic role of this component and its antiresorptive effect has also been related with a positive effect on the bone, and, although there are no prospective studies in humans, a recent work carried out in a model

using oophorectomised rats showed that those rats treated with resveratrol had a BMD significantly higher than the untreated rats[14].

At all times we are referring to a moderate intake of alcohol since in populational studies, it has been observed that people drinking large quantities of alcohol, including beer, had a reduction in BMD in relation to the population of non-drinkers[15].

Beer and bone health

A moderate intake of beer has been shown in a study carried out with ultrasound to have a positive effect on bone mass in postmenopausal women, as an independent variable[16]. The authors evaluated bone mass measured by ultrasound in the phalanx in a group of 1,697 healthy women, 710 premenopausal, 176 perimenopausal and 811 postmenopausal (average age 48.4 years), with a body mass index (BMI) of between 19 and 32 kg/m^2. A positive and independent relationship was found between those parameters determined by ultrasound and the following variables: age, BMI, status of gonads and intake of beer, but not with consumption of wine. The positive relationship between the ingestion of beer and bone mass may be due to various factors:

A) The role of alcohol

As we have already said, the alcohol which some beers have has a beneficial effect on bone, related to the aforementioned polyphenols which the alcohol contains.

B) Role of silicon in bone[17]

Silicon (Si), an important component of beer, appears to be the main determining factor, acting on bone formation, since the differences were minor when the results were adjusted to its consumption.

Silicon is a non-metallic element in the periodic table with a molecular weight of 28. It is the second most abundant element in the earth's crust, but it is rarely found as a free element since, due to its affinity to oxygen, it forms silica and silicate, as well as organic forms such as silicones.

The ingestion of Si varies, in most Western countries, between 20 and 50 mg per day, greater than the intake of iron or zinc. It is usually ingested as orthosilicic acid, and the most significant source of this in infancy is cereals and in adults, beer, whose intake is higher in men than in women, its concentration being low in the water we drink, although somewhat higher in rocky regions.

In a study carried out in the United Kingdom, the higher concentrations of Si in foods were found in cereals, especially in less refined cereal, and among drinks, in beer, whose cereal origin (barley) is well known[18].

The content of silicon in beers varied between 6.4 and 56.5 mg/l, with an average of 30 mg per litre, being greater in those beers made form barley than in those made from wheat[19]. With two beers usually being the equivalent of a little less than half a litre in our country, an individual may obtain 15 mg of this nutrient by drinking two beers. Hops, a normal component of beers, has more Si than the grain, thus making a greater contribution of silicon[19]. During the brewing process, the great majority of the silicon stays in the grain which is used; however, when the grain is subject to aggressive manipulation during the brewing process, this may facilitate a greater extraction of silicon from the grain which is then incorporated into the beer. The authors also say that the lightest malts had more silicon than the darker ones, such as black or toasted malt.

Effect of silicon on bone

There are many studies, both experimental and in humans, in which a positive effect of Si in the bone has been observed, and showing that its administration produces in a positive effect on bone mass. Since the initial findings of Schwartz et al.[20] on the potential role of silicon in bone and connective tissues, there has been much research carried out into the potential role of dietary silicon[17]. Numerous cell and tissue culture studies have attempted to study the action mechanism of silicon in bone. Carlisse et al.[21], using chondrocites and tibial epiphysis from chicken embryos, showed that the silicon increases the synthesis of the bone matrix, and that the increase the activity of prolyl hydroxylase, the enzyme related to the synthesis of collagen, was dependent on the dose of silicon. A study carried out in human osteoblasts[22] has confirmed that silicon increases osteoblast proliferation, the synthesis of the extracellular matrix, the activity of alkaline phosphatase and the synthesis of osteocalcin. More recently, using orthosilicic acid, a positive effect on bone formation has been observed, with a positive action by silicon on cell differentiation and the synthesis of type 1 collagen, as well as an increase in mRNA of these proteins, suggesting a potential role in genetic transcription[23,24].

In the aforementioned Framingham cohort study[25] the association between the ingestion of silicon and BMD in the lumbar spine and in four locations in the hip was examined in 1,251 men and 1,586 pre- and postmenopausal women, aged between 30 and 87 years, adjusting the results for all factors which it is known may have an influence on BMD and on nutritional intake. It was observed that the dietary intake of silicon was associated positively, and significantly, with BMD in the hip in men and premenopausal women, but not in postmenopausal women. It was also observed that there was a significant association with BMD in the lumbar spine in men, concluding that a high ingestion of silicon in younger men and women could have a positive impact on the health of the skeleton, especially in cortical bone.

However, the authors warned that many other studies have shown that the consumption of more than one or two alcoholic drinks a day may be

damaging to health. The advice of the authors would be "drink beer, but in moderation" since this consumption "contributes to an improvement in silicon levels, and thus, in your health".

We know also the beneficial role of bone implants which contain silicon in the repair of bone. Implants which contain silicon secure themselves better than those that don't, due to the spontaneous formation of a biologically active layer of a substance similar to apatite on their surface[26].

Oral supplements of silicon have been shown to be beneficial in patients with low bone mass and who are osteoporotic, with a tendency being observed to an increase in markers for bone formation, especially P1NP (N-terminal propeptide of type I collagen), as well as an increase in femoral BMD[27].

C) Role of the phytoestrogens

Another of the mechanisms related to the beneficial effects of the moderate ingestion of beer on the bone could be due to its phytoestrogen content. Due to the similarity both structural and functional of these with 17-beta estradiol, interest in these substances has increased recently. In a study carried out in the United Kingdom the amount of phytoestrogens in various foods, including tea, coffee, alcoholic drinks, nuts, seeds and oils was quantified. It was found that beers, except bitter, were the foods which contained the most phytoestrogens, around 71µg/100g, especially lignane[28].

Finally, there is a conviction that beer drinkers are, generally more obese, but when the BMI is adjusted for other risk factors for obesity it is found that it is highly improbable that the intake of beer is related to BMI[29].

In summary, we have available various experimental and clinical studies in which it is concluded that the moderate ingestion of beer, due to its high flavonoid and silicon content, could have a positive effect on BMD, causing it to increase. More prospective studies are necessary in order to evaluate its possible effect in reducing fractures.

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Symptomatic hypocalcaemia after the administration of bisphosphonates

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Summary
The bisphosphonates are widely and very safely used both for the prevention and the management of metastatic bone disease in tumoral processes. In spite of this, its use if not free of complications, of which hypocalcaemia, which is usually light, is one of the most frequent. There are various factors which increase the risk of this occurring, some of which are not yet well known, but which should be taken into account in all patients before the administration of these drugs to avoid serious cases of symptomatic hypocalcaemia.

Key words: *bisphosphonates, zoledronic acid, prostate carcinoma, bone metastasis, hypocalcaemia.*

Introduction

The bisphosphonates have a well-established role in the management of tumoral hypercalcemia and other skeletal complications of neoplasms. Their usually good levels of tolerance may make us forget that on occasion they have serious secondary effects. It should be of interest, therefore, for us to communicate the case of a patient recently treated in our centre, as a warning in this matter.

Clinical case.

A 74 year old male with a history of post-traumatic epileptic crisis, in treatment with phenytoin and phenobarbitol; high digestive haemorrhage secondary to duodenal ulcer and carcinoma of the prostate with bone affectation, as well as urethrovesical and ganglion carcinoma, in treatment with bicalutamide and leuproreline for the past four months. Admitted due to pain and increased diameter of the right inferior member, which was related to compression of the iliac vessels by lytic metastases in the right iliac bone, extending into the soft tissues. Also present were multiple osteoblast metastases. Notable among the complementary tests were anaemia of chronic disease (Hb, 10.8 g/dl), a moderate deterioration in renal function (urea, 81 mg/dl; creatinine, 1.6 mg/dl) alkaline phosphatase, 1,150 U/l (normal, 129 U/l) and calcium at the lower limit for normality (8.1 mg/dl; normal: 8.4-10.4 mg/dl), with albumin of 3.7 g/dl and corrected calcium of 8.4 mg/ml.

As part of the palliative treatment, and with the aim of reducing the tumoral affectation of the bone and the osteoporosis derived from the hormonal blockage, local radiotherapy was administered as well as a dose of 4 mg of zoledronic acid by short intravenous infusion. Five days later, he began to feel a sensation of "numbness" in both arms. Notable from the physical examination was a positive Trousseau sign after 1 minute. The total calcemia was 4.8 mg/dl, and of ionic blood calcium 2.7 mg/dl (normal: 4.6-5.4 mg/dl). In the ECG there was a prolongation of QT (0.48 sec). The magnesemia and phosphatemia were normal (2.3 mg/dl and 2.7 mg/dl respectively). The concentration of 25 (OH) vitamin D was very low (7 ng/ml; normal:20-60 ng/ml) and the parathormone (PTH) was raised (526 pg/ml; normal 0-65 pg/ml).

Treatment was initiated with endovenous calcium and vitamin D derivatives (calcifediol 266 µg/24h and calcitriol 2 µg/24h), with which the tetanic manifestations disappeared after 48 hours. At the time of discharge, six days later, the calcemia was 7.9 mg/dl. Outpatient treatment was continued with calcium and vitamin D supplements at the same dose as during the admission, and it was decided to suspend indefinitely the treatment with zoledronic acid. A month later the calcemia was 7.9 mg/dl.

The bisphosphonates inhibit bone resorption and, apart from their use in osteoporosis, have a well-established role in the prevention and management of skeletal complications of neoplasms. Due to its potency and ease of administration zoledronic acid is the bisphosphonate most frequently used. In general, its tolerance is good, with the most frequent non-specific manifestations being pseudo-flu.

Hypocalcemia is another frequent secondary effect. In recent clinical trials hypocalcemia has been observed in approximately 5-10% of patients treated with zoledronate[1]. In studies of normal practice it reaches 30-40%[2-4]. However, it is light and without clinical repercussions. Thus, in the series of Zuradelli, 48% of patients with hypocalcemia had levels of calcium of between 8 and 8.5 mg/dl; 40% between 7 and 8 mg/dl; 11% between 6 and 7 mg/dl and only 1% had calcemia lower than 6 mg/dl[2]. Symptomatic cases are fortunately rare, given the tendency of hypocalcemia provoked by the inhibition of bone resorption induced by the bisphosphonates to be compensated for by an increase in the secretion of PTH, which reduces the renal elimination of calcium and increases its intestinal absorption through the stimulation of the renal hydroxylation of vitamin D. Cited also among those factors which increase the risk of developing serious hypocalcemia, have been the previous existence of hypocalcemia, various disorders which tend to impede this compensatory response, such as renal insufficiency, hypomagnesemia, vitamin D deficiency, hypoparathyroidism and treatment with loop diuretics, which increase calciuria[3,5-9]. Our patient had a number of these risk factors, including the deterioration of renal function and vitamin D deficiency, as well as treatment concomitant with anti-epileptics, which, unfortunately, were not corrected before the administration of the bisphosphonate. Whether on not the risk of hypocalcemia is higher in those patients with osteoblast metastasis is open to discussion[2]. In principle, this would be expected, given their greater tendency to deposit calcium in the skeleton and the frequency with which these patients have spontaneous hypocalcemia[10]. In fact, some studies have even found a lower frequency of hypocalcemia after the administration of zoledronic acid in patients with prostate cancer[3].

In order to limit the risk of hypocalcemia, all the aforementioned factors should be discounted, for which reason an earlier analytical determination of vitamin D, PTH, phosphatemia, magnesemia, and calcemia, and the correction of any alterations before initiating treatment with bisphosphonates would be recommended. In the case of vitamin D deficit, high doses may be used (for example, 10,000-20,000 U/day) over 2-4 weeks, since vitamin D and calcium supplements in "physiological" doses are not always efficacious, which is not surprising, given that at this dose vitamin D may take months to normalise. In any case, bisphosphonates should be avoided in patients with earlier hypocalcemia, hypoparathyroidism or renal insufficiency with creatinine clearance lower than 30 ml/min (except in the case of tumoral hypercalcemia).

In short, zoledronic acid and other powerful bisphosphonates are useful in the treatment of skeletal complications of neoplasms. However,

although they are usually well tolerated, they are not free of potentially serious secondary effects. Therefore, clinicians should be scrupulous in identifying and dealing with those factors which may increase their toxicity prior to their administration, which is rarely urgent. The case which we present is a warning of the consequences which may result if this is not carried out.

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