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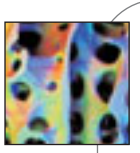

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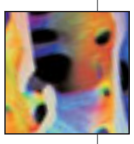

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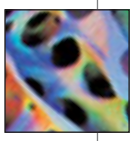

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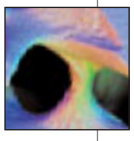

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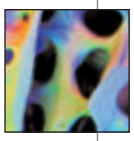

Letter to the Editor

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Bone pathology of Gaucher disease

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Gaucher disease (GD) is a congenital fault of the metabolism due to a deficiency in the lysosomal enzyme glucocerebrosidase, also called acid beta glucosidase. This enzyme deficit results in the accumulation of non-metabolised substrate in the lysosomes of various cell lines of the monocyte-macrophage system. The deposit of non-degraded material, a glucocerebroside called glucosylceramide, is an intermediate metabolite in the synthesis and breakdown of glucosphingolipids. These macrophages laden with lipids, called "Gaucher cells" , are involved in the pathogeny of the disease[1]. GD is a sphingolipidosis, which constitutes the most frequent liposomal deposition disease. GD is a multiethnic disorder which is inherited in a recessive autosomic way[1]. The Gaucher Registry is the largest co-operative observational register in the world. Up to January 2007, 4,585 patients from 56 countries had been registered (www.gaucherregistry.com). It is estimated that there are currently around 300 diagnosed cases in Spain, although it is calculated that there are many more. In the majority of case, the molecular basis of the disease is made up of mutations in the gene GBA (Glucocerebrosidase beta acid) located in chromosome 1 (1q21) which codes for glucocerebrosidase. GD has three clinical forms, and in all of these there is bone, bone medullar and visceral affectation. The Neuronopathic Gaucher Disease Task Force of the European Working Group on Gaucher Disease classifies the disease as: type 1, or non-neuropathic; type 2, or acute neuropathic; and type 3, or chronic neuropathic[2]. Type 1 GD is the most common, making up 94% of all cases. Type 2 GD is the form called infantile cerebral. Type 3 GD is very rare and is only seen in the Norrbottnian region in the north of Sweden. For this reason we are always here refering to type 1 GD. GD, as with other rare diseases is characterised by being multisystemic. Notable among its multiple clinical manifestations are osteopenia, bone pain, bone fractures, anaemia, thrombopenia, haemorrhages, delayed growth, hepatomegaly, splenomegaly and changes in liver function tests. The prognosis of GD depends on the degree of affectation of these clinical manifestations. GD is a disease which starts in infancy but which is not usually diagnosed until the age of 16 years[2]. Even in those patients diagnosed as adults, the signs and symptoms begin in infancy[3]. This is why each patient is different in terms their age of presentation, symptomology, diagnosis and progression of the disease. Although there is a fulminant presentation form in infancy, the disease may be asymptomatic and diagnosed by chance in adults, in whom it usually takes an insidious and progressive course. Despite being treated as a hereditary disease, the diagnosis of type 1 GD is carried out in 74% of cases at an adult age. And 10% of cases of GD are even diagnosed at over 50 years of age. If it is not brought to mind, it is almost impossible to diagnose. It initially presents as a combination of symptoms such as bone pain, haematomas and asthenia. For this reason it is usually wrongly labelled as a non-specific viral infection, "growing pains", a crisis of acute bone pain with local inflammation and/or fever with necrosis in the hip categorised as Perthes disease, accidental fractures, recurrent epistaxis due to non-specific alterations in coagulation and splenomegaly. The patient with established type 1 GD is usually pallid, with a distended abdomen, thin extremities and valgus knees. They may die in the aftermath of sever bone disease, haemorrhages, infections, liver insufficiency or lung complications. In addition, these patients also have a higher risk of multiple myeloma.

The skeletal affectations are accompanied by osteopenia, bone pain crisis similar to those of drepanocitic anaemia, osteolitic lesions, pathological fractures, vertebral compression and osteonecrosis (avascular necrosis) of the proximal and distal extremes of the femur, and the proximal extremes of the tibia and humerus[4]. The data in the International Register of GD from 1,698 patients shows that 94% have type 1 GD, and of

these, 63% suffer from bone pain, 33% have a crisis of bone pain, 8% have required joint replacement and 94% had radiological evidence of skeletal disease. A radiological study may prompt a diagnosis of GD and/or its complications. X-rays of the large bones can show in 46% of cases a deformity in the Erlenmeyer flask in the distal extreme of the femur, caused by anomalous metaphyseal remodelling[3]. This failure is suggestive but not pathognomonic, requiring a differential diagnosis from osteopetrosis, Nieman-Pick disease, heavy metal poisoning and fibrous dysplasia. The X-rays may also show fractures and lytic lesions, which are present in 15% and 8%, respectively, in the patients on the GD Registry[3]. Bone densitometry with DXA shows generalised loss of bone mass in all the patients[5]. Osteopenia is present in 42% of the patients on the GD Registry[3]. Gammagraphy with technetium detects the presence of ischemia during bone pain crises. The infiltration of the bone medullar, present in 40% of the patients, can be detected through magnetic resonance. Bone infarcts and osteonecrosis, present in 25% of cases, can also be detected through magnetic resonance[3]. The bone affectation can also cause an increase in acid phosphate. The diagnosis is based in a high index of suspicion based on clinical, radiological and laboratory signs described above. The confirmation diagnosis is based on the demonstration of a deficit in the activity of the glucocerebrosidase enzyme (beta glucosidase) in the leukocytes of peripheral blood (enzymatic diagnosis). It is also possible to carry out a study of the mutations of DNA in the cells of the patient, which serve to classify and diagnose their carrier status. They also serve to predict clinical signs and to identify familial cases and heterozygote carriers. The evolutionary control of GD includes blood analysis (chitotriosidase, haemogram and haematic biochemistry), bone densitometry, following the recommendations of the ICGG (www.gaucherregistry.com/)[3]. Chitotriosidase is a marker for the stimulation of the macrophages. It is increased in Gaucher patients and reduces in response to replacement therapy. It is used in the diagnosis and follow up of the disease. GD is one of the few rare diseases which has a treatment. This consists of treatment to replace the deficient enzyme, glucocerebrosidase, through the administration of recombinant glucocerebrosidase imiglucerase (imiglucerase)[3]. Early treatment can prevent or delay the progression of bone, and other, complications , hence the importance of early diagnosis of the disease. Once developed, osteoschlerosis, osteonecrosis and vertebral compression are irreversible[3]. In summary, GD is a rare multisymptomatic disorder which requires a high index of suspicion on the part of the doctor. Given that it affects multiple organs and systems, any professional caring for patients should be aware of it. GD is one of the few hereditary metabolic disorders which can be treated through enzyme substitution therapy with recombinant enzyme. Since early treatment can prevent the development of irreversible physical disabilities, early diagnosis is essential to improve the patient's development. This is why observational epidemiological studies are of help to the doctor in their approach to the diagnosis and treatment of this disease.

We have established in SEOMM a working group on Gaucher disease. To all those associates who are interested in the study of this disease - come and join us!

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The paradox of vitamin D deficiency in sunny regions, in young people or in osteoporotic patients treated with vitamin D, could be explained by common genetic variations. Have we found the Rosetta Stone of this apparent contradiction?

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The "epidemics" of rickets which devastated humanity appeared to have ended with the discovery of vitamin D at the start of the last century. However, severe and prolonged deficiency of vitamin D, with clinical manifestations of rickets and osteomalacia is rising again, above all in ethnic minorities, in Western countries[1].

At present, vitamin D deficiency constitutes a pandemic which affects more than half the population of the whole world[2], and is a significant factor in age-related loss of bone and muscle mass , falls and fractures[2,3].

In addition, in developed societies, vitamin D deficiency is associated with a higher risk of degenerative and chronic diseases, such as autoimmune diseases: diabetes mellitus, multiple schlerosis; cancer: colon and breast; infectious diseases, such as tuberculosis and seasonal flu; cardiovascular diseases, cardiac insufficiency, hypertension, and acute myocardial infarction, and even a higher risk of cardiovascular death, or death by any other cause[2,3]. Although, the great majority of the studies are associative and not interventional, the biological plausability generated by knowledge of non-hormonal actions, intracrines and paracrines of the endocrine system of vitamin D, give consistency to the potential problem which, for the public health system, a deficiency or insufficiency of vitamin D may constitute[3].

"Vitamin D" in circulation is made up of vitamin D_3 and D_2, the first mainly acquired by subcutaneous formation by ultraviolet B radiation, and in smaller qualities by ingesting the few natural dietary sources which contain it, as well as fortified foods or supplements, the second solely from these last two sources[4]. Once acquired, the vitamin D, and later its metabolites, are transported by means of a vitamin D transporter protein, also known as "gc-globulin (group-specific component)", which also participates in transport within cells[2,3].

In the liver, by the action of, above all, the microsomal enzyme CYP2R1, the "vitamin D" is converted in to 25 hydroxyvitamin D (calcifediol), the most stable and abundant metabolite, biomarker for the status of the organism of vitamin D[2,3].

An adequate blood level of calcifediol is critical for human health because it is a substrate for the formation of 1-25-dihydroxyvitamin D_3 (1-25$(OH)_2D_3$ or calcitriol), through the action of the enzyme CYP27B1-hydroxylase in the kidneys. This enzyme is stimulated by the parathyroid hormone and inhibited by phosphorus and by the fibroblastic growth hormone 23 (FGF23), produced by the osteoblasts and osteocytes.

Calcitriol is a key hormone in the homeostasis of bone and calcium which controls the regulation

of the transcription of the genes involved by binding them to a high affinity receptor (HAR) in the classic target organs: intestine, kidneys, bone (osteoblasts-osteocytes)[2,3].

Calcitriol is also synthesised in other organs and tissues, such as muscle, heart, brain, breast, colon, pancreas, prostate, skin, immune system. Those which possess the enzyme CYP27B1-hydroxylase activator for the synthesis of calcitriol and the inactivator enzyme (24-hydroxylase, CYP24A1), which favours its catabolism, and the HAR receptor.

Calcitriol regulates approximately 3% of the human genome, with three generic effects: regulation of hormonal secretion, inhibiting rennin, stimulating the secretion of insulin and its action; it regulates the growth and proliferation of cells and modulates acquired and innate immunity[2].

At present, there is a significant controversy regarding three aspects related to calcifediol. Its quantification; the establishment of minimum adequate, and optimum, levels; and the apparent paradox of vitamin D deficiency in sunny regions, in young people from these regions, and in osteoporotic patients, treated, or not, with vitamin D.

Despite its importance, the measurement of 25(OH)D has always been problematic and even now generates concerns[5]. In fact, until relatively recently it was restricted to research centres, which used methods based on protein competition or high resolution liquid chromatography (HRLC). At the end of the last century other methods were validated for use in care, such as RIA, ELISA or chemiluminescence. The spread of availability of the CLAR technologies, coupled in tandem with mass spectrometry (LC-MS/MS) has improved the performance of the measurement of 25(OH)D and is allowing the standardisation of the result obtained with conventional techniques[6].

Even nowadays, there is no unanimous consensus on the recommended minimum blood levels of 25(OH)D to ensure bone health, and other health objectives mediated through vitamin D. Last October, in Bruges, Belgium, during the 14th "Workshop" on vitamin D a round table was convened to reach a consensus on this matter[7].

The debate became focussed around two options, the European one, led by Roger Bouillon and Paul Lips, who proposed minimum blood levels of calcifediol of 20 ng/ml, and the American, defended in presentations by Robert Heaney and Reinold Vieth, both proposing levels of 25(OH)D higher than 40 ng/ml[7], without an agreement being reached. In any case, these levels should always be higher than 20ng/ml, which would suppose average blood levels in the population to be higher than 30 ng/ml. Surprisingly, a target for minimum levels was proposed, but not one for maximum blood levels.

The upper limit for vitamin D in the blood is also not clearly established. But in populations highly exposed to the sun, blood levels of 25(OH)D are not usually found above 60 ng/mL, and no complications of hypercalcaemia or hypercalciuria are found[8]. Therefore, reaching blood levels of calcidiol of between 20 and 30 (higher than 20 in any case) and 60 ng/mL, seems recommendable from a physiological point of view. Surprisingly, even in a country as sunny as Spain, and independently of the region we consider, the insufficiency and even the clear deficiency in vitamin D, is that described in scientific publications[9,10,11], and that which we find in normal clinical practice. On the other hand, in patients treated with calcium and vitamin D in postmenopausal osteoporosis there is evidence of insufficiencies in calcium and vitamin D in more than 60% of the population, both in Spain[11] as well as in other countries[12,13].

In this edition of the Review of Osteoporosis and Mineral Metabolism[14] a higher prevalence of insufficiency or deficiency in vitamin D is described in a group of medical students from Las Palmas de Gran Canaria, which confirm the data found in young junior doctors (Residentes) who began their specialisation at the 12th October Hospital in Madrid[15]. These data coincide with the descriptions of young people in countries or geographical regions which are sunny and have a good climate, such as Hawaii[16], or of colder and less sunny regions[17].

These descriptions and observations of low levels of vitamin D even in situations favourable to finding adequate levels, produce great perplexity among researchers and medical practitioners, because, at least theoretically, exposure to sunlight or a sufficient intake of vitamin D should be enough to maintain the status of adequate vitamin D.

We know that personal habits and socio-cultural factors, which can modify the diet and exposure to sun, are the main determinants of the availability of vitamin D in the blood. The concentration of 25(OH)D is higher in summer and autumn, and lower in spring and winter[18]. However, only a quarter of the variability in blood levels of 25(OH)D can be attributed to the season, latitude and intake of vitamin D[19,20]. Association studies of families and twins suggested that genetic factors contributed the most to the individual variability observed, with more than 50% of this variability being inherited[21]. In fact some rare Mendelian alterations, such as the Smith-Lemli-Optiz syndrome are associated with vitamin D deficiency[22].

Almost at the same time that this edition of the Review of Osteoporosis and Mineral Metabolism 14 published the apparent contradiction of being young, knowing the importance of taking sun and living in a sunny region of Spain, and having low levels of vitamin D, Wang et al. in The Lancet, give a possible explanation[23]. By means of a large consortium of experts ("SUNLIGHT consortium"), a study of some 30,00 persons in five selected epidemiological cohorts, which were then increase to 15, stated that at least 3 or 4 genes contribute to the variability in concentration of 25(OH)D in the blood[23].

The genes involved code for three key enzymes in the metabolism of vitamin D: 7-dehydro-

cholesterol (7-DHC), reductase (responsible for the availability of 7-DHC in the skin); hepatic 25-hydroxylase CYP2R1 (involved in the conversion of vitamin D to 25-hydroxyvitamin D) and CYP24A1 (key enzyme in the catabolism of vitamin D). In addition, the GC gene which codes for the vitamin D transporter protein. The polymorphisms in GC had the greatest effect on the blood concentration of vitamin D[24].

The authors propose that those patient found in the higher quartile of a "score" constructed with those genotypes studied multiply by two their risk of having vitamin D insufficiency.

This finding could constitute the Rosetta Stone to start deciphering the hieroglyphics of the variability in blood concentrations of 25(OH)D in patients who, according to environmental factors, should have high levels and "surprisingly" have low levels. If confirmed, it would help us to understand the "inexplicable" variations in the corporal status of vitamin D cited earlier, and would demonstrate that some polymorphisms could protect or accelerate the step to deficiency or insufficiency in vitamin D. Posing the following question: do these genes modify the response to supplementation with vitamin D?, the answer has important pharmacological or nutrigenomic implications.

In any case, the battle against vitamin D deficiency continues, and while we know, in depth, the mechanisms involved, we should propose as an unrenounceable public health objective, the correction of vitamin D deficiency, from infancy and throughout life, to prevent its impact on bone and to achieve other health objectives, and in osteoporotic women treated with anticatabolic drugs, to optimise their therapeutic response[25].

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Factors related to vitamin D deficiency in students of medicine in Gran Canaria

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Summary

Introduction: The bone-related and non bone-related functions of vitamin D are becoming better known by the day. As a result, levels of 25 hydroxyvitamin D (25-HCC) above 30 ng/mL are considered optimum.
Objectives: To study in a population of medical students in Gran Canaria what nutritional and lifestyle factors are associated with high levels of 25-HCC.
Material and method: A transverse study carried out in 98 Medical students of both sexes at the University of Las Palmas de Gran Canaria. All completed a questionnaire about their lifestyles and nutritional habits. A general physical examination was carried out and blood in fasting was taken to determine various biochemical parameters, including markers for remodelled bone, PTH and 25-HCC. In addition, bone mineral density was determined by dual X-ray absorptiometry and using ultrasound parameters in the calcaneum.
Results: We did not find statistically significant differences between thestudents who had levels of 25-HCC higher than 30 ng/mL and those with levels below this figure, in any of the variables studied, with the exception of male sex and the consumption of vitamin supplements.
Conclusions: Male gender in students of medicine in Gran Canaria, and the consumption of vitamin supplements, are associated with levels of vitamin D lower than 30 ng/mL.

Key words: *Vitamin D, Optimum levels, Young people, Students, sun, Exercise, Canary Islands, 25 hydroxycholecalciferol.*

Introduction

Vitamin D has a crucial role in bone metabolism, being responsible for the intestinal absorption of calcium and for bone mineralisation[1]. However, in recent years, in addition to being recognised as having an important role in the prevention and treatment of osteoporosis, many extra-bone actions have been described: reduction in risk of infections and autoimmune diseases, increase in muscle power, reduction in risk of suffering neoplasias of the colon, breast and prostate, improved control of diabetes, and preventing the appearance, or improving the course, of other diseases[2-13].

In the last few years a debate has developed around what are the optimum levels of vitamin D. Its metabolite, 25 hydroxyvitamin D (25-HCC) is considered to be the best indicator of the state of vitamin D reserves. Some authors have come to recommend optimum figures of 75 ng/mL of 25-HCC[14]. Others, such as Heaney, consider optimum levels to be those higher than 32 ng/mL[15], and as a consequence of this, a broad current of opinion has developed which situates the optimum levels of vitamin D as those in which 25-HCC is above 30 ng/mL, especially when referring to its extra-bone actions[1,16-19].

Nowadays, levels of vitamin D (25-HCC) are considered to be optimum when the values of 25-HCC are above 30 ng/mL, with the majority of authors considering that there is an insufficiency when these levels are below 30 ng/mL and a deficiency when levels are lower than 20 ng/mL[1,17-19]. However, in a high proportion of the population, both in patients and in healthy subjects, levels are found below these values.

The students of medicine of the University of Las Palmas de Gran Canaria (ULPGC) would theoretically be in ideal conditions for having optimum values of vitamin D, given that the climate of Gran Canaria is very sunny, with an annual average for sunshine of 2,750 hours, with the total average daily level of solar radiation in January being 3.1 kWh/m^2 and in July, 5.5 kWh/m^2,[20], and that the students are young, healthy and have theoretical knowledge regarding the vitamin D metabolism and the consequences of a deficit. However, in a previous study[21], we found that only 38.8% of the students of medicine of ULPGC, (42.1% of the males and 44.9% of the females), showed values of 25-HCC higher than 30 ng/mL, observing a deficiency in vitamin D in 32.6%, and an insufficiency in 61.2%, of the students.

Thus, in this work we have tried to identify what nutritional and life style variables could be associated with optimum levels of vitamin D.

Material and method

This is a transversal study, carried out in students of medicine in the Faculty of Health Sciences of ULPGC. The universe consisted of the totality of medical students in this faculty (620 in the 2007-8 course). All were invited to participate in this study, without restrictions. 103 students signed up, from all the courses, and gave their informed consent at the moment of completing the questionnaire, described later. There were two students from whom it was not possible to take blood, and another three who were not included because they did not complete the questionnaire or did not attend an appointment to determine their bone mineral density. 98 students completed the study.

Questionnaire. Physical examination

All the participants were asked to complete a questionnaire, which was self-completed, in which data was gathered on nutritional and lifestyle habits, with special attention paid to activity related to exposure to sun. All were weighed in light clothes and their height measured. The collection of data and the extraction of blood were completed over three days in May 2008.

The body mass index (BMI) was obtained using the formula: BMI: weight/height^2 (kg/m^2).

Collection of samples and laboratory techniques

The blood and urine samples were collected in the morning between 8.00 and 9.00 hours, after a night of fasting. The blood was collected in the correct tubes for each specific test, with the least vein compression possible, centrifuged at 1,500 g for 10 minutes, the serum separated in aliquots and stored within an hour of extraction at -20° C until the biochemical analysis was carried out, although most of these were carried out on the same day as the extraction.

The glucose, urea, creatinine, calcium, inorganic phosphorus, total protein, total cholesterol and its fractions and triglycerides were measured using automated techniques in an auto-analyser (Kodak Ektachem Clinical Chemistry Slides).

The blood calcium was corrected in accordance with the total proteins, by means of the formula:

Corrected calcium: Previous calcium (mg/dl)/[0.55 + total proteins (g/L)/16]

The tartrate resistant acid phosphatase (TRAP) was determined by spectrophotometry. Parathyroid hormone (PTH), 25-HCC, beta-crosslaps, osteocalcin, and PINP were determined through immunochemiluminscence.

Measurement of bone mineral density

The bone mineral density (BMD) was measured in the lumbar spine and in the proximal extremity of the femur with a Hologic QDR 1000 (Hologic Inc. Waltham, USA) densitometer. All the measurements were carried out by the same technician so that there were no interobserver variations. The coefficient of variation in our centre is 0.75 ± 0.16% with a range which varies between 0.6-1.13%[22]. The T-score values were calculated using the values of normality previously established for the Spanish population[23].

Determination of ultrasounds in the calcaneum

The ultrasound parameters in the calcaneum in the dominant foot were estimated by means of a Sahara© Hologic©(Bedford, MA, USA) ultrasound machine. This apparatus measures both the broadband ultrasound attenuation (BUA) and the speed of sound (SOS) in the area of interest of the calcaneum. The BUA and SOS combine in a single parameter called the quantitative ultrasound index (QUI), known also as the consistency index, which is obtained by means of the formula:

$$QUI = 0.41(SOS) + 0.41(BUA) - 571$$

Statistical study

This study has as its aim the identification of those factors which are associated with optimum levels of vitamin D. To this end, starting with the determination of the marker (blood levels of 25-HCC), the subjects were classified as having, or not having, an ideal level, according to whether the level of the marker was or was not above 30 ng/mL. In each of the groups in the study, the numerical variables were summarised as an average and SD, or as a median and IQR, according to whether or not they assumed normality, while the categorical were summarised as percentages.

In order to identify factors associated with the main objective, a multidimensional logistic regression analysis was carried out. Included in the analysis were all the variables which showed an association with $p< 0.1$, and all those which were related to frequent exposure to the open air (hiking, sport and walking in the open air). A retrospective selection of variables was carried out using the likelihood ratio test. A variable was kept in the model when the corresponding p-value was less than 0.1. The logistic model obtained is summarised in p-values and adjusted odd-ratios which were estimated by means of confidence intervals at 95%. The results of the analysis are then summarised in tables.

Results

Table 1 shows the number of students included in each group. A total of 60 subjects had levels of 25-HCC lower than 30 ng/mL, forming group I, or the group with insufficient levels. The 38 remaining, whose levels of 25-HCC were equal to, or greater than, 30 ng/mL made up the non-deficit group, or the group with optimum levels. The study was carried out in the month of May. There were no statistically significant differences between the two groups in terms of age, weight, height, BMI or waist measurement. The proportion of males who had insufficient levels of vitamin D was statistically higher ($p= 0.05$).

Table 2 lists the descriptions of the nutritional and lifestyle habits of the students who formed part of the study. There were no statistically significant differences in the distribution of these parameters between the students who had levels of 25-HCC higher than 30 ng/mL and those in whom it did not reach these levels. The only difference found was a tendency to a higher use of vitamin complexes among those students who had levels of 25-HCC lower than 30ng/mL, $p= 0.07$.

Table 3 shows the results obtained by comparing a series of biochemical parameters: kidney function, liver function, lipids, cholesterol and its fractions, triglycerides, glucose and ions. No statistically significant differences were found in any of these cases.

In Table 4 we present the data corresponding to the biochemical markers for remodelled bone (MRB), as well as p¡aratiroidea hormone (PTH) and the stimulating hormone of the thyroid (SHT).

In Table 5 we present the densitometric values. The bone mineral density (BMD) was estimated in the lumbar spine (L2-L4) and in the proximal extremity of the femur, in the femoral neck, the trochanter, the intertrochanter and the whole hip. In all these cases there were no statistically significant differences in the values obtained for the students in the two groups.

Table 6 shows the results of the logistic regression analysis. It can be observed that both the male sex and the consumption of vitamin supplements are inversely associated with optimum levels of vitamin D. Although the consumption of coffee appears to be protective and there seems to be a higher number of hikers the differences do not reach statistically significant levels.

Discussion

At present, there is a notable controversy about what are the optimum levels of vitamin D. Not many years ago values below 8 ng/mL of 25-HCC were considered as "severe deficiency", but more recently, optimum values of vitamin D have been considered to be those which prevent an increase in PTH and the development of secondary hyperparathyroidism (HPT)[25,26]. Even though up until now no consensus document has been published which advises on minimum desirable values of 25-HCC, there is a current trend to consider this to be 30 ng/mL[1,2,14-16,19,25].

We carried out the current study in a population of medical students of the ULPGC, because we consider that it could be considered as a "model" population for having optimum levels of vitamin D, for various reasons. First, because they are young and healthy, second, because due to their studies they know about the physiology of vitamin D and the ways of obtaining it, and third, because the place in which they reside, Gran Canaria, with its geographic proximity to the equator, situated at a latitude of 27 57 31 N°, has many hours of sun a year[20]. However, in analysing the prevalence of hypovitaminosis D in Canarian students, we found that only 38.8% of the students of medicine of the ULPGC, (42.1% of the males, and 44.9% of the females) showed values of 25-HCC higher than 30 ng/mL, with an insufficiency in vitamin D (less than 30 ng/mL) being observed in 61.2% of the students and vitamin D deficiency (less than 20 ng/mL) in 28.6% of them[21].

Table 1. Baseline characteristics of the population studied, classified as a function of blood levels of 25-HCC

	Insufficient levels n = 60	**Optimum levels n = 38**	**Value of p**
Age (years)	22.2 ± 3.3	22.4 ± 3.9	0.781
Man/woman (%)	36.7 / 63.3	18.4 / 81.6	0.054
Weight (Kg)	65.1 ± 11.7	62.0 ± 9.9	0.185
Height (cm)	168 ± 7.9	165 ± 8.2	0.092
BMI (Kg/m^2)	22.2 ± 2.9	21.7 ± 2.1	0.372
Waist (cm)	74.5 ± 8.9	71.9 ± 7.0	0.134

Table 2. Comparison of a series of parameters related to nutritional and lifestyle habits, depending on levels 25-HCC in the blood

	Insufficient levels n = 60	**Optimum levels n = 38**	**Value of p**
Coffee (%)	46.7	65.8	0.064
Alcohol (%)	30.0	26.3	0.694
Tobacco (%)	3.3	2.6	0.844
2 or more glasses of milk (%)	55.0	63.2	0.425
Meat 2 or more times/week (%)	76.7	76.7	0.534
Fish 2 or more times/week (%)	70.0	60.5	0.334
Butter (%)	8.3	5.3	0.565
Margarine (%)	6.7	15.8	0.146
Nº of salads weekly*	4 (3-5)	4 (2-5)	0.577
Nº of vegetables weekly*	3 (2-4)	3 (2-5)	0.950
Nº of fruits weekly*	6 (2-7)	7 (4-7)	0.223
Diet* in the last year (%)	18.3	23.7	0.522
Vitamin supplements (%)	21.7	7.9	0.072
Vitamin supplements in the last 3 months (%)	25.0	21.1	0.635
30 minutes walk daily (%)	76.7	76.3	0.968
Open air (walking) (%)	65.0	65.8	0.936
Sport (%)	51.7	55.3	0.728
Sport in open air (%)	11.7	21.1	0.408
Beach (last 3 months) (%)	71.7	63.2	0.377
Protective cream (%)	83.3	89.5	0.397
Hiking (%)	8.3	13.2	0.442
Rural living (%)	16.7	26.3	0.248
Chronic disease (%) ‡	30.0	31.6	0.869

(*) Median (IQR) ‡ The chronic diseases recorded were basically allergies (rhinitis, asthma), acne and migraine

Table 3. Biochemical parameters. Kidney function, liver function, blood lipids and ions

	Insufficient levels n = 60	Optimum levels n = 38	Value of p
Glucose (mg/dl)*	85 (82-88)	86 (81-91)	0.532
Urea (mg/dl)*	25 (22-28)	25 (22-32)	0.669
Creatinine (mg/dl)*	0.96 (0.89-1.07)	0.96 (0.89-1.04)	0.881
Uric acid (mg/dl)*	4.4 (3.7-5.3)	3.9 (3.3-4.8)	0.092
Total protein (g/L)*	7.5 (7.3-7.8)	7.6 (7.4-7.9)	0.478
Sodium (mEq/L)*	141 (140-142)	141 (140-142)	0.153
Potassium (mEq/L)*	4.3 (4.2-4.5)	4.3 (4.1-4.6)	0.921
HDL (mg/dL)	55.6 ± 14.0	58.3 ± 11.1	0.313
LDL (mg/dL)	104.8 ± 28.1	103.3 ± 24.7	0.779
Triglycerides (mg/dl)*	68 (53-107)	75 (59-91)	0.974
GPT (UI/L)*	15.7 (12.9-19.6)	15.3 (12.7-18.7)	0.904
GOT (UI/L)*	21.9 (20.0-24.6)	20.4 (16.6-23.2)	0.073
GGT (UI/L)*	15.6 (12.1-20.2)	14.3 (11.7-18.8)	0.314

(*) Median (IQR)

Table 4. Biochemical markers for remodelled bone. PTH and TSH

	Insufficient levels n = 60	Optimum levels n = 38	Value of p
FATR (UI/L)	2.1 (1.9-2.4)	2.0 (1.8-2.3)	0.255
Beta-crosslaps	0.46 (0.38-0.60)	0.46 (0.35-0.57)	0.699
P1NP (μg/L)	57.7 (44.3-74.3)	49.2 (41.4-68.7)	0.185
Osteocalcin (ng/mL)	24.3 (20.7-28.6)	24.2 (19.0-29.0)	0.930
TSH (UI/L)	1.84 (1.31-2.32)	1.60 (1.15-2.27)	0.284
PTH (ng/ML)	27.7 (20.1-34.8)	24.1 (16.0-34.1)	0.380

Median (IQR) in all cases

In this work we have studied what could be the factors which contribute to the existence of levels of 25-HCC below 30 ng/mL. Therefore, we have grouped the students according to their being below or above the cut-off point. The baseline characteristics of both groups are shown in Table 1. The same table shows that that is a higher number of males with low levels of vitamin D, the difference being statistically significant (p= 0.05), and confirmed in the logistic regression analysis (p= 0.047), Table 6. We did not observe statistically significant differences in any of the other variables shown in Table 1: age, height, weight, BMI or waist measurement. We do not know the reason why sex could play a role in the attainment, or not, of optimum levels of vitamin D. In a study carried out in healthy subjects, specifically 116 doctors starting their specialism (MIR), Calatayud et al.[27], confirmed the high prevalence of vitamin D insufficiency, since only 4.3% of the males and 12% of the females had levels of 25-HCC higher than 30 ng/mL. In another study carried out in Hawaii in young people, Binkley et al.[28], did not analyse the influence of sex on levels of 25-HCC, neither do they make reference to it, although the study included 60 males and 30

Table 5. Densitometric values in lumbar spine and proximal extremity of the femur. Ultrasound parameters in the calcaneum. Expressed as Z-score and T-score

	Level of vitamin D		
	Insufficient n = 60	**Optimum n = 38**	**Value of p**
DXA. Lumbar spine and proximal extremity of femur			
T-score lumbar	-0.125 ± 0.919	-0.135 ± 1.340	0.970
Z-Score lumbar	-0.104 ± 0.821	-0.103 ± 1.188	0.994
Z-Score femoral neck	0.209 ± 1.015	0.194 ± 1.209	0.947
T-Score femoral neck	0.151 ± 1.037	0.202 ± 1.194	0.823
T-Score total for hip	0.366 ± 1.114	0.265 ± 1.201	0.675
T-Score Trochanter	0.311 ± 1.073	0.314 ± 1.176	0.987
T-Score intertrochanter	0.368 ± 1.161	0.149 ± 1.147	0.364
Ultrasounds. Calcaneum			
Z-Score BUA	0.904 ± 0.774	0.904 ± 0.826	0.998
Z-Score SOS	1.372 ± 0.805	1.235 ± 0.823	0.483
Z-Score QUI	1.407 ± 1.032	1.184 ± 0.852	0.270
T-Score-BUA	-0.211 ± 0.781	0.903 ± 0.146	0.625
T-Score SOS	-0.097 ± 0.767	-0.199 ± 0.798	0.531
T-Score QUI	0.017 ± 1.030	-0.171 ± 0.858	0.351

The values express averages ± SD

Table 6. Multidimensional logistic analysis

Factor	Value of p	OR (95% CI)
Consumption of coffee	0.081	2.23 (0.91;5.50)
Hiking	0.058	4.51 (0.95;21.5)
Male sex	0.047	0.319 (0.103;0.985)
Vitamin supplements	0.048	0.233 (0.055;0.987)

females.

We did not find statistically significant differences in the distribution of lifestyle or nutritional habits between the two groups. In the comparison of averages or frequencies, we found only one "bias" in the consumption of coffee, which was less among students who had insufficient levels of 25-HCC, p= 0.064, and in the consumption of vitamin supplements which was higher in those students who had lower levels of vitamin D, p= 0.072. We did not find a single bibliographical reference around the possible reasons why a lower consumption of coffee is associated with lower levels of vitamin D, or the inverse, reasons why a higher consumption of coffee is associated with higher levels of vitamin D. Perhaps the only plausible explanation is that, in our culture, coffee is usually accompanied by milk, and the milk could be supplemented with vitamin D, but against this, we also observed in Table 2, that the consumption of milk was similar between the two groups, without statistically significant differences. We only found one study in the bibliography, published by Haney et al.[29], which, contrary to our

study, associated the consumption of vitamin supplements with higher levels of 25-HCC.

Curiously, we did not find statistically significant differences between the students who had higher levels of 25-HCC with those who showed lower values, in any of the following variables: walking daily for 30 minutes, walking in the open air, practicing sport, practicing it in the open air, having been to the beach in the last three months, and use of protective creams. These findings surprised us, since we expected that the students who had higher levels of 25-HCC would have greater physical activity in the open air or have spent more time at the beach. In the study carried out with young people in Hawaii, the authors obtained results similar to ours[28].

We did not find statistically significant differences in any of the biochemical parameters which we analysed, which were measured, basically, to detect any asymptomatic pathology. The study by Hinkley et al.[29], also found no differences in the values of creatinine in the two groups of young people with higher and lower values of vitamin D. By being a population of healthy adults, in whom the existence of chronic disease was scarce, with a lower pathology (allergies, headaches, etc), there were no statistically significant differences in values of bone mineral density measured by double X-ray absorptiometry (DXA) or in ultrasound parameters in the calcaneum, as can be seen in Tables 3 and 5. For the same reason, no differences were found in the biochemical markers for remodelled bone, either for formation or for resorption, Table 4, or in levels of PTH.

Lastly, we carried out a logistic regression analysis, studying what variables are associated with levels of 25-HCC below 30 ng/mL, and we found a statistically significant association with the male sex (p= 0.04).

Among the limitations of our study we include the fact that it was a transversal study, with a relatively small population, as well as the fact that the collection of data on exposure to sun, lifestyles and nutritional habits was through self-completed questionnaires. It is possible that some students gave incorrect information on these matters. Finally, the 25-HCC was determined by immunochemiluminescence, which is the technique we had available, instead of high pressure liquid chromatography, which is considered to be the ideal technique for the measurement of this metabolite[30].

In conclusion, Canarian medical students, although being in ideal conditions for having optimum levels of vitamin D, showed high levels of insufficiency and deficiency, without our having been able to identify what factors are associated with this, with the exception of male sex. Therefore more studies are needed on this matter.

So, we need to look deeper into the causes which result in this "paradox", that in situations advantageous to the acquisition of vitamin D are found instances of insufficiency or deficiency in vitamin D.

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The effect of zoledronic acid on the markers for remodelled bone in Paget's disease

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Summary

Background: The arrival of the biphosphonates signified an advance in the treatment of Pagets's disease of bone (PDB), but agents which are more efficacious and easier to use are needed to improve the complement of treatments. Zoledronic acid, a biphosphonate administered in the form of a single intravenous perfusion, could satisfy these requirements.
Method: We administered a perfusion of 15 minutes in duration of 5 mg of zoledronic acid to patients with PDB. The principal criterion for evaluating efficacy was the rate of therapeutic response at 6 months and 12 months, defined as a normalisation of the levels of alkaline phosphatase (AP), of amino-terminal propeptide of procollagen type 1 (P1NP), as markers for formation, and of carboxy-terminal telopeptide of collagen type 1 (CTx) as marker for resorption. We also evaluated the response of AP, CTx and P1NP at 18 months and 24 months.
Results: At 6 months and 12 months all the patients who received zoledronic acid presented a therapeutic response with normalisation of levels of AP, P1NP and CTx. The response was maintained at 18 and 24 months, although only one patient showed raised levels of AP at 24 months, coinciding with an elevation of hepatic gamma-glutamyl transpeptidase.
Conclusions: A single perfusion of zoledronic acid produces a rapid, complete and sustained response in PDB.

Key words: *Paget's disease of bone, Zoledronic acid, Bone markers.*

Introduction

Paget's disease of bone (PDB) is a process with an unknown cause which affects approximately 3% of the population over 55 years of age. It is the second most frequent cause of bone metabolism disease after osteoporosis. Around 2% of the United States population over 60 years of age[1], and between 6% and 7% of older people in western Europe suffer from PDB[1,2].

It is characterised by being a localised affectation of remodelled bone which starts with an increase in bone resorption mediated by the osteoclasts, with a later compensatory increase in the formation of new bone. The result is a disorganised mosaic pattern in the trabecular and cortical bone. This structural change produces bone which is increased in size, less compact, more vascular and more susceptible to deformation and fracture than normal bone.

To assess the activity of the disease and to supervise the response to treatment biochemical markers for bone turnover are used.

Although a viral origin of the disease, or the existence of immunological changes[3], have been invoked, the true aetiology of this disease is not known, and we cannot count on an appropriate therapy for its cure and must use pharmacological agents which suppress the activity of the pagetic osteoclasts, essentially the antiresorptives. On the one hand, the group of calcitonins, of salmon, of human, or of eel, administered principally intramuscularly or subcutaneously and, in some cases, intranasally, and on the other, the group of biphosphonates[4].

The indications for treatment and the choice of a therapeutic agent for the treatment of PDB even now continues to be debated. Improving the symptoms and preventing future complications should be the logical objectives of treatment for PDB. It has been clearly demonstrated that the suppression of the pagetic process by any of the agents can reduce certain symptoms, such as bone pains due to locally increased heat, headache due to the affectation of the skull, secondary lumbago due to pagetic changes in vertebrae, and a number of neural compression syndromes, in the majority of those patients. Pain due to secondary arthropathy in the spine, hip, knee or arm does not usually respond to antipagetic treatment. Although it is possible that osteolitic lesions can partially recuperate, deformities of the extremities do not improve after treatment, and deafness is almost impossible to reduce, although some studies certainly suggest a slow improvement in auditory ability after treatment.

In asymptomatic patients, the indications for treatment are less clear. There is no proof that a substantial reduction in the biochemical indices of the activities of PDB might prevent future complications. However, Meunier et al. have observed a conversion to a normal pattern of layered bone in bone biopsies after suppression of pagetic activity[5]. We also know that the active disease if untreated, may lead to the maintenance of a persistent degree of abnormal remodelled bone over many years, and develop complications in the bone or surrounding tissue. Therefore, the presence of moderate asymptomatic activity, such as FA two or three times above the upper limit of normality, is an indication for treatment. The biphosphonates, the treatment most used for PDB, often normalise the biochemical markers for bone turnover, achieves the substitution of chaotic fibrous bone for normal layered bone[6], and can also reduce bone pain[7]. The oral biphosphonates which are used nowadays should be administered daily, orally, over a period of two to six months; in addition, the patients need to fast before and after the treatment due to the low bioavailability of these drugs, and to stay upright for 30 minutes after their administration, in order to reduce the high risk of gastrointestinal complications. Another, intravenous, biphosphonate, pamidronate, is also used, which is a little impractical for patients because it is usually administered in a number of slow intravenous perfusions, which last some hours, and which require multiple visits. The development of drugs which are more comfortable to use, more efficacious and with a more prolonged effect could resolve these problems. Among the biphosphonates which have been used in clinical trials, zoledronic acid was highly efficacious in preclinical models[8,9]. Administered as a single perfusion lasting 15 minutes, its effects on the bone mineral density in postmenopausal women are similar to those achieved with 12 months of treatment with oral biphosphonates[10]. A recent study has shown its efficacy in the treatment of PDB[11]. This medicine offers the possibility of significant improvements in terms of its ease of use and therapeutic accomplishments, which, along with its higher efficacy, could increase the rate of response and the duration of periods of remission.

In this study we have assessed the effects of zoledronic acid on the biochemical indices of the disease's activity.

Method

Patients

18 patients (12 males and 6 females) in the Polyclinic for Bone Metabolism Diseases in our hospital, older than 30 years of age and diagnosed with PDB through traditional methods (bone gammagraphy and biochemical markers for bone turnover), were studied. The average age of the patients was 74 years (with a range of 50-91 years), two patients (11%) presented a monostotic form, and 16 patients (88%) corresponded to a poliostotic from. The exclusion criteria were the existence of primary hyperparathyroidism; data indicative of liver or kidney disease; history of uveitis, iritis or diabetic nephropathy or retinopathy; and the use of treatments for PDB in the preceding 180 days.

Treatment

The patients received an intravenous perfusion of 5 mg of zoledronic acid over a period of 15 minutes. In the background they were administered orally 1g of calcium a day and between 400 and 1,000 UI of vitamin D a day.

Assessment criteria

At the baseline, and at 6 and 12 months, levels of were determined of creatinine and FA using an autoanalyser (modular Roche DDPP), and of P1NP aminoterminal propeptide (ELISA) as another marker for formation, and CTx telopeptide (ELISA) as marker for resorption. In six patients an assessment was carried out at 18 months and in four at 24 months. All except three patients had raised levels of FA (average: 192 UI/l, normal, up to 129 UI/l). All had raised levels of P1NP (168.7 ug/l, normal, up to 62 ug/l. In 16 patients the values of CTx were raised (average: 0,895 ng/ml, normal, up to 0.548 ng/ml).

The principal criterion for the assessment of the therapeutic response was the proportion of patients in whom were obtained a normalisation of levels of FA, P1NP and CTx.

Results

At 6 months and 12 months from the infusion of zoledronic acid, a normalisation of the levels of FA, P1NP and CTx was observed in 100% of the patients. At 6 months a reduction was observed in FA of 64%, in CTx, of 78.4%, and in P1NP, of 83.2%. The response at 12 months was similar, maintaining normality in these parameters in 100% of the patients. The reduction in FA was 62%, in CTx, 75.4% and in P1NP, 83.5% (Figures 1,2 and 3). The response to the infusion of zoldronic acid was a significant reduction in blood levels of biochemical markers, although higher for P1NP and lower for CTx.

The number of patients studied at 18 and 24 months was small (6 and 4 patients), but it was observed that the levels of FA, CTx and P1NP stayed normal in all of them, except in one case in which a discrete elevation in the level of FA was produced, which coincided with an increase in hepatic GGTP. The reduction in FA was 64.21% at 18 months and 52.05% at 24 months, that of CTx was 81.9% at 18 months and 75.2 at 24 months and P1PNP was75.0% at 18 months and 80.7% at 24 months.

All the patients had an acceptable clinical response without significant secondary effects, although some presented light flu-like symptoms, without other significant biochemical changes, except a patient who developed hypocalcaemia after the infusion.

Discussion

The study corroborated the safety and efficacy of a therapy in a single dose for PDB, already demonstrated in an earlier study[11]. A single perfusion of 5 mg of zoledronic acid, administered over a period of 15 minutes, produces changes in various biochemical markers for bone activity.

Alendronate, taken orally, produces a reduction in concentrations of FA of 73% to 79% at 6 months[6,12,13], with normalisation of this index in 48-63% of patients. Other trials with risedronate have shown a reduction in concentrations of FA of 69% to 77% at 6 months, with normalisation in its blood levels in up to 73% of patients[7,14]. Tiludronate reduces the concentrations of FA by 49-59% at 6 months, with normalisation of levels in 11-44% of patients[15]. Ibandronate administered intravenously reduces levels of FA by 70% after one or two doses[16].

Figure 1. Changes in blood levels of alkaline phosphatase at 6 and 12 months after i.v. infusion of 5 mgrs of zoledronic acid

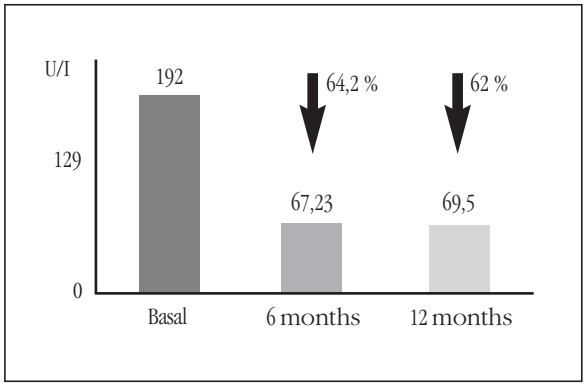


Figure 2. Changes in blood levels of procollagen type 1 aminoterminal propeptide (P1NP) at 6 and 12 months after i.v. infusion of 5 mgrs of zoledronic acid

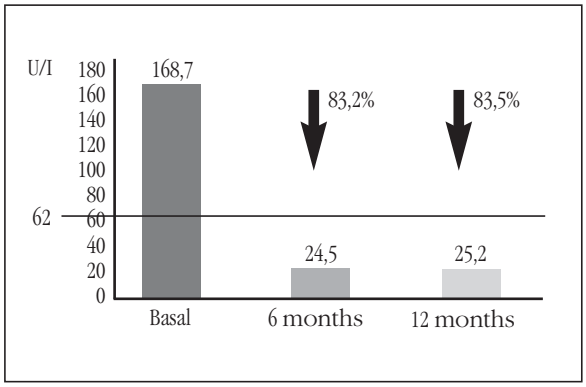
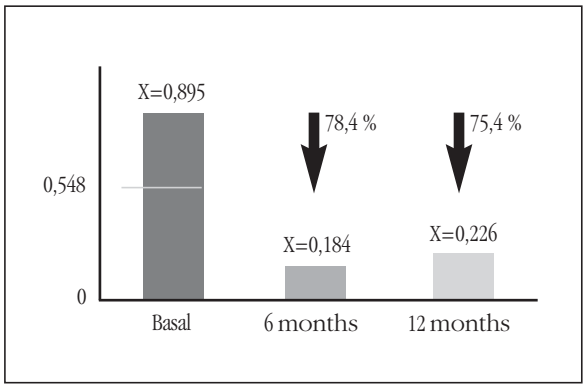


Figure 3. Changes in blood levels of collagen type 1 carboxyterminal telopeptide (CTx) at 6 and 12 months after i.v. infusion of 5 mgrs of zoledronic acid



When PDB is treated with biphosphonates, the duration of the remission depends to a great extent on the nadir reached by the metabolic turnover of the bone, for which reason it is probable that the interval between treatments is very prolonged with this drug.

This fact could bring benefits for patients both in reference to the ease of treatment and the risk of long term complications, such as degenerative arthropathy.

In an earlier study, zoledronic acid had shown a normalisation of FA of 88.6% at 6 months after infusion[11]. In our case, the normalisation was 100%, with a reduction in levels of 64.21%. The normalisation of levels of P1NP and CTx were also 100%, with a reduction in P1NP of 83.2%, higher than that achieved for FA, probably due to the fact that blood concentration of P1NP is a more specific index for osteoblast activity.

The bone resorption, assessed using the blood concentration of CTx, showed reductions of a similar magnitude, although somewhat less, as P1NP. Reid et al.[11] found a higher reduction in CTx than in P1NP in the first weeks after the infusion, consistent with the fact that the osteoclasts are the principal target for the biphosphonates.

In the aforementioned study, in the follow up after the trial (median: 190 days), only one of the 113 patients treated with zoledronic acid presented a loss in the therapeutic response. In our series, 100% of the patients maintained their normal levels of FA, P1NP and CTx at 12, 18 and 24 months, although one patient showed a slight increase in FA at 24 months coinciding with an increase in hepatic GGT. These data are in accord with the series of Hosking et al.[17], who studied the follow up up to 2 years of the patients included in the group of Reid et al.[11], in whom the response achieved at 6 and 12 months was maintained.

Our results confirm the efficacy of zoledronic acid in patients with PDB and add information to the data available by observing that prolonged remissions can be obtained.

The magnitude and duration of the effect of zoledronic acid are, probably, the result of its administration in a single dose, the great affinity of the drug with the minerals in the bone, and its powerful inhibition of the enzyme farnesyl diphosphate synthase[18-20]. The persistence of its effect makes it especially appropriate for the treatment of PDB, in that the necessity of frequently repeating a treatment is a big clinical problem.

In order to achieve a reduction in the incidence and the seriousness of complications in the long term, a persistent normalisation in bone turnover over many years may be necessary, and this now appears to be a realistic possibility with the use of zoledronic acid.

The flu-like symptoms are frequent after the intravenous administration of aminbiphosphonates and have been noted in two thirds of patients treated with pamidronate for Paget's disease.

Asymptomatic hypocalcaemia is frequent after the use of intravenous biphosphonates in patients with Paget's disease[21] and rarely require therapeutic intervention, although those patients with pre-existing hypocalcaemia or vitamin D deficiency should be treated before receiving these drugs. Our results indicate that the use of calcium supplements is fundamental for reducing to the minimum the appearance of asymptomatic hypocalcaemia.

In conclusion, we have established that a single perfusion of zoledronic acid can achieve rapid and prolonged remission, obtaining an excellent biochemical response at 6 and 12 months, achieving normalisation of raised levels of the markers for remodelled bone, a normalisation which is maintained at 18 and 24 months.

The effect is maintained for up to two years after treatment. The long duration of the remission could give way to a more complete control of the activity of the disease than has been possible up until now.

P1NP is the marker which has the better response to the administration of this compound and may serve as the outstanding marker in the diagnosis and follow up to treatment of Paget's disease.

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Vertebroplasty and kyphoplasty as treatment for osteoporotic vertebral fractures

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Summary
Over the last decade vertebroplasty and kyphoplasty have become popular as therapeutic options for the treatment of vertebral fractures. In fact, numerous non-controlled studies have indicated that both procedures are very efficacious for the control of pain associated with fractures. However, some recently published randomised trials have cast doubt on the true effectiveness of these procedures. On the other hand, certain observations have suggested that the increase in the rigidity which is produced by the injection of metacrylate into a vertebral body could facilitate the collapse of the adjacent vertebra. Therefore, vertebroplasty and kyphoplasty should not be considered as a routine theraputic measure, but should be limited to carefully selected patients, in whom the potential benefits surpass the risks and costs of the procedure. In any case, the patients should be put on a global treatment programme which includes pharmaceutical measures and non-pharmaceutical care to reduce the risk of future vertebral and peripheral fractures.
Various clinical trials have recently been published which were supposed to be an important contribution to knowledge regarding the effectiveness of vertebroplasty. The results have been rather contradictory both within themselves, and with earlier observational studies. For this reason it is worth reviewing this questions with the intention of helping clinicians who need to take decisions on the treatment of patients with osteoporotic fractures. We have not dealt with the possible utility vertebroplasty in other processes, such as fractures caused by tumours or by trauma.

Key words: *Vertebroplasty, Kyphoplasty, Vertebral fractures, Osteoporosis.*

Non-controlled studies

In the last decade vertebroplasty has been popularised for the treatment of acute or sub-acute vertebral fractures. This technique consists of the injection of a mixture of polymethacrylate (PMMA) and radio-opaque contrast by means of metallic trocars which are introduced through one or both vertebral pedicles (Figure 1). This compound, initially liquid, later solidifies in the interior of the vertebral body. It is assumed that this augments the resistance and provides mechanical stability to the fractured vertebral body, thereby avoiding its progressive collapse. In addition, since the initial studies it has been observed that many patients report a notable improvement in pain immediately after the procedure, due to a mechanism which is unclear, perhaps related to the chemical or thermal ablation of the nerve endings. These factors resulted in the establishment of the technique in many centres. The procedure requires a general anaesthetic or deep sedation. It is a demanding technique, which needs to be carried out by trained persons and with high resolution fluoroscopic equipment. Generally it is well tolerated and has few secondary effects. The main complication in the short term comes from the escape of PMMA into adjacent structures. If this happens in the direction of the intervertebral disc it may cause pain and result in a lesion in the adjacent vertebra. But if it is a small amount it does not usually have consequences. More serious is an escape towards the medullar canal or towards the foramens, causing medullar or radicular compression which may require surgical decompression[1]. Escapes into the venous blood flow may provoke local problems, pulmonary embolisms or arrhythmias[2].

Later, a modification in the initial technique arose, called kyphoplasty (Figure 2). With this, the injection of material is not made directly into the spongy vertebral bone, rather, a cavity is first created by inflating one or two balloons in the central region of the vertebral body[3,4].

In a search of Pubmed carried out in September 2009, 1,100 works were found on vertebroplasty or kyphoplasty. In the initial studies, with series of patients with osteoporotic or tumorous vertebral fractures, very favourable results were seen, such that 80% of patients had a significant improvement in pain. The refractory pain due to medical treatment was precisely the principal indication for treatment. However, in some patients the indication was prophylactic, that is to say, with the intention of "strengthening" a vertebra which had a small loss of height, and thereby avoiding the progression of its collapse. It has been suggested that the presence of bone oedema in the magnetic resonance (as a marker for acute or sub-acute fracture) is associated with a higher clinical efficacy of this procedure. However, a study by Voormolen et al. observed an improvement in pain in 94% of patients who had oedema, and 71% in those whom it was not present[5]. This suggests that the presence of oedema is associated with a greater efficacy of vertebroplasty, but that its absence does not exclude its use. However, it being a non-controlled study, means it is difficult to assess the influence the results could have on the spontaneous evolution of pain after fractures, which means that it is not possible to draw definitive conclusions.

It is notable that the growing establishment of vertebroplasty took place in the absence of appropriate clinical trials which demonstrate its efficacy. Hence, although observational studies suggested that the procedure was highly efficacious, it remains unclear up to what point the natural history of the disease is modified, nor what was the placebo effect component of the intervention. Also, it must not be forgotten that the pain of vertebral fractures tends to improve after a few weeks in the majority of patients, even in the absence of treatment. On the other hand, there have been doubts as to the long term safety of the procedure, since some authors have observed a higher rate of appearance of new fractures in adjacent vertebrae[6]. In fact some biomechanical models predicted that the increased rigidity of a vertebra increased the stress to which the neighbouring vertebrae were subject, which in theory increased the risk that they would fracture. Subsequently, in various series of cases, a higher incidence of new fractures in patients treated with vertebroplasty or kyphoplasty than in those subject to non-invasive treatment, has been found[7]. However, these not being randomised trials, the two groups are not necessarily comparable, which means that these studies do not allow definitive conclusions to be drawn in this respect.

Although the widespread use of vertebroplasty in the absence of trials which have demonstrated its efficacy may have been facilitated by some aggressive commercial practices, it should be taken into account that it is very difficult to carry out randomised blind trials with this type of treatment, in which invasive interventions are analysed. Fortunately, some researchers have made a serious effort in recent years to establish controlled studies which try, better, to assess the real efficacy of the intervention.

Non-randomised controlled trials

Between the years of 2003 and 2005 4 controlled, but not randomised, studies have been published. This is to say, patients were offered the possibility of having vertebroplasty (or kyphoplasty) and the development of those who accepted the procedure was compared with those who rejected it (which became the control group).

One of these studies (published preliminarily in 2003 and then in 2005), included patients with recent osteoporotic fractures, of less than 6 weeks standing. In comparison with the control group, the group treated with vertebroplasty experienced an improvement in pain from the following day and after 6 weeks. However, at the end of 6-24 months there were no differences[8,9].

A Spanish group, Alvarez et al.[10], carried out a similar study, but with patients with fractures and

pain of longer standing, between 6 weeks and 12 months. They also found that the group treated by vertebroplasty reported less pain than the control group on the following day, and after 3 to 6 months. Again, at the end of a year there were no differences between them. On the other hand, the treated group had a higher rate of new vertebral fractures.

On their part, Kasperk et al. assessed the usefulness of kyphoplasty in patients with vertebral fractures of more than 1 year's standing. They found that the procedure was associated with less pain and a better quality of life in the measures taken during the 6 months of follow up. They found no differences in the risk of suffering new vertebral fractures[11].

The assignment to the treatment groups not being randomised in these studies, the two groups, treated and control, are difficult to compare. In various cases the authors show that there are no differences in their baseline characteristics (one exception is the work of Alvarez in which the group subject to vertebroplasty had more serious characteristics of disease than the control group). But this means that it is impossible to say to what degree the patients were comparable on account of aspects related to their perception of the disease, their aversion to risk or tolerance of pain, all of these very important when the measure of the results is essentially subjective, such as it the case with pain and quality of life. On the other hand, the fact that there is no masking, means it is difficult to know the extent to which the result may have some involuntary bias, originating from the patients or the evaluators. In addition, it is certainly not possible to separate the real effect from the placebo effect.

Figure 1. Fracture of L3 treated by vertebroplasty

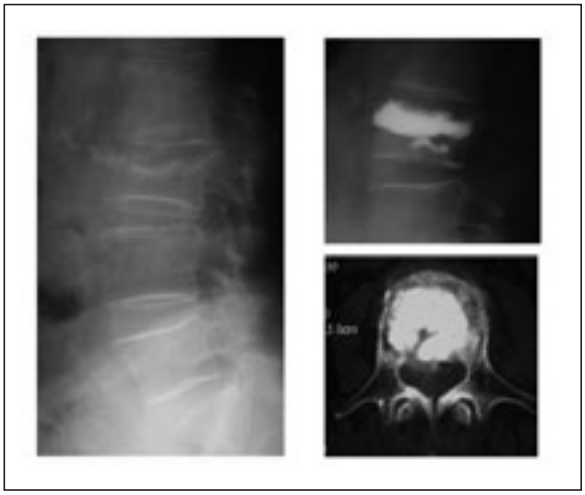
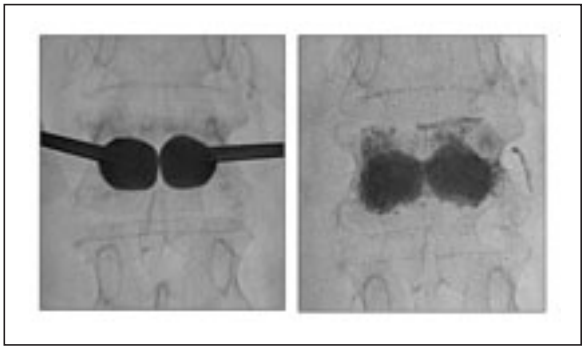


Figure 2. Kyphoplasty. Balloons inflated in the interior of the vertebral body (left) and control at the end of treatment (right)



Randomised controlled trials

The first randomised trial was published by Voormolen et al., who compared the evolution of a small group of patients with osteoporotic fractures, which had developed over between 6 weeks and 6 months[12]. From the day following the procedure the intensity of the pain was significantly less in those treated with vertebroplasty than in the controls. At 2 weeks there continued to be a definite trend in the same direction, but the difference was not statistically significant. However, given the small number of patients, the power of the study was limited. Interestingly, in this brief follow up period, two new fractures appeared in the treated group and none in the control group.

Another randomised study of 49 patients with recent osteoporotic fractures and refractory pain found similar results: the group subject to vertebroplasty had less pain 24-48 hours after the procedure, but the differences had disappeared after 3 months[13].

More recently, Wardlaw et al. published a randomised trial on the effect of kyphoplasty in 149 patients, who were compared with 151 patients subject to non-invasive treatment[14]. Differently from other studies, these authors included both osteoporotic and tumorous fractures (although the latter only made up 2% of cases). All these were relatively recent (according the indication of the presence of oedema in the RM), but which had been developing over more than 3 months. During the 12 months of follow up the patients who had received kyphoplasty had less pain, and higher points on the quality of life scale, than those subject to medical treatment. The differences were established early and then later tended to decrease a little. Although they did not reach statistically significant levels, the incidence of new morphometric vertebral fractures was higher in the kyphoplasty group than in the control group (33% against 25%). Similarly, 14% of this group presented new clinical fractures (against none in the control group).

Compared with those covered earlier, these three studies present the advantage of being controlled, which tends to ensure the comparability of the treated and control groups. However, in not being masked, it is difficult to know if the subjectivity of the patients or the evaluators has an influence in the analysis of the development. So, this means that other studies published more recently

are especially interesting, in which for the first time an effort was made to mask the treatment applied. One of these, published by Kallness et al., included 131 patients with fractures of more than one year in development. Of these, 68 were randomly allocated to have vertebroplasty and 63 to have medical treatment, preceded by a simulation of vertebroplasty, including sedation and the injection of a local anaesthetic in the periosteum[15]. There were no differences between the two groups in terms of pain or in the quality of life scales during the 3 months follow up. This was the case independently of the period of development of the fracture. However, the study's protocol allowed the patients to request another intervention in cases where significant symptoms persisted: which 43% of those in the control group and 12% of the group treated with vertebroplasty ($p< 0.001$), requested.

In a similar study, Buchbinder et al., compared 38 patients treated with vertebroplasty with 40 controls, in whom the procedure was simulated. Again, no differences were found between the two groups with respect to pain or quality of life scales, neither in the group as a whole, nor in the sub-groups resulting from dividing the patients according to their period of development (more or less than 6 weeks). Neither were there differences in the incidence of new fractures[16].

Vertebroplasty, kyphoplasty and biomaterials

In theory, kyphoplasty can present some advantages over simple vertebroplasty. On the one hand, it reduces the escape of material from the vertebral body. On the other, the inflation of the balloons lifts the vertebral platelets, which, to a greater or lesser extent, recovers the vertebral collapse, so, attempting to correct the angle of vertebral kyphosis. Although, theoretically, the re-establishment of the height of the vertebral body is beneficial, its practical clinical repercussions continue to be unclear. In a systematic review of 69 studies, Hulme et al. did not find clear differences in the degree of correction of the height of the vertebral body obtained with vertebroplasty and kyphoplasty, but the escapes of material from the vertebral body were less frequent with kyphoplasty (9% against 41%)[17]. However, it should be taken into account that only a small number of studies carried out a direct comparison between the two techniques, and there were no randomised studies. It has been suggested that that the higher number of escapes of cement towards the intervertebral disc which happens with vertebroplasty could be associated with a higher frequency of fractures in adjacent vertebrae[18]. In the controlled studies on which we have commented earlier, there seems to be a tendency to better results in those in which a kyphoplasty has been carried out than in those which assessed vertebroplasty. This question has also been analysed in another review of 168 studies of vertebroplasty and kyphoplasty, in which a lower rate of escape of cement was observed with kyphoplasty (7% against 20%), as well as a lower rate of new fractures (14% against 18%), although paradoxically, the improvement in pain was somewhat higher after vertebroplasty 18. However, most of the studies reviewed did not carry out a direct comparison between the two procedures, and as a consequence, the patients included are not necessarily comparable. This, therefore, makes a recent study by Lui et al. very interesting, in which they randomly assigned 100 patients with fractures of the thoracic-lumbar union to vertebroplasty or kyphoplasty. In the latter, an improvement in vertebral height and of angle of kyphosis was observed, but no differences were found between the two groups in terms of pain over 6 months of follow up[19]. Similar results (lower incidence of escape of cement and improvement in kyphosis, but without differences in terms of pain) were found after another study which compared kyphoplasty with vertebroplasty, after an assignation by suitability, not randomly[20].

There have scarcely been any studies carried out into the cost-effectiveness of these procedures[21]. But, in all cases, it is necessary to take into account the fact that the cost of materials for kyphoplasty are notably higher than those for vertebroplasty.

In recent years, biomaterials based on calcium phosphate (CaP) have been used as an alternative to PMMA. Some authors have suggested that these materials are reabsorbed over time and could induce a powerful osteogenic response. Our personal experience does not support this idea and neither do the studies of other authors. Thus, Grafe et al. studied a series of patients treated by kyphoplasty and compared the results of an injection with PMMA with that of CaP (20 patients in each group). They found no significant differences at 6, 12 and 36 months with respect to pain, physical function, the restoration of the height of the vertebral body, or the frequency of new fractures[22]. On the other hand, Blattert et al. analysed the effects of kyphoplasty with PMMA or CaP in a prospective study of 60 osteoporotic fractures with randomised assignment. They found a higher rate of failure in cement based on CaP in burst fractures, which suggests that its biomechanical properties do not make it recommendable for this type of fracture[23]. However, it has been suggested that biomaterials based on CaP would be preferable to PMMA in young patients, with traumatic fractures and good bone quality, in whom are expected a good bone-forming response, and who wish to avoid the presence of an inert foreign substance in the long term.

Conclusions

In view of the these studies it is evident that we still have significant gaps in our knowledge around the real benefits of vertebroplasty and kyphoplasty in terms of their capacity to modify the natural history of vertebral fractures. However, it is possible to draw some, at least provisional, conclusions, which go towards defining the role of these procedures in the therapy for vertebral fractures, and to guide clinical practice:

Table 1. Summary of controlled studies. VP: vertebroplasty. CP: Kyphoplasty

Author, year	Promoted by industry	Inclusions	Period of evolution	Randomised	Masked	Groups (n)	Results
Buchbinder 2009	no	Recent fractures (oedema or linear frx in RM) Average age: 76 Sex: 80% women	< 1 year	yes	yes	VP (n=38) Puncture (n=40)	• No differences in pain or quality of life at 1 week, 1, 3 o 6 months. • No differences in new fractures
Kallmes 2009	no	Clinical fractures with bad response to analgesics (VAS > 3/10) Average age: 74 Sex: 75% women	< 1 year	yes	yes	VP (n=68) Puncture (n=63)	• No differences in pain or quality of life at day 3, 14, 30 or 90. • More changes to the other intervention in the control group (43 against 12%)
Rousing 2009	no	Recent fractures with refractory pain Average age: 80 Sex: 82% women	< 2 months	yes	no	VP (n=25) Control (n=24)	• Less pain in VP at 24 hrs, without differences at 3 months. • 3 new fractures in VP and 1 in control
Wardlaw 2009	yes	Recent fractures (oedema in RM), primary or secondary, with intense pain (VAS > 4/10) Average age: 73 Sex: 77% women	> 3 months	yes	no	CP (n=149) Control (n=151)	• Less pain and improvement in quality of life in CP at 1 and 12 months. • Tendency to more frx in CP (clínical 14 vs 0%; Rx 33 vs. 25%)
Voormole 2007	¿	Recent fractures (oedema in RM), with refractory pain Average age: 73 Sex: 82% women	6 weeks-6 months	yes	no	VP (n=18) Control (n=16)	• Less pain in VP at day 1; no significant trend at day 14 • 2 new fractures in VP
Kasperk 2005	yes	Fractures Average age: 69 Sex: 82% women	< 1 year	no	no	CP (n=40) Control (n=20)	• Less pain at 3 and 6 months and quality • No difference in new fractures
Diamond 2006	no	Recent fractures Refractory pain Average age Sex	< 6 weeks	no	no	VP (n=88) Control (n=38)	• Less pain at day 1 and 6 weeks, but not at 6, 12 and 24 months. • No difference in new fractures
Álvarez 2006	no	Recent fractures with refractory pain Average age: 72 Sex: 80% women	6 weeks-1 year	no	no	VP (n=101) Control (n=27)	• Less pain day 1, month 3 and 6, not at end of 12 months. • Initial functional improvement, but not later. • More fractures in VP

• The pain of vertebral fractures tends to improve with time, independently of the treatment applied.

• The trials with higher methodological quality with random assignment and masking, do not demonstrate a clear benefit of vertebroplasty as against conventional treatment in the treatment of osteoporotic fractures. Therefore vertebroplasty should not be recommended as a standard treatment. These patients should receive appropriate treatment with analgesics, education on activities to be undertaken, measures for the prevention of falls, and drugs aimed at increasing bone resistance. On occasions they may benefit from physiotherapy or orthosis which limit flexion, with the aim of allowing early mobility for the patient, this avoiding secondary bone loss through being bedridden.

• In two randomised but not masked trials, promoted by the industry, kyphoplasty has shown symptomatic benefits in patients with osteoporotic fractures, together with a tendency to an increase in the number of new fractures. As a consequence, neither is it possible, at this moment, to recommend kyphoplasty, generally, as a standard treatment.

• In comparison with vertebroplasty, kyphoplasty improves the angle of kyphosis and presents a lower risk of escape of contrast, but there is no evidence that it brings a clear benefit from a clinical point of view.

• There is no definitive evidence as to whether these procedures increase, or not, the incidence of new fractures. Neither are there studies which demonstrate their preventative value. Therefore, at present, there is no justification for their use with the sole objective of preventing the progression of vertebral collapse in patients without significant pain.

• Numerous observational studies (in addition to the personal experience of many doctors, including the authors of this article) indicate that in some patients these procedures achieve a rapid and acute alleviation of symptoms. As a consequence, we think that they can be a therapeutic alternative for some specific patients, such as:

- Those who have recent fractures, with intense pain which persist for more than 6 weeks despite appropriate analgesic treatment (including opiates).

- Those who have intolerance or contraindications to powerful analgesics

- Those who have concomitant diseases which make immobilisation or the limitations of respiratory excursions especially inadvisable.

- In pseudoarthrosis of vertebral fractures of more than three months standing in which is progressive and painful kyphosis is confirmed.

• Vertebral fractures are a well known marker for a heightened risk of other fractures. As a consequence, invasive treatment should always be accompanied by other therapeutic measures which reduce the possibility of suffering new vertebral or peripheral fractures.

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The importance of the type of preparation of calcium and vitamin D in the prevention and treatment of osteoporosis

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Summary
Most Europeans do not meet the adequate intake for calcium and vitamin D; supplementation of both can help to meet requirements. Inappropriate intake can lead to reduced calcium absorption, higher bone remodeling rates and increased bone mass loss. Also, vitamin D deficit has been linked to reduced muscle function and increased risk of falling. Calcium from carbonate is the most common form, due to its cost-effectiveness profile, of calcium supplement for choice. Calcium lactate and gluconate are less concentrated forms of calcium and are not practical oral supplements. The purpose of the present article is to examine the importance of the combination calcium-vitamin D its role in the prevention and management of osteoporosis and the most common and useful formulations for its clinical use.

Key words: *Calcium supplements, Vitamin D, Osteoporosis, Postmenopause.*

1. Introduction

The prevention of osteoporosis continues to be one of the areas of unfinished business in public health and which will become more relevant as the population ages. This disorder is characterised by a greater bone fragility and an increase in the risk of having fractures, located most frequently in the spine and hip, although any bone may become affected[1]. In our country, the annual incidence of hip fractures for this reason is 400 cases per 100,000 women over 50 years of age. The vertebral fracture is the osteoporotic fracture with a higher incidence in women and in males. Its annual incidence is 1,250 cases per 100,000 women and has been demonstrated to be a significant risk factor for other osteoporotic fractures and even for mortality[2].

Calcium and vitamin D are nutrients of great importance and absolutely essential for the acquisition and maintenance of bone health. The supply of calcium and vitamin D is critical throughout life; first to achieve a bone mass which is both quantitatively and qualitatively adequate and, subsequently, when from the age of 30 it begins to diminish. Unfortunately, at least within the population of the theoretically developed countries, 85-90% of the female population doe not ingest sufficient calcium and more than 50% of postmenopausal osteoporotic women have inadequate levels of vitamin D[3,4].

At the margins of its relevance in bone metabolism, calcium is essential for neuromuscular activity, coagulation of blood and adequate cardiac function. As has already been indicated, it is a vital component of bone architecture and necessary for the correct deposition of minerals throughout life. More than 99% of calcium present in the organism is found in the bones and teeth, exercising the function of reservoir. When the dietary supply is not sufficient for the maintenance of the levels of extracellular liquid (ECL) and plasma necessary for the maintenance of the vital functions in which it participates, mechanisms for obtaining calcium from the bone reservoir are activated, by which negative balance is produced in the bone. Calcium is absorbed in the small intestine with the help of vitamin D. It is eliminated through renal excretion, although a small proportion is found in the faeces. The kidney does not only participate in the excretion, but in the joint management of calcium, increasing the excretion or the re-absorption as is appropriate to the body's homeostasis of calcium.

2. Calcium

2.1.Calcium deficit in nutrition

The needs of calcium for an adult are reflected in the daily supply necessary for the maintenance of calcium homeostasis and the integrity of the skeleton. The amount necessary for a European adult is 800 mg daily[5]. After the menopause, the requirements increase up to at least 1,000 mg/day[6,7], although the WHO quantify their recommendation for European women at 1,300 mg/day[8,9].

Those people who do not obtain a sufficient intake of dietary calcium should supplement this, with the aim of reaching the daily minimum requirements. The evidence suggests that the average citizen does not even reach the low end of the daily recommended consumption. Women who are in their forties report a calcium intake of 50% of that recommended for postmenopausal women 10. In a study carried out in nine European countries to confirm the dietary requirements of European postmenopausal and osteoporotic women, the results indicate that only 37.2% of the population studied were found to be in treatment with calcium supplements (64.2% of the Spanish population). However, only 19.1% of those surveyed reached the daily intake of 1,300 mg/day recommended by the WHO (in our country, 50% of patients in treatment with supplements receive between 800 and 1,300 mg and 28%, doses higher than 1,300 mg/day). Among the population aged over 75 years, only 17.1% reached the recommended requirements, while among those younger than 75 years that figure was 20.5%[4].

2.2. Calcium supplements

With the aim of assuring a correct intake of calcium, there is a range of supplements currently on sale in our country (Table 1). The most commonly used, and best studied, are the carbonate and citrate forms, although the latter is not available in our environment; but it is true that some preparations contain citric acid as an excipient which may facilitate the transformation of carbonate into citrate. The bioavailability of the calcium ion is 20-30%, being absorbed in the duodenum and jejunum, by means of a mechanism of passive diffusion. The mechanism for the absorption of calcium is a saturable process, which means that at a dose of around 500 mg, absorption diminishes intensely. The absorption has a variable efficiency since it depends on a multitude of factors, among others: diet, age, rate of growth, vitamin D contained in the diet and the requirements for calcium. Foods rich in glucose, lactose or galactose increase notably the bioavailability of calcium. The absorption of a specific salt of calcium depends on the one hand on its capacity for disassociation, different for the various salts and slightly favouring the citrate and pidolate forms, and on the other, its capacity for dissolution. There are marked differences in the dissolution of the preparations of calcium supplements, in principle supposedly due to differences in pharmaceutical formulation, although experience shows that not all preparations of the same salt exhibit equivalent absorption[11-13].

Calcium carbonate possess the highest proportion of calcium element among the different salts[14]. This translates, from the patient's perspective, into the need for a lower daily requirement of tablets to reach the dose to be provided. The way of ensuring an optimum absorption is to take the tablets with meals, dividing the doses higher than 500 mg into a number of smaller amounts. The

Table 1. Formulations of calcium supplements currently marketed in our country

Formulation	Calcium content (%)	Recommended daily dose (mg de Ca)	Interval of administration (h)	Dose contained in a unit (mg de Ca)	Form: in association with vit. D, or not
Carbonate	40	500-1,200	24	500	- Without association - Vitamin D
Phosphate	38.8	1,200	24	1,200	- Without association - Vitamin D
Pidolate	13.5	1,000-1,500	8-12	500	- Without association - Vitamin D
Lactate	12.9	500-1,000	6-12	250	- Vitamin D
Lactogluconate	6.8	1,000	24	500	- Carbonate + Vitamin D
Glubionate	6.5	500-1,500	24	500	- Carbonate

secretion of gastric acid and, definitively, gastric pH, plays a very important role in the intestinal disassociation of the carbonate, with the bioavailability of the carbonate and citrate forms being equivalent in these circumstances[11,15]. However, other studies indicate that the citrate form, even under these circumstances has a higher bioavailability than the carbonate, although authors themselves indicate that these differences may be due to deficiency in the secretion of gastric acid in the individuals participating in the study[16]. This fact acquires relative importance in older patients, with hypo- or achlorhydria, or gastrectomy, in whom the bioavailability of the carbonate form may be found to be diminished. One study, crossed and randomised, shows that the inhibitor of the proton pump, omeprazol, notably reduces the fraction of calcium absorbed from calcium carbonate in postmenopausal women after 12 hours of fasting[17]. In addition, another case-controlled study shows that the long term treatment with proton pump inhibitors, especially when high doses are used, are associated with an increase in the risk of hip fracture[18].

Calcium supplements are generally well tolerated. However, a recent report of the Women's Health Initiative (WHI) from the US reveals the absence of statistically significant differences in the incidence of adverse gastrointestinal effects (gases, abdominal distension, constipation) between patients to whom had been administered a placebo and those administered a calcium/vitamin D combination[19]. In clinical practice, up to 50% of patients report gastrointestinal symptoms - constipation, flatulence and abdominal distension – after the ingestion of these preparations. Usually the carbonate form is associated with a higher frequency of these adverse affects, although it is also recommended that the citrate form be substituted by the carbonate form if the symptoms appear in association with the former[20]. Additionally, in patients who report poor tolerance, it is recommended that the combined calcium/vitamin D be started at a low dose, increasing it later until the required dose is reached after 1-2 months.

Certain studies on the safety of those compounds[19] have generated controversy as to at what point calcium and vitamin D supplements increase the risk of renal lithiasis. Patients randomly chosen to receive 1,000 mg/day of calcium and 400 UI/day of vitamin D_3 had a 17% higher risk of suffering renal lithiasis with respect to the placebo group. However, apparently the women included in the group treated with supplements self-medicated to a significant degree, reaching a daily calcium intake of 2,000 mg. Recent studies suggest that a diet poor in calcium can increase the risk of lithiasis[21]. It is recommended that supplements be taken with food to facilitate the union of calcium with oxalates in the intestine, and not to exceed the maximum recommended dose.

A recently published study has given the supplements a dubious role in being transformed into an independent cardiovascular risk factor, although this assertion is far from being demonstrated[22] and has subsequently been challenged by other authors[23] . In all cases, it should not be forgotten that the total dietary intake of calcium for each patient should be considered before calculating the necessary dose of supplements.

3. Vitamin D

3.1. Physiology

Vitamin D has a significant influence on good bone health. Among its functions can be highlighted: the regulation of the intestinal absorption of calcium and the stimulation of resorption in cases where it is necessary to increase the concentration of blood calcium; it also contributes to the maintenance of normal levels of calcium and phosphorus in the blood. UVB radiation is absorbed by 7-dehydrocholesterol which exists in the skin, to form previtamin D_3. Previtamin D_3, is, on the other hand an unstable compound and it rapidly transforms by the action of heat, into vitamin D_3[24]. Vitamin D_3 reaches the extracellular space and from here, the capillaries, where it combines with the vitamin D transporter protein (DBP)[25]. Once in the capillaries, the vitamin D reaches the liver, where it undergoes hydroxylation which results in the formation of 25-hydroxyvitamin D [25(OH)D]. The 25(OH) once again bonds with the DBP and progresses to the kidney where it is transported to and released in the tubular renal cell and again hydroxylated, forming 1.25-dihydroxyvitamin D[1.25 $(OH)_2$ D][26]. This the biologically active form of vitamin D and that which is responsible for the homeostasis of calcium. The vitamin D contained in foodstuffs reaches the lymphatic system through the chylomicrons, entering the bloodstream and binding themselves to the DBP[26]. From here they will later reach the liver and kidney to be transformed into the active form of vitamin D.

The main natural sources of vitamin D are sunlight and diet (< 10%) which essentially includes: blue fish, such as salmon, mackerel, tuna, bonito, horse mackerel, sardines and other fortified foods such as milk, yoghurts and some cereals[25,27]. Despite a high consumption of blue fish, surveys of dietary habits in Spain show that the intake of vitamin D is notoriously insufficient, 208 ± 4 UI/day, whilst in postmenopausal women it is 168 ± 14 UI/day, when it should be reaching 800-1,000 UI/day[28]; and what is more significant, it is insufficient since infancy. In Calatan and Canarian children the daily intake of vitamin D is 120-96 and 60-75 UI/day, respectively[29].

Blood calcidiol [25 (OH)D] is the best metabolite of vitamin D to be dosed and its blood levels are considered to be a very useful index for the management of optimum levels of vitamin D. It is considered by different authors that the lower limit of normality is 10 to 15 ng/ml, although it is known that levels of PTH increase with values of calcidiol from 25 to 30 ng/ml; from all this it can be concluded that desirable values of calcidiol would be above 40ng/ml[30,31].

Approximately 95% of Spanish people over 70 years of age do not have a sufficient intake of vitamin D from the diet. Fortunately, our benign climate with a generous exposure to sunlight, reduces the deficit to 56% in winter and even down to 28% during the summer months[32].

However, in recent times, the emphasis put on the necessary use of sun protection has counteracted to some extent the potential benefits spelled out by various authors of a sunny country such as ours, and this could be one of the causes of the high prevalence of vitamin D deficit. Currently, the recommendations of the panels of experts are centred on recommending an intake of 1,000-2,000 UI/day to prevent the development of this deficiency[33].

Figure 1 represents the biological activity of vitamin D, responsible for the homeostasis of calcium.

3.2. Calcium-vitamin D interaction

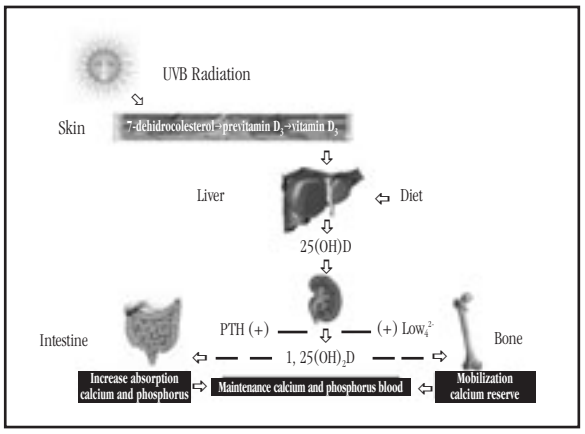
The increase in the administration of vitamin D and of 25-hydroxyvitamin D (25(OH), brings with it a consequent increase in the metabolites 1,25-dihydroxyvitamin D and 24.25-dihydroxyvitaimin D and as a consequence, of the absorption of calcium and of the concentration of calcium ions in circulation[34]. When the levels of parathormone (PTH) are low, the stimulus which facilitates bone resorption ceases, which for a long time was thought to be an essential mechanism by which vitamin D contributes to the improvement in mass and strength of bone. However, it has been observed that on other occasions, it is the increase in the supply of calcium in the diet, that is responsible for the increase in the gastrointestinal absorption of this ion, and of the concentration of circulating calcium ions, while the rates of PTH and biochemical markers for bone turnover diminish. A recent study has contributed to the clarification of the relationship that exists between calcium, PTH and 25(OH)D[35]. According to the results of this study, it seems clear that the suppression of PTH depends more on the levels of blood calcidiol than on the intake of calcium, and therefore, when the levels of blood calcidiol are sufficient (≥ 25 nM) an ingestion of calcium of 800 mg may be sufficient for the maintenance of the homeostasis of calcium. On the other hand, another study corresponding to the same period of time, indicates that the supply of calcium does not have any impact on the relationship between the supply of vitamin D and the blood concentration of 25(OH)D[36].

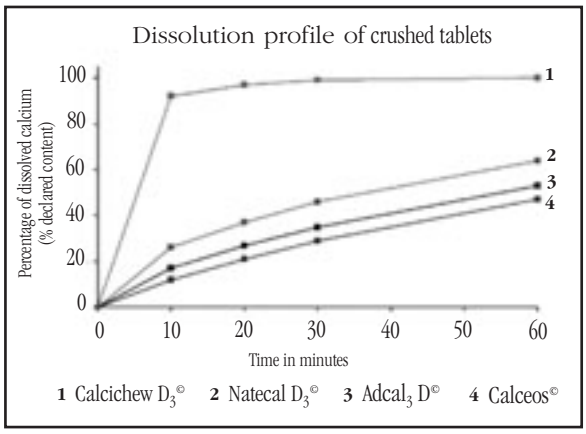
This evidence therefore suggests that the requirements for vitamin D are independent of the external supply of calcium. The levels of vitamin D are related to the bone mass in young adults, and their supplementation diminishes the percentage of loss of bone mass in adults[37].

4. Administration of calcium combined with vitamin D

As has been referred to earlier, the association between calcium carbonate and vitamin D in the great majority of commercial preparations brings with it an increase in the absorption of calcium as a result of the interaction of these two compounds. In fact, a study carried out with the objective of quantifying the absorption promoted by cholecalciferol, found an up to 16.6% higher accumulated secretion of this ion when administered

Figure 1. Biological activity of vitamin D.
Figure 2. Dissolution profile of Mastical D© (Calcichew D_3©) and other reference products (Natecal D©, Adcal D© and Ideos©) once crushed. The composition of the 4 formulations is: vitamin D_3, 400 UI and calcium carbonate, 500 mg per Mastical D© and Ideos© and 600 mg for Natecal D© and Adcal D©).





in combination with vitamin D supplements[38]. However, it has also been mentioned in another chapter of this article, that there may be marked differences in the dissolution of the preparations of calcium supplements, supposedly due to their pharmacological formulation, although not all the preparations of the same salt exhibit the same absorption. Among other factors, the possibility that the combined tablets are chewable gives the USP (United States Pharmacopeia) dissolution test an availability which is perfectly correlatable, *in vitro-in vivo*[39,40]. These results motivated the authors to carry out a second study in the same conditions as the first, which has not yet been published, comparing the dissolution profile of this formula with 2 lots of 2 other formulations, among which were included a flash release form, mouth-dispersible, which, when placed on the tongue rapidly dissolved in the mouth, releasing the microgranules contained inside the enteric covering (Figures 3 and 4; Tables 2 and 3). Once more, minimal differences were found in the dissolution profile between the intact and crushed forms, with the chewable formulation of calcium carbonate. The release of calcium from the tablet was also considerably more rapid with this formulation during the first hour, by which the speed of absorption and, possibly, the quantity of bioavailable calcium is higher in human beings.

5. Clinical evidence on its use

Supplements of calcium, combined with vitamin D should be considered for all people who: do not have an adequate ingestion of calcium, have oestopenia or osteoporosis, peri- and postmenopausal women, mothers in natural state of lactancy after a multiple birth, vegetarians, amenorrheic women, institutionalised older people, those intolerant of lactose, patients subject to chronic corticotherapy and who are suffering an inflammatory intestinal disease. Specifically, the association of calcium carbonate and vitamin D is indicated in: treatment of calcium and vitamin D deficiency in older people and as an adjuvant for specific treatment for osteoporosis in patients with risk of calcium and vitamin D deficiency.

During infancy and adolescence, it has been shown that the suboptimum supply of calcium is due to the replacement of the ingestion of milk with an excessive consumption of fizzy drinks[42]. In 2006, the American Academy of Pediatrics published a report which provided a guide for the optimisation of bone health in children and adolescents[43]. This report recommended the ingestion of daily sources of calcium, due to their prevalence in the diet, and other nutrients they contain. Supplementation is suggested as an alternative form to this daily food intake. In any case, a special emphasis is made for paediatricians to remind families of the benefits which calcium and vitamin D bring in the reduction of risk of suffering osteoporosis in the future, as well as suffering fractures during infancy and adolescence.

Calcium supplements can stimulate the growth of bone to a significant extent in young women[44]. In a randomised and controlled study or 4 years duration, 352 women at stage 2 of puberty were studied, this being the group in which the calcium supplements showed significant bone growth during the most accelerated phase of development. The authors conclude with the possible implication of these supplements in the prevention of osteoporosis and secondary fractures due to bone fragility during growth.

The need for calcium is exacerbated in menopause. The low levels of oestrogen favour bone resoprtion, at the same time as reducing the efficiency of the intestinal absorption of calcium and its renal conservation. At 65 years of age, the absorption of calcium is 50% of that seen in adolescence. An inadequate level of vitamin D may also limit the absorption of calcium and negatively

Figure 3. Dissolution profile of Mastical D© (Calcichew D_3©) and other reference products [Natecal D© (lots 06384 and 07073) and Natecal D Flas© (lots 08051 y 08057)] in the form of intact tablets. The composition of the 3 formulations is: vitamin D_3, 400 UI and calcium carbonate 500 mg for Mastical D© and 600 mg for Natecal D© and Natecal D Flas©).

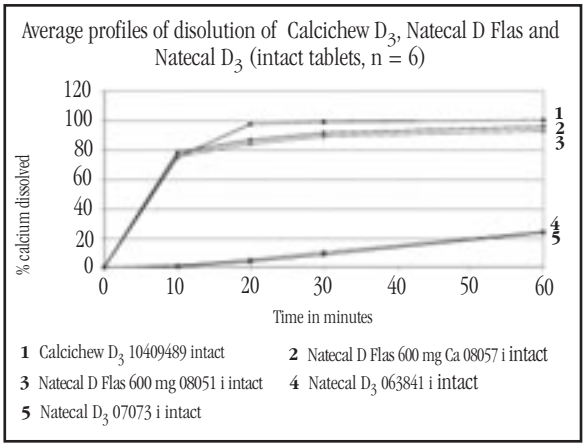
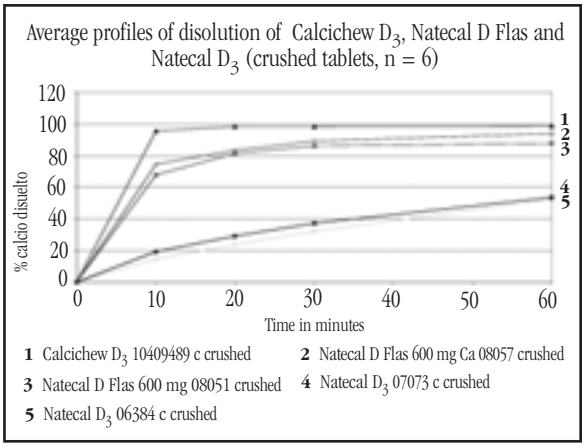


Figure 4. Dissolution profile of Mastical D© (Calcichew D_3©) and other reference products [Natecal D© (lots 06384 and 07073) and Natecal D Flas© (lots 08051 and 08057)] in the form of crushed tablets. The composition of the 3 formulations is: vitamin D_3, 400 UI and calcium carbonate, 500 mg for Mastical D© and 600 mg for Natecal D© and Natecal D Flas©).



affect bone health. With age, a number of factors contribute to its blood concentration being inadequate: inadequate intake, little exposure to the sun, reduction in the efficacy of renal transformation of vitamin D to 1.25 (OH) D and reduction in the intestinal response to vitamin D.

The North American Menopause Society (NAMS) recommends 1,200 mg of calcium daily and a sufficient supply of vitamin D, which allows blood levels of 25(OH)D ≥ 30 ng/ml. The primary source of calcium recommended is through food, but as alternatives supplements and reinforced foods are also recommended[45]. Calcium and vitamin D are recommended as adjuvants for all types of pharmacological treatments for osteoporosis. Clinical trials with calcium supplements, although of short duration, show a reduction in the loss of bone mass in women who are postmenopausal and at risk of fracture. A meta-analysis of 15 clinical trials which includes a total of 1,806 patients randomly allocated to calcium supplements or to its normal dietary intake over a period of 2 years shows an increase in bone density in the lumbar spine of 1.66%, 1.64% in the hip and 1.91% for the distal radius in the groups supplemented with calcium[46].

An analysis of 20 clinical trials in postmenopausal women also shows that supplementation with calcium (500-1,200 mg/day) reduces the annual loss of bone mass from 1% in women not receiving supplements to 0.014% in those receiving them[47].

A meta-analysis which includes 29 randomised clinical trials (n= 63,897) concludes that the evidence supports the use of calcium or calcium/vitamin D in the preventative treatment for osteoporosis in patients over 50 years of age. The reduction in risk of fracture is significant in those studies in which the degree of compliance of patients is highest. Also, the therapeutic effect is higher when a dose ≥ 1,200 mg/day of calcium and ≥ 800 UI/day of vitamin D is used[48].

Similarly, a double blind study controlled by placebo of 1,460 women over 70 years of age, followed for 5 years concluded that supplements of calcium carbonate at a dose of 1,200 mg/day, as means of public health intervention, are only efficacious in those patients who show a sufficient therapeutic compliance in the long term[49]. Beyond this, another meta-analysis of 7 prospective trials concludes that the intake of calcium alone, does not only demonstrate a reduction in risk of hip fracture, but that it may increase it[50].

The Women's Health Initiative (WHI), a long term randomised trial which studied more than 36,000 women of ages of between 50 and 79 years, over a period of 7 years, has assessed the effects of calcium and vitamin D on the prevalence of fractures[20]. The researchers have found a relative reduction of 29% (per protocol) in hip fractures in those women who complied daily with the treatment of 1,000 of calcium carbonate and 400 UI of vitamin D. Another interesting meta-analysis is that carried out on randomised clinical trials in patients (9,083) in whom oral vitamin D, with or without calcium supplements, was administered, when compared with a placebo. According to the results of the 4 clinical trials (9,083 patients), the balanced relative risk of hip fracture is 1.10 (CI 95%: 0.89-1.36) with monotherapy with vitamin D. For another 6 clinical trials (45,509 patients) with the calcium/vitamin D combination, the relative risk reduces to 0.82 (CI 95%: 0.71-0.94), which suggests that vitamin D only

Table 2. Percentage of calcium released in the dissolution study of Mastical D© (Calcichew D_3©) and other reference products [Natecal D© (lots 06384 and 07073) and Natecal D Flas© (lots 08051 and 08057)] in the form of intact tablets. The composition of the 3 formulations is: vitamin D_3, 400 UI and calcium carbonate, 500 mg for Mastical D© and 600 mg for Natecal D© and Natecal D Flas©).

	% calcium dissolved (average)		
Time (min)	**Mastical©**	**Natecal D©**	**Natecal D Flas©**
10	75.1	0.8-1.5	75.7-78.3
20	97.7	4.1-5.4	84.1-87.0
30	98.9	8.8-10.4	89.1-91.7
60	100.4	23.5-24.2	92.8-96.1
120	100.0		95.2-97.8

Table 3. Percentage of calcium released in the dissolution study of Mastical D© (Calcichew D_3©) and other reference products [Natecal D© (lots 06384 and 07073) and Natecal D Flas© (lots 08051 and 08057)] in the form of crushed tablets. The composition of the 3 formulations is: vitamin D_3, 400 UI and calcium carbonate, 500 mg for Mastical D© and 600 mg for Natecal D© and Natecal D Flas©)

	% calcium dissolved (average)		
Time (min)	**Mastical©**	**Natecal D©**	**Natecal D Flas©**
10	95.6	14.7-19.3	67.7-74.6
20	98.5	23.8-29.3	81.5-83.2
30	98.3	32.2-37.2	86.1-89.2
60	98.6	52.3-53.6	87.7-93.8
120	99.0		90.5-95.2

reduces the risk of hip fracture in association with calcium[51].

However, an additional effect which is found with an adequate supply of vitamin D is a reduction in the incidence of falls. Individuals with higher levels of 25(OH)D are able to walk and get up from a chair more quickly. These actions improve rapidly when the levels of 25 (OH)D increase from very low levels to the middle of the range of reference. The improvement continues, although more slowly, until the levels are at the higher limit of the said range[52,53].

More recently, at the 31st Annual Meeting of the American Society for Bone and Mineral Research, were revealed the results of a meta-analysis carried out on 5 European randomised clinical trials, in which it was concluded that calcium accompanied by vitamin D does not only reduce the mortality of older patients by diminishing the risk of hip fracture, but it even reduces the mortality within the group of patients who have already suffered one[54].

6. Conclusions

Calcium supplements play an important role in bone health throughout the cycle of life. For all those in whom we treat loss of bone mass, the aim is not only to ensure an adequate intake of calcium but also of vitamin D and other nutrients which are indispensible for bone health. The best way, from the pharmacological point of view, of supplementing calcium is in its carbonate form. Calcium carbonate is cost-effective, although it should be taken with meals, with the aim of optimising its bioavailability, and the dose for the most immediate absorption should not exceed 500 mg (which does not mean that this dose could be exceeded in preparations of sustained release). However, what should not be forgotten is the fact that there may be marked differences in the dissolution of the different preparations of calcium supplements, supposedly due to the pharmaceutical formulation, although not all the preparations of the same salt exhibit the same absorption.

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Cardiovascular disease, diabetes mellitus type 2 and osteoporosis

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Summary
In recent years various epidemiological studies have shown an independent association of age between type 2 diabetes and osteoporosis, as well as an increase in cardiovascular mortality in patients with a reduction in BMD and/or osteoporotic fracture. The most recent research has focussed on factors involved in the physiopathology of the two diseases. In general, the studies which have investigated the relationship between cardiovascular risk factors, bone metabolism, bone mass and risk of fracture have shown inconclusive and contradictory results. In patients with DM2 there is an increase in risk of fractures in spite of a higher BMD, caused essentially by an increased risk of falls associated with the presence of vascular complications, although changes in bone quality are also a determining factor. Knowledge of the physiopathological mechanisms common to these pathologies will not only help better management of patients, but also could contribute to the development of drugs which would act on the two processes.

Key words: Type 2 diabetes *mellitus, Osteoporosis, Cardiovascular disease.*

Abbreviations
BMD: bone mineral density; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG triglycerides; BMI: body mass index; AHT: arterial hypertension; DM2: diabetes mellitus type 2; PTH: parathormone; NO: nitric oxide; MGP: matrix Gla protein; OPN: osteopontine; IMT: carotid intimal-medial thickness; OPG: osteoprotegerin; CVD: cardiovascular disease; SD: standard deviation; OR: odds ratio; HbA1c: glycosylated haemoglobin; GIP: gastric inhibitory polypeptide ; GLP-1: glucagon-like peptide-1.

Diabetes mellitus type 2 and osteoporosis are two entities with significant socio-health repercussions at a global level, derived essentially from the appearance of cardiovascular disease in the former, and fragility fractures in the latter. Although traditionally both diseases, and their associated complications, have been considered to be independent processes, in recent years a great deal of interest has been sparked in the study of common factors and mechanisms between the two.

1. Cardiovascular disease and osteoporosis

In recent years various epidemiological studies have shown an association independent of age between the two processes[1], and an increase in cardiovascular mortality in patients with a reduction in BMD and/or osteoporotic fracture. Although for some time it has been known that the two diseases share risk factors which could justify their association, such as age, oestrogenic depletion, sedentariness , consumption of alcohol and tobacco, and dietary factors[2], the most recent research has centred on those aspects implicated in the physiopathology of both diseases.

1.1. Cardiovascular risk factors

Studies which have investigated the relationship between cardiovascular risk factors, bone metabolism, bone mass and risk of fracture, have shown inconclusive and contradictory results in most cases.

Dyslipidemia
In *in vitro* studies, HDL-C appears to show an inhibitory effect on osteoblast activity induced by inflammatory cytokines in the vascular wall[3], and raised concentrations of oxidised LDL-C has an apoptotic effect on osteoblastic cells[4], inhibiting their differentiation and promoting osteoclastic activity[5]. Most of the studies carried out have not found a relationship between LDL-C and BMD, although in a recent study the values of TC and LDL-C showed a positive correlation with hip and lumbar BMD in males[6]. In addition, high values of TG after adjusting for BMI have been positively associated with BMD[7]. In terms of the association between lipids and vertebral fractures, the results of the studies differ as a function of sex. Thus, in postmenopausal women with vertebral fracture the levels of TC, LDL-C and TGs were lower than in those women without fracture[8], although in other cases no association has been demonstrated[9]. Studies carried out in males have not shown an association between lipids and vertebral fracture[6,9]. In the study carried out by Hernandez et al. in a Spanish cohort of males, levels of LDL-C and TC were lower in those subjects with non-vertebral fractures. The discrepancy between studies could reflect the influence of genetic, dietary or geographic factors on this association.

Arterial hypertension
In AHT, a higher rate of bone loss in relation to an increase in the excretion of calcium in the urine has been described, which raises the levels of PTH[10]. A positive relationship has been proposed between BMD and the presence of AHT[11,12], while other authors describe a negative or independent association[13]. In respect of fractures, the data are more consistent, and we know that AHT is a risk factor for hip fractures in women[14], and for other locations in both sexes[15], with one of the possible pathogenic factors being the increased risk of falls caused to a great extent by the hypotensive effect of the antihypertensive drugs. Other authors have described, as a class effect of hypotensive drugs, a discrete reduction in the global risk of fractures which could be related to a reduction in the urinary excretion of calcium[15].

The influence of different hypotensive treatments on BMD and other related factors has also been evaluated. Thus, in postmenopausal women with AHT in treatment with tiazides, the levels of markers for remodelling were lower with respect to the control group, and the lumbar BMD, higher[12].

Obesity
The pathogenic mechanisms responsible for the relationship between fat and bone are multiple: gastrointestinal peptides such as GLP-1 and GIP, levels of insulin in circulation and adipokynes. On many occasions this relationship is complex and discordant results have been found. Leptin, the adipokyne increased in obesity, in the hypothalamus slows the formation of bone by inhibiting the proliferation of the osteoblasts[16], whilst in the bone it stimulates osteoblastic, and inhibits osteoclastic, differentiation[17]. The results of clinical trials are also contradictory, finding a positive relationship between blood levels of leptin and BMD in women[18], and negative in males[19]. On the other hand, adiponectin halts osteoclastogenesis in *in vitro* studies[20], and in DM2 its blood levels are negatively related to BMD[21].

Different studies have shown a positive relationship between body weight and BMD. This relationship is greater in women, both postmenopausal and sedentary[22]. Similarly, a recent meta-analysis shows a protective effect of obesity on the global risk of fracture[23]. Analysing the different types of fracture, this protective effect is shown on hip and vertebral fractures[24], but not in distal radius fracture[25].

Hyperhomocysteinemia
Hyperhomocysteinemia is a marker for cardiovascular risk which has been associated with a higher rate of bone resorption[26], and a higher risk of fractures[27]. However, active therapy to control its blood levels was not able to reduce the incidence of fractures[28].

Metabolic syndrome
One of the fundamental components of metabolic syndrome is hyperinsulinemia and insulin resistance. Insulin has been demonstrated to stimulate the proliferation of osteoblasts and the secretion of other factors implicated in bone formation, such as BMPs and IGF-1, from which one would expect a higher BMD in these patients. Thus, in patients with metabolic syndrome a higher BMD in the hip has been described[29]. The presence of metabolic syndrome has also been related to a lower risk of non-vertebral fractures, both in men and women in a transversal study[30], while in a prospective study incidental clinical fractures were 2.6 times more frequent in those patients with metabolic syndrome compared with the controls[31]. In patients with DM2, the added presence of other components of metabolic syndromes was associated with a lower prevalence of vertebral fracture[32].

1.2. Factors involved in bone metabolism and cardiovascular disease
Oestrogens
The protective effect of oestrogens on the vascular system of postmenopausal women, and the increase in vascular disease after menopause suggests a role for oestrogenic depletion in the development of atherosclerosis in women. In relation to this fact, it has been observed that the gene for the alpha oestrogenic receptor is associated with a higher risk of cerebrovascular disease[33], and in turn, certain polymorphisms of the beta receptor appear to be a risk factor for acute myocardial infarction in Spanish males[34].

Vitamin D
The relationship between vitamin D and vascular disease has been studied in depth, with contradictory results. In experimental animals high concentrations of vitamin D in the diet favoured the development of coronary and aortic arteriosclerosis[35]. In humans, various studies have found an association of risk between certain variants of the vitamin D receptor gene and the presence of coronary disease[36], while others show no such association[37]. An epidemiological study in the US showed that supplementing foods with vitamin D increased the incidence of arteriosclerotic disease. However, other works have put the relationship the other way round, and have associated the deficit in vitamin D with the presence of peripheral arterial disease[38] and myocardial infarction[39], thus, as an inverse relationship between 1-25 dihydroxyvitamin D and the degree of coronary calcification[40].

Parathormone (PTH)
Receptors for PTH have been confirmed in cardiac and smooth muscle cells, attributing to them a trophic effect and suggesting that it could be responsible for the hypertrophy of the left ventricle observed in patients in dialysis. On the other hand, in mice with acute myocardial infarction treatment with PTH favours the migration of angiogenic progenitor cells to the damaged area, which could attenuate the ischemic damage[41], and recently it has also been found that PTH increases the endothelial expression of NO[42].

Parameters of remodelling
A deficit in MGP encourages the presence and the extent of vascular calcification in experimental animals and specific polymorphisms are associated with a high risk of myocardial infarction in humans [43], which suggests that it has a role in the inhibition of vascular calcification[44]. In turn, osteocalcin is expressed in the vascular tissue and its blood levels have been related with parameters for arteriosclerosis in patients with DM2[45]. Osteopontin (OPN) is expressed in calcified atheromatous lesions, and mice with high levels of OPN have a higher IMT[47]. The type 2 bone morphogenetic protein and its osteogenic mediator CbFa-1 (core-binding factor α 1) are increased in human arteriosclerotic lesions, but not in healthy vessels[47]. Catepsin K, the main enzyme involved in bone resorption, could be involved in the destabilisation of the plaque, since it has been observed that in ApoE knockout mice the catepsin K deficit preserves arterial stability and integrity, and diminishes vulnerability to artherosclerotic plaques[48].

OPG
OPG is expressed in the smooth muscle cells and in the endothelial cells of the arterial wall where they appear to be an autocrine survival factor of the endothelial cells[49]. The increase in the levels of OPG in blood have been associated with the presence and severity of arterial calcification in various locations and in different pathologies: renal insufficiency in haemodialysis[50], coronary calcification in rheumatoid arthritis[51] and abdominal aortic calcification in peripheral arthropathy[52]. If the raise blood levels of OPG is simply a marker for vascular damage, represents a defence mechanism or, on the contrary, is an active mediator for the progression of the disease, remains to be clarified.

The predictive value of blood levels of OPG in the incidence and mortality of CVD has been confirmed in different populations studied. Thus, it has been shown that the increase in blood levels of OPG is a risk factor for cardiovascular morbimortality in conditions of accelerated atherosclerosis such as in women of advance age[53], haemodialysed patients[54], and diabetes type 1[55], but also in the general population[56]. Raised blood levels of OPG are associated with the presence and severity of coronary disease[57], and with the severity of peripheral arthropathy[58]. OPG has also been related to

surrogate markers for sub-clinical arteriosclerotic disease. In postmenopausal women without CVD high levels of OPG are positively related to endothelial dysfunction, arterial rigidity and ITM[59].

1.3. Surrogate markers for CVD and osteoporosis

The majority of transversal studies carried out have described an inverse association between the presence, severity and progression of arterial calcification and BMD, both in menopausal women[60,61], and in males[62], as well as an increased risk of fracture in postmenopausal women with aortic calcification[63]. Carotid atheromatosis, another surrogate marker for CVD, is associated with a lower lumbar bone mass in postmenopausal women[64], and a higher risk of fracture[65]. The presence of osteoporosis and/or fracture have also been related to an increased risk of sub-clinical arteriosclerotic disease[66].

1.4. Cardiovascular events and osteoporosis

In osteoporotic women or those with vertebral fracture there has been described a relative risk of 3.9 and 3 respectively, of cardiovascular events, this risk being proportional to the severity of the osteoporosis at diagnosis[67]. In the same way, the lumbar BMD is reduced in patients with cardiovascular disease independently of age[68], and the presence of peripheral arterial disease and/or ischemic cardiopathy is associated with a higher risk of hip fracture[69]. There has also been a significant association found between the presence of myocardial infarction and low bone mass[70], and between the presence of osteoporosis/osteopenia and an increased risk of obstructive coronary disease in both sexes[71]. On the other hand, a decrease of 1 SD in the BMD in the calcaneum and femoral neck increases the risk of cerebrovascular disease by 1.3 and 1.9 respectively[72].

2. Diabetes mellitus type 2, osteoporosis and risk of fracture

2.1. Diabetes and bone mass

The deleterious effect of DM on the bone varies as a function of the type of diabetes. In patients with DM2, although the results are odd, there appears to be an increase in the risk of fractures despite a higher BMD, caused fundamentally by an increased risk of falls associated with the presence of vascular complications, as well as alterations in bone quality, which are also a determining factor[73].

Studies which have assessed BMD in patients with DM2 show discordant results. In the lumbar region, positive[74], negative[75], and neutral[76] effects have been described. In the hip, the results are somewhat more uniform, with a higher BMD for both sexes being observed in the majority[77], and in the distal third of the radius, negative[76], or neutral[78] effects have been described. The result in the studies indicated above mostly confirm that the main determinants of BMD in patients with DM2 are age and BMI. Although not all, some of these studies have found a negative relation between the degree of metabolic control[76] and the duration of the disease[71]. In the Spanish population with DM2 exercise, BMI and the adequate consumption of calcium appear to be factors protective of osteoporosis, on the other hand, age, and the consumption of zinc are risk factors[79,80].

2.2. Risk of fractures in patients with DM2

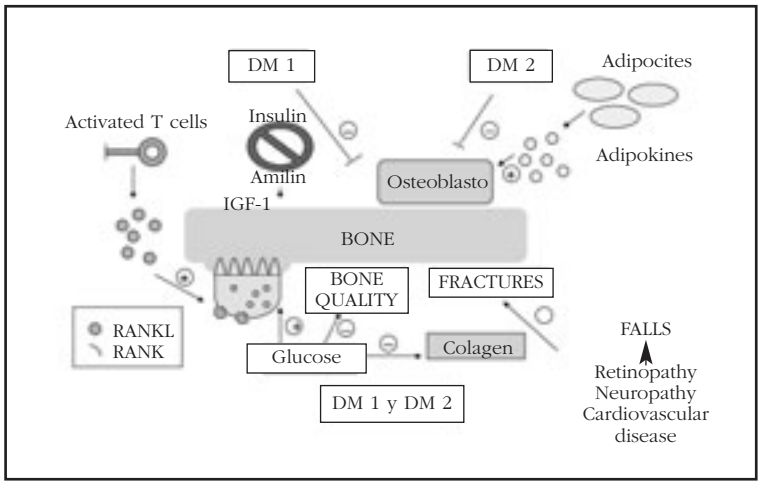
Most of the studies show an increase in risk of fracture in spite of a higher BMD. Thus, an incidence of fractures in patients with DM2 has been described which is similar to the control group despite a higher BMD[81]. And an increase in the risk of non-vertebral fractures of 69% for both sexes in the diabetic population[74]. The fact that in this study the increase in risk is circumscribed in those patients with DM2 in treatment, and that they suffered a higher percentage of falls, makes one think that the higher risk of fracture in these patients is due to a higher rate of falls. In fact it has been corroborated that the risk of falls is increased only in those patients treated with insulin (OR 2.76) and that the principal risk factors for this increase are age, alterations in balance, diabetic neuropathy and retinopathy, and coronary disease[82]. Another risk factor for falls in this group of patients is the high prevalence of hypovitaminosis D which they suffer[83]. A recent review has demonstrated a global increase in the risk of any fracture of 30%, and 70% for hip fractures[84]. The results were consistent in Europe and the US, and there was a relationship with the follow up, since those with disease of more than 10 years standing had an even higher risk of hip fracture. On the other hand, no increased risk of vertebral, proximal humeral or in the distal third of the radius was found, although there was a 30% increase in risk for the bones of the feet. Against these results, a retrospective cohort study did find an increased risk of vertebral and proximal humeral fracture, the main risk factors being age, previous fracture, neuropathy and treatment with insulin, with exercise, BMI and the use of biguanides being protective factors[85]. The same as with BMD, the majority of the studies did not observe an association between the degree of metabolic control, determined by HbA1c, and the risk of fracture, save for one Japanese study where the presence of HbA1c > 9% was associated with an increase in the risk of vertebral fractures[21]. On the other hand, blood levels of pentosidine (a product of non-enzymatic glycation) is an independent risk factor for vertebral fracture in both women and men with DM2[82]. In Spain, the GIUMO study carried out in postmenopausal women with obesity and DM2 did not observe an increased prevalence in vertebral or hip fractures, nor in conjunction with non-vertebral fractures[86]. Finally, a biphasic effect has been proposed regarding the risk of having a hip fracture, since patients with hydrocarbonate intolerance, or with a recent diagnosis of DM2, have shown a lower risk of fractures[74,87], while those with disease of longer duration have an increased risk[85,87].

On the basis of this theory, initially overweight and obesity will play a protective role, while subsequently the development of complications due to diabetes will raise the risk of fracture.

2.3. Potential pathogenic mechanisms of osteoporosis in DM2

Hyperglycemia has direct adverse effects on bone metabolism in both types of DM (Figure 1). In being the principal source of energy for the osteoclasts, it increases, dose-dependently, their activity *in vitro*[88]. On the other hand, non-enzymatic glycosylation of various bone proteins, including collagen type 1, alters and reduces bone quality[89]. Thus, in animal models of diabetes, the content of pentosidine in bone increases during the course of the disease, reducing the biomechanical properties of the bone, in spite of maintaining a stable BMD[90]. The increase in glycemia also has indirect effects on the skeleton since it favours hypercalciuria and interferes with the PTH/vitamin D system. On the other hand, the improvement in glycemic control in poorly controlled DM2 reduces the urinary excretion of calcium and phosphorus[91]. In addition, in recent years, interest has grown in research into the effect of the incretins on bone metabolism. It has been suggested that GIP and GLP-2 could be responsible for the inhibition of bone resorption after the ingestion of food, and it has been observed that those patients with DM2 have a reduction in this effect after an oral overload of glucose[92]. A Spanish study, carried out in diabetic rats has found that GLP-1 has an anabolic effect on bone, independently of insulin[93]. However, if the alterations in the incretin system present in DM2 are responsible for the changes in BMD in this group of patients, it is still to be elucidated.

Figure 1. Potential mechanisms responsible for osteoporosis and osteoporotic fracture in both types of diabetes (adapted from Hofbauer et al. 2007)



3. Conclusion

Atherosclerosis and osteoporosis are chronic degenerative diseases with a high incidence in developed countries and whose prevalence increases with age. Both are silent processes with a high economic cost, especially when there are acute complications which include cardiovascular disease and fractures. The OPG/RANKL system has been suggested as a common mediator for both processes, but its precise significance is unknown. Knowledge of the common physiopathological mechanisms of these two pathologies will not only help in better management of patients but it could also contribute to the development of active drugs for both processes. Research into type 2 diabetes may bring important data regarding this complex association.

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Determination of the principal metabolites of vitamin D in blood by means of on-line solid phase extraction with liquid chromatography-tandem mass spectrometry

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Summary

The determination of metabolites of vitamin D is very important in bone metabolism, in coronary disease, cancer, innate immunology, etc. Unfortunately, variation in methods for determining the metabolites of vitamin D limits the ability of clinicians to monitor the status, supplementation and toxicity of vitamin D. In this work, an automatic method of determining the most important metabolites of vitamin D is presented. 0.2 ml of serum is injected into an XLC-MS/MS (eXtraction Liquid Chromatography-tandem Mass Spectrometry) platform to be cleaned and preconcentrated through extraction in the solid phase (SPE). The analytes retained in the SPE cartridge are eluated directly by the mobile chromatographic phase containing 10% water in methanol, with 5 mM of ammonium formate as ionizing agent, at a flow of 0.3 ml/min for the separation of the analytes, and their later detection through triple quadrupole mass spectrometry (MS/MS).

The limits of detection varied between 3.5 and 8.2 pg/ml. The coefficients of variation within the trial varied between 1.5 and 2.3% during the same day, and between 2.5-3.9% over a week. The recuperation varied between 97 and 99.7% for all analytes. The total time taken for the analysis was 20 minutes.

Thus, the proposed method is robust, cheap and appropriate for use in clinical and research laboratories.

Key words: *Metabolites of vitamin D, Healthy population, Deficiency in vitamin D.*

Introduction

Lack of vitamin D constitutes one of the most prevalent deficiencies in the world. It affects more than half of the population: babies, young people, adults, postmenopausal women and older people, in whom, if they have osteoporotic fractures, the prevalence of low levels of vitamin D reaches 100%[1]. In Spain, in spite of its geographical position and climate which facilitate adequate sunshine, this situation is faithfully reproduced.

Deficiency in vitamin D, in addition to its role in the etiopathology and treatment of rickets or osteomalacia, contributes to multiple extra-skeletal pathologies[1,2]. In fact vitamin D deficiency is associated with an increased risk of suffering diabetes mellitus[4], arterial hypertension[5], cardiac insufficiency[6], cardiovascular disease[7], peripheral arterial disease, acute myocardial infarction[8], cancer[9], as well as risk of suffering infections[10], autoimmune and inflammatory diseases 11, and mortality[12,13]. In addition, the taking of the usual doses of treatment of osteoporosis is associated with a reduction in rates of mortality[14]. All of which has increased interest in the metabolism of the endocrine system of vitamin D, and the quantification of its key metabolites.

The state of vitamin D is determined by the blood concentration of 25-hydroxyvitamin D [25(OH)D][1,2], which includes the concentrations of $25(OH)D_3$ and $[25(OH)D_2$, although it is not clear if both have the same activity, or the same weight as vitamin D_2 or D_3 in the maintenance of the state of 25-(OH)D in humans[15,16]. For its quantification, methods based on liquid chromatography (LC)[17,18], on protein competition tests by chemiluminescence[19], on high and low frequency radioimmunoassay by sampling[20,21], on automatic methods of chemiluminscence[19], and on liquid chromatography tandem mass spectrometry (LC-MS/MS)[22,23], are normally used. These new methods have generated much controversy due to the fact that the interlaboratory studies carried out have not shown concordant results between these different methods , nor among those based on immunoassay[24-26]. The clinical application of LC-MS/MS has improved the selectivity in the determination of 25(OH)D, although the coefficients of variation continue to be high (20%) among the different laboratories which use it, due to the nonexistence of standardised procedures for the analysis of vitamin D, since they are methods developed by and, generally very dependent on, the operator. The implementation of international measures of standardisation which certify the quality of the methodology, such as those conducted by DEQAS (vitamin D External Quality Assessment Scheme)[27], have shown the differences in the determination vitamin D and have developed measures for their standardisation, such as the use of the same standards for the calibration of the method[28].

The determination of $1,25(OH)_2$ dihydroxyvitamin D_3 is necessary in cases of renal insufficiency, hypoparathyroidism, pseudohypoparathyroidism, screening for hypercalcemia, etc. This determination is more complicated than with 25(OH)D because the concentration is much lower and its stability less. Most of the methods use I^{125} as a marker in radioimmunoassay after a process of extraction. The structural similarity between the metabolites of vitamin D means that the specificity of the method is always in question, since there is no robust study of interference, due to the difficulty of finding a reference method. LC-MS/MS is used for the determination of calcitriol, using precipitation of proteins and previous extraction in solid phase[29] and, recently, applying a derivation of Diels-Alder to improve the ionisation efficiency[30]. In spite of the fact that current methods based on LC-MS/MS provide high sensibility and sensitivity, a consistent platform, totally automated, with high frequency of sampling, precision and exactitude, which makes unnecessary the presence of expert operators, is demanded. From the experience of the group it is thought that the most appropriate platform is XLC-MS/MS (eXtraction Liquid Chromatography-tandem Mass Spectrometry), since it is a closed system which avoids the loss of analytes, and is totally automatic, for which reason it has low coefficients of variation.

This research seeks to fill this gap through the development of an automatic method based on the on-line coupling of solid phase extraction with liquid chromatography and tandem mass spectrometry to determine vitamins D_3 and D_2, the metabolites 25-hydroxyvitamin D_3 and D_2, $24,25(OH)_2$ dihydroxyvitamin D_3, and $1,25(OH)_2$ dihydroxyvitamin D_3, and their application in serum from blood donors.

Material and method

Solvents and standards

Ammonium formate, 25-hydroxyvitamin D_3 (25(OH) D_3) and 25-hydroxyvitamin D_2 ($25(OH)D_2$), vitamin D_2 and vitamin D_3, were obtained from Sigma (Sigma–Aldrich, St. Louis, MO, USA). 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$), were provided by Roche (Basle, Switzerland). Methanol, acetonitrile and formic acid were obtained from Scharlau (Barcelona, Spain).

The reserve solutions were prepared by dissolving a known quantity of analytes ($25(OH)D_2$, $25(OH)D_3$, vitamin D_3, vitamin D_2, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$) in methanol. From the reserve solutions the working solutions were prepared by dilution of an appropriate volume in methanol, calculating the exact concentration by photometry.

Instrumentation

The separation chromatography was carried out in reverse phase mode with an Agilent 1200 Series (Palo Alto, CA, USA) chromatograph, followed by electrospray ionisation (ESI) in positive mode, and detection by means of mass spectrometry in tandem (Agilent 6410 Triple Quadrupole). The analyses were processed through the MassHunter Workstation Software (Agilent) programme for qualitative and quantitative analysis. The automa-

Table 1. Method of extraction in solid phase

	Flow rate	Volume	Solvent	Commentary
New cartridge				
Autosampler				Load sample
Solvation	5 mL/min	2 mL	Methanol	
Solvation	5 mL/min	4 mL	30% ACN-0.2% FA	
Equilibration	0.4 mL/min	0.4 mL	30% ACN-0.2% FA	
Load of sample	0.4 mL/min	2 mL	30% ACN-0.2% FA	
Washed	2 mL/min	4 mL	30% ACN	
Elution			Mobile phase	7 min
Tube cleaning	5 ml/min	2 mL	Methanol	
Tube cleaning	5 mL/min	2 mL	Water	

tic solid phase extractor used was a Prospekt2 (Spark Holland, Emmen, Netherlands) system, and (Midas) autosampler with a sample loop of 0.2ml. The solid phase extraction cartridge was a Hysphere C18 (Spark Holland) of 10 x 2 mm. The analytic column used was Synergi Hydro-RP (Phenomenex, Torrance, CA, USA) of 2.5 µm particle size, 100 x 2,0 mm.

The extraction of blood was carried out using the standard process. Once obtained the blood was centrifuged at 4° for 10 minutes, aliquoted and frozen at -80°C until used.

Procedure

The autosampler fills the sample loop (0.2ml) and initiates the sequence of operations described in Table 1. Basically, the process of extraction starts with the preparation of the cartridge by means of an activation of the stationary phase with methanol, conditioner and equilibration with an aqueous solution of 0.2% formic acid in 30% acetonitrile. With this same solution, the sample is pulled towards the cartridge. In these conditions the analytes are retained in the absorbent contained in the cartridge, and then 30% acetonitrile is used as a washing solution for interferences. Subsequently, the stages of chromatographic elution and separation commence, putting the mobile phase in contact with the extraction cartridge by the twisting of a valve. The elution time is 7 minutes. The initial mobile phase was 5 mM of ammonium formate contained in 90% methanol at a flow rate of 0.3 ml/min. In minute 2 a lineal gradient of 5 minutes was programmed to obtain 5 mM of ammonium formate in 100% methanol. The temperature of the column was 15° C. The total time for the analysis was 20 minutes.

The eluite of the chromatographic column was ionised using ESI in positive mode and monitored by MS/MS in multiple reaction monitoring (MRM) mode. The flow and temperature of the drying gas (nitrogen) in the ESI was 13 l/min and 350° C, respectively. While the pressure of the nebulizer was 35 psi and the capillary voltage 4,000 V. The scanning time for each transition of MS/MS was 50 minutes.

Results

The limit of detection (LOD) according to the definition of the IUPAC (International Union of Pure and Applied Chemistry) is the minimum quantity of analyte detectable, and is calculated experimentally as the concentration which corresponds to 3 times the standard deviation of the noise to signal ratio calculated in 10 samples. The limit of quantification (LOQ) according to IUPAC is the minimum quantifiable quantity of analyte, generally corresponding to the smallest concentration of the calibration line and is calculated as the concentration which corresponds to a signal 10 times the standard deviation of noise to signal ratio in 10 samples. The individual values of these limits and the regression coefficients are found in Table 2.

Evaluation of the precision of the method

The coefficients of variation, intra- (repeatability) and inter-trial (reproducibility) were calculated over seven days, carrying out daily measurements with replicas in a serum of known concentration[31]. The results are shown in Table 2.

Evaluation of the exactitude of the method

The exactitude of the method and the matrix effect was studied using samples with or without being fortified with standard solutions. The recuperation was calculated with two configurations of the Prospekt2, a double cartridge for non-fortified samples and a single cartridge for fortified samples[32]. This was done because the recuperation may not be adequate for two reasons: first, due to

Table 2. Figures of merit. *Expressed as a percentage of the relative standard deviation

Analyte	LOD (ng/mL)	LOQ (ng/mL)	Coefficient of correlation	Repeatability* (%)	Reproducibility* (%)
$24,25(OH)_2D_3$	0.055	0.184	0.9978	1.6	2.5
$1,25(OH)_2D_3$	0.0035	0.012	0.9977	1.8	2.9
$25(OH)D_3$	0.082	0.272	0.9987	1.5	3.1
$25(OH)D_2$	0.080	0.267	0.9973	1.7	2.8
Vitamin D_2	0.084	0.284	0.9943	2.3	3.9
Vitamin D_3	0.085	0.281	0.9915	2.1	3.5

LOD: limit of detection; LOQ: limit of quantification

a poor retention in the cartridge of the compound under study, which can be seen in the double cartridge, because what is not retained in the first one is retained in the second; the second reason is a low elution, which may be probed by means of a fortified sample whose concentration is known.

The recuperation in the double cartridge system is calculated as the quantity in the first cartridge/[quantity in cartridge 1+quantity in cartridge 2]. The configuration of a single cartridge is calculated as [final concentration - initial concentration/concentration added evaluated in a single sample in five repetitions on the same day under identical conditions. It is understood that the initial concentration is the concentration of analyte present in the sample before adding a known quantity of it, that is to say, the quantity of a blank sample. The results are found in Table 3.

Application of the method

Samples were analysed from 92 blood donors. A representative chromatogram of a sample fortified with standard solutions appears in Figure 1. Levels of vitamin D_3 were detected: 10.4 ± 4.8 ng/ml; but levels of vitamin D_2 were not. Blood levels of 25(OH)D (21.3 ± 5.7 ng/ml) correspond to the sum of $25(OH)D_2$ and $25(OH)D_3$. The share of $25(OH)D_2$ out of a total 25(OH)D is 1.9 %. 5% of the population studied had blood levels of 25(OH)D < 10 ng/mL, 42% < 20 ng/ml, 40% between 20 and 30 ng/ml, and only 18% were > 30 ng/ml. Blood levels of $24,25(OH)_2D_3$ were 4.1 ± 1.6 ng/ml and of $1,25(OH)_2D_3$, 48.2 ± 11.4 pg/ml.

Discussion

The analysis of vitamin D and its metabolites represent a great challenge, due to the high lipophilic nature of these compounds, which means that they are found strongly bonded to their transporter proteins; bonds which need to be broken for their analysis by liquid chromatography. The cleaning of the extracts is essential, since other endogenous lipids will be co-extracted along with the vitamin D metabolites, which results in unclean extracts which may distort the shape of the chromographic peaks and curtail the life of the column. This makes necessary the use of selective and highly sensitive systems such as mass spectrometry for an exact quantification. It also means that the use of a closed system is critical, which avoids the breakdown of the metabolites of vitamin D by light. The advance in methodologies to ensure the determination of vitamin D has not improved the variations previously shown in the measurement of 25(OH)D[24,25].

The disparity in the results affects all laboratories using the same, or different, methodologies. The use of methods with a low level of automisation makes the measurement of vitamin D highly dependent on the user, and requires rigorous quality control to assure the results. Methods based on RIA are not all equal, given different specificity for $25(OH)D_3$ and $25(OH)D_2$; therefore, there are some which overestimate levels of 25(OH)D and others which give lower values for some of the metabolites[26,33].

HPLC is commonly recognised as the gold standard for the determination of vitamin D metabolites[3,17,18,34], but it has high equipment costs and possesses a low frequency of samples, given the obligatory precipitation of proteins and/or liquid-liquid extraction using the methods described above, which has made its establishment in laboratories as a routine technique difficult. The methods which exist for the quantification of $1,25(OH)_2D_3$ are laborious and take much time; therefore, more rapid, cheaper and simpler methods are required, which also reduce the risk to health associated with the use of radioisotopes. In recent studies an EIA kit had a poor correlation with a typical analysis by RIA35. Kissmeyer et al.[29], published in 2001 an method using LC-MS/MS for determining $1,25(OH)_2D_3$, but this required 1-mL of serum, in addition to an earlier precipitation of proteins and drying stage in a flow of nitrogen, and later reconstitution, which leads to low fre-

quency of samples and relatively high coefficients of variation, because all the stages of treatment of the sample before the chromatographic analysis were manual.

In conclusion, the proposed method is an improvement on existing methods since it allows the rapid and automated determination of the concentrations of vitamins D_3 and D_2, and the metabolites $24,25(OH)_2D_3$, $1,25(OH)_2D_3$, $25(OH)D_3$ and $25(OH)D_2$ using a small quantity of serum, permitting both research into the physiology and physiopathology of the endocrine system, and clinical association studies, or their use in normal practice.

The use of an on-line system for the extraction means that there is no loss of analytes by degradation, and the total automation of the process of analysis means that the precision and accuracy is improved and the need for an expert user is avoided.

Therefore, the proposed method is rapid, with a high sensitivity, exactitude and precision. The main inconvenience it presents is the high cost of the equipment used, although the application of the method is cheap.

Figure 1. Chromatogram of a fortified blood sample
(1) $24,25(OH)_2D_3$; (2) $1,25(OH)_2D_3$; (3) $25(OH)D_3$; (4) $25(OH)D_2$; (5) vitamin D_2, (6) vitamin D_3

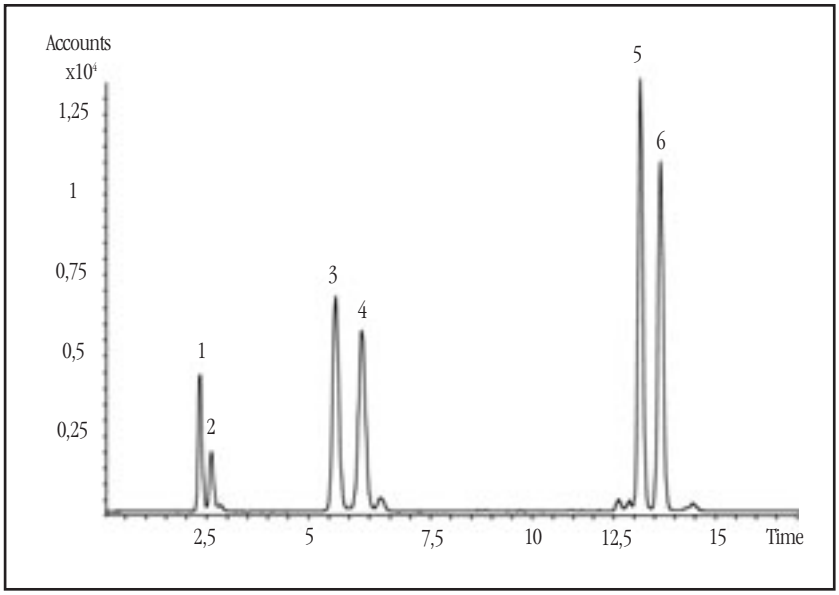

Table 3. Recuperation of each analyte: (1) two-cartridge configuration, (2) single cartridge configuration

Analyte	Recuperation (1)	Recuperation (2)
$24,25(OH)_2D_3$	97.0	96.5
$1,25(OH)_2D_3$	100.2	99.5
$25(OH)D_3$	99.8	99.3
$25(OH)D_2$	98.9	99.0
Vitamin D_2	99.1	99.4
Vitamin D_3	98.3	98.4

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Current perspectives on the role of vitamin D and calcium in the patient care for osteoporosis: an expert panel discussion

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Summary
Background: A better knowledge of the wide variety of actions of vitamin D is an essential step to improve the quality of osteoporosis care. This review of the current evidence of the binomiun 'vitamin D-osteoporosis' is the result of a one-day expert panel meeting held in Madrid in 2008. The panel consisted of experts in osteoporosis and mineral bone metabolism pertaining to a range of clinical disciplines and drawn from throughout Spain.
Method: A literature search was performed on the MEDLINE database for clinical trials, randomized clinical trials, systematic reviews and meta-analyses for articles published between 2007 and 2008, using the terms osteoporosis, vitamin and calcium. The resulting articles were the material used for small-group discussions at the meeting.
Findings: Oral alendronate and risedronate are the aminobisphosphonates of choice because of their proven efficacy in vertebral, nonvertebral and hip fractures. The adequate dose of vitamin D could be defined as 800 IU/day for healthy adults and as 1000 IU/day for osteoporotic patients, and the adequate amount of calcium intake is 1000-1200 mg/day. The dose required for correct functioning of extraskeletal actions of vitamin D may be higher. Calcium supplementation could be secured through the diet but drug administration is required when vitamin D supplementation is given.
Conclusions: Optimization of the nutritional supply of vitamin D and calcium is the first step in the care of the patient with osteoporosis. Vitamin D supplementation does not exclude the intervention on other factors that may influence the risk of falls.

Key words: *Vitamin D, Calcidiol, Calcitriol, Calcium, Osteoporosis, Vitamin D receptor, Risk of fracture, Aminobisphosphonates, Alendronate, Risedronate, Muscular weakness.*

Introduction

It has been known for a long time that vitamin D intervenes in the regulation of blood calcium and phosphorus levels, and that a lack of vitamin D leads to rickets. However, recently it has become necessary to revise our knowledge of this vitamin, since the existing evidence indicates that vitamin D also exerts extraskeletal actions of great relevance, and which reflect its fundamental role in relation to musculoskeletal health. At present, however, we lack data of sufficient quality to firmly define the intervention of vitamin D in both the origin and treatment of osteoporosis. In view of this situation, and with the purpose of specifically addressing some points of controversy, the decision was taken to create a group of experts in osteoporosis and mineral metabolism pertaining to a range of disciplines (Internal Medicine, Endocrinology, Rheumatology, Traumatology and Orthopedic Surgery, Gynecology, Primary Care, Rehabilitation or Health Economics), in order to examine the role of vitamin D from the broad perspective of their different fields. In March 2008, Merck Sharp & Dohme Spain sponsored a one-day symposium in Madrid as a forum for discussion to allow a panel of experts to identify the current challenges of the binomium "vitamin D-osteoporosis", supported by an analysis of the evidence-based literature, and with the aim of establishing a series of final conclusions based on consensus. The synthesis of this work is the subject of the present review.

Methods

In order to review the topics addressed in this meeting, a literature search was performed on the MEDLINE database. English-language and Spanish-language articles from January 2007 to February 2008 were included. Search terms used were 'osteoporosis', 'vitamin D' and 'calcium' (MeSH major topics). Other limits placed were 'clinical trial', 'meta-analysis', 'randomized controlled trial' and 'review' as type of article, and 'all adult: 19+ years' as ages.

Key experts from various areas belonging to different scientific societies, including the Spanish Society of Bone and Mineral Metabolism Research (SEIOMM), Spanish Society of Internal Medicine (SEMI), Spanish Society of Rheumatology (SER), Spanish Society of Endocrinology and Nutrition (SEEN), Spanish Society of Family and Community Medicine (SEMFYC), Spanish Society of Primary Care Physicians (SEMERGEN), Spanish Society of Traumatology and Orthopaedic Surgery (SECOT) and Spanish Association for the Study of Menopause (AEEM) were invited to attend one-day symposium to develop the current consensus document. Before the meeting, printed articles obtained from the literature search were distributed among the participants. At the time of the meeting, attendees were divided into small groups and discussed the topics of interest previously assigned to each group on the basis of the literature provided. Then, the leaders of the groups presented the conclusions reached in the different sub-meetings to the general audience and an open discussion was initiated. Final statements were accepted by consensus of all participants.

The literature search has been updated with any relevant publications that have been published from April 2008 to April 2009.

Discussion

Efficacy of aminobisphosphonates and vitamin D in reducing osteoporotic fractures

Osteoporosis is a very common condition in elderly people, and is associated with an increased risk of fracture. Osteoporotic fractures constitute an enormous public health problem, not only because of the healthcare costs involved, but also because of the increased morbidity and mortality, and decrease in the patient quality of life. Likewise, its increasing prevalence – due in part to the gradual aging of the population – has renewed interest in the efficacy and safety of the drugs available for treating the reduction in bone mineral density associated with osteoporosis[1].

Regarding the efficacy of antiresorptive therapy, and in addition to the clinical practice guide to the treatment of postmenopausal osteoporosis developed by the study group of the Sociedad Española de Investigaciones Óseas y Metabolismo Mineral (SEIOMM)[2], some systematic reviews and meta-analyses summarize the evidence derived from clinical trials and other type of studies.

In a now classical meta-analysis of randomized clinical trials and systematic reviews published by Cranney et al.[3], the aminobisphosphonates alendronate and risedronate exhibited the greatest effect in terms of the reduction of vertebral fractures compared with vitamin D, calcitonin, raloxifen and etidronate. Likewise, a positive effect of hormone replacement therapy was demonstrated in terms of the incidence of vertebral fractures, though the existence of selection bias in the analyzed studies may have led to overestimation of the magnitude of the effect of treatment. Regarding nonvertebral fractures, convincing evidence was only recorded in favor of risedronate and alendronate. The magnitude of risk reduction was estimated to be 50% for alendronate in relation to both vertebral and nonvertebral fractures, versus a little over 33% for vertebral fractures and 25% for nonvertebral fractures in the case of risedronate. Another later meta-analysis has confirmed the efficacy of alendronate in reducing the risk of hip fractures (45-55%) in different populations of postmenopausal women[4]. On the other hand, a review of randomized, placebo-controlled studies of the efficacy of different antiresorptive agents[5] again found alendronate to offer great efficacy, with a reduction in the risk of hip and nonvertebral fractures of 45-55%. Efficacy was also demonstrated for hormone replacement therapy (25-36%) and risedronate (26-27%). Lastly, another recent systematic review6 also supports the efficacy of alendronate, risedronate and estrogens in preventing hip fractures among males and females with osteoporosis or diminished bone mineral density.

In relation to vitamin D, an extension of the findings of the meta-analysis conducted by Bischoff-Ferrari et al.[7] (in which a reduction in femoral fracture risk was recorded in individuals over 60 years of age administered a daily vitamin D dose of 700-800 IU), published by Boonen et al.[8], demonstrated that oral treatment with vitamin D only proved effective in reducing the risk of hip fracture (and of any nonvertebral fracture) when associated to a daily supplement of 1,000-1,200 mg of elemental calcium. To further increase uncertainty as to the effects of calcium, there also have been meta-analyses suggesting an increased risk of hip fracture when using calcium supplements[9], as well as an increased risk of cardiovascular events[10], or more recently, meta-analyses and controlled trials showing independent beneficial effects of vitamin D[9,11].

In summary, clinical trials published in the literature and their combined evaluations in the form of systematic reviews and meta-analyses offer conclusive results on the efficacy of the aminobisphosphonates alendronate and risedronate in reducing osteoporotic vertebral, nonvertebral, and hip fractures. Regarding the safety of long-term treatment, the best available data correspond to alendronate. The extension of the FIT (Fracture Intervention Trial) to 10 years (FIT Long-term Extension, FLEX)[12] has demonstrated that the continuation of alendronate treatment in postmenopausal women (both 5 and 10 mg/day) during 10 years does not increase fracture risk, maintains bone mass, and reduces bone remodeling compared with discontinuation of treatment after 5 years. The data of the FLEX study have led to the recommendation to continue alendronate therapy for more than 5 years in women at high risk of suffering osteoporotic fractures.

There is consensus regarding the indication of aminobisphosphonates, including advanced age (over 65 years), in the presence of significant fracture risk. As regards calcium and vitamin D supplements, the existing evidence does not allow us to draw firm conclusions as to their effects in reducing the risk of osteoporotic fractures. On the other hand, since 2008, the Internet offers a new tool (the FRAX index) for evaluating the absolute osteoporotic fracture risk, developed by experts of the World Health Organization (WHO)[13]. The FRAX tool, which can be found at http://www.shef.ac.uk/FRAX/index_SP.htm, uses individual models that combine and integrate clinical risk factors with the bone mineral density of the femoral neck (if known), with evaluation of the following factors: age, sex, body mass index (BMI), previous fracture, hip fracture in the parents, active smoking, treatment with corticosteroids, rheumatoid arthritis, secondary osteoporosis, high daily consumption of alcohol, and bone mineral density of the femoral neck. The FRAX algorithms estimate the probability of hip fracture and of the most important osteoporotic fractures (clinical vertebral fracture, fracture of the proximal humerus, forearm and hip) after 10 years. This tool probably will have a significant impact on the evaluation of osteoporotic patients and on the indication and selection of treatments.

Regarding the influence of vitamin D deficiency as a risk factor for osteoporotic fractures, in a study of 2,546 postmenopausal women with osteoporosis that had been included in the placebo group of three prospective controlled studies of risedronate1[4-16], six risk factors present at baseline showed a significant association with the risk of nonvertebral fracture in the logistic regression analysis, among which serum concentration of 25-hydroxy-vitamin D, which exhibited a strong impact similar to that of very advanced age (over 80 years)[17]. In the LASA (Longitudinal Aging Study Amsterdam)[18], conducted in a representative cohort of 1,311 Dutch men and women in which vitamin D was measured and fractures were recorded during 6 years of follow-up, levels of ≤≤12 ng/mL were associated to increased fracture risk in the 65-75 years age group, but not in the 75-89 years age group. No statistically significant associations were recorded for other cutoff points (<10 ng/mL, 10-19.9 ng/mL, 20-29 ng/mL, ≥ 30 ng/mL) after adjusting for confounding variables.

In the group of 159,579 women between 50-79 years of age included in the Women's Health Initiative (WHI), collected from an observational study and three clinical trials involving hormone therapy, diet modifications and treatment with calcium and vitamin D supplements, in which risk factors for fracture were examined, treatment with calcium/vitamin D failed to show a beneficial effect – probably because calcium intake was high and there were a very few women with calcium consumption of < 400 mg[19]. However, a meta-analysis of five clinical trials on femoral fractures (n = 9,294) and seven clinical trials on nonvertebral fractures (n = 9,820) concluded that oral supplementing with 700-800 IU/day of vitamin D reduced the risk of hip fracture by 26%, and the risk of any nonvertebral fracture by 23% versus calcium or placebo in institutionalized or outpatients elderly subjects[7]. A 400 IU oral dose of vitamin D per day did not seem to suffice to prevent fractures. For this reason the authors recommended increasing the usual vitamin D dose of 400-500 IU/day to 700-800 IU/day.

The data of two meta-analyses confirm the efficacy of vitamin D supplements in preventing fractures only when combined with the administration of calcium. In the meta-analysis published by Boonen et al.[8], in which the cohorts of the RECORD study (Randomized Evaluation of Calcium OR vitamin D)[20] and of the Women's Health Initiative[21] of calcium and vitamin D were analyzed, the combination of calcium and vitamin D resulted in a reduction of 18% in the risk of hip fracture compared with placebo or no treatment, and of 25% compared with the administration of vitamin D alone. In order to optimize the clinical efficacy of this treatment, the authors recommended a vitamin D dose of 700-800 IU/day and a total elemental calcium dose of 1000-1200

mg/day. In the meta-analysis published by Tang et al.[11] in which 29 randomized clinical trials were identified with a total of 63,897 subjects aged 50 years or older, treatment with calcium or with the combination of calcium and vitamin D led to a 12% reduction in the risk of all types of fractures, being significantly higher (24%) in those trials in which treatment compliance was high. The effect of treatment was better in case of daily doses of calcium of ≥ 1200 mg and daily doses of vitamin D ≥ 800 IU *versus* lower doses of both compounds. The authors concluded with the recommendation of a combined treatment of calcium (800 mg/day) and vitamin D (800 IU/day) for the prophylaxis of osteoporosis in people over 50 years of age. The convenience of combined treatment of calcium and vitamin D is also supported by the results of the meta-analysis of Bischoff-Ferrari et al.[7]

In a recent meta-analysis on the efficacy of oral supplemental vitamin D in preventing nonvertebral and hip fractures in subjects ≥ 65 years of age, 12 double-blind randomized controlled trials for non-vertebral fractures (n = 42,279) and 8 for hip fractures (n = 40,886) comparing oral vitamin D, with or without calcium, with calcium or placebo were assessed[22]. To incorporate adherence to treatment, the dose was multiplied by the percentage of adherence to estimate the mean received dose for each trial. The pooled relative risk (RR) was 0.86 (95% confidence interval [CI], 0.77-0.96) for prevention of non-vertebral fractures and 0.91 (95% CI, 0.78-1.05) for the prevention of hip fractures, but with significant heterogeneity for both endpoints was observed. Including all trials, antifracture efficacy increased significantly with a higher dose and higher achieved blood 25-hydroxyvitamin D levels for both end points. For the higher dose (> 400 IU/day), the pooled RR was 0.80 (95% CI, 0.72-0.89) for non-vertebral fractures and 0.82 (95% CI, 0.69-0.97) for hip fractures. The higher dose reduced non-vertebral fractures in community-dwelling individuals and institutionalized older individuals and its effect was independent of additional calcium supplementation. The authors conclude that non-vertebral fracture prevention with vitamin D is dose dependent, and a higher dose should reduce fractures by at least 20% for individuals aged 65 years or older.

In a population of community-dwelling women and men (older than 20 years of age) U.S. NHANES III population-based survey, vitamin D status seems to be the dominant predictor of body mass density relative to calcium intake. Only women with vitamin D concentrations < 50 nM (19.4 ng/mL) seem to benefit from a higher calcium intake[23]. In another study, treatment with anti-resorptive agents over 13 months was associated with for three to fivefold lower bone mineral density changes and 1.5-fold increased risk of incidence fracture in vitamin D insufficient as compared to vitamin D repleted postmenopausal osteoporotic women[24]. Finally, in a prospective cohort of 175 previously bisphosphonate-responsive patients, 39 had a significant decrease of bone mineral density at follow-up. Twenty (51%) of these patients had vitamin D insufficiency. Correction of vitamin D insufficiency (100,000 IU/week for 5 weeks) was associated with significant increases in bone mineral density at the lumbar spine and the femoral neck[25].

Influence of dietary calcium in the treatment of osteoporosis

The relationship between calcium and osteoporosis can be systematized by five points: Is the administration of calcium necessary for the treatment of osteoporosis? And if so, how much should be administered? What amount of calcium do Spaniards consume? Should calcium be administered as a drug supplement or as food? And finally, can a guiding regimen be suggested?

Regarding the need to administer calcium for the treatment of osteoporosis, the results of many studies can be used as arguments both in favor and against such administration. In a study of 1,471 postmenopausal women treated with 1 g of calcium citrate a day for 5 years, no reduction in fracture risk was noted, though the bone mineral density increased26. In a series of 208 postmenopausal Afro-American women, the administration of 1,200 mg of calcium a day, with or without 800 IU of vitamin D, did not modify bone mineral density[27]. In a double-blind, placebo-controlled trial with a duration of 5 years, in which 1,460 women over 70 years of age were randomized to 1,200 mg/day of calcium carbonate or placebo, the treatment proved ineffective in preventing clinical fractures, though the authors attributed this result to poor compliance[28]. A double-blind, randomized controlled trial for a 2-year period carried out in 323 healthy men, the administration of 1,200 mg/day of calcium had beneficial effect on bone mineral density comparable with those found in postmenopausal women but a dosage of 600 mg/day was ineffective[29].

The analysis of prospective cohort studies included in a meta-analysis[9] showed that the administration of calcium was not associated with the risk of fracture in males or females, while the analysis of controlled clinical trials showed that the use of calcium supplements did not reduce the risk of hip fracture but rather increased such risk. In the case of nonvertebral fractures, the effect observed in the clinical trials proved neutral. In fact, these and also some other studies[30,31] show that calcium increases bone mineral density in postmenopausal women, but that calcium alone does not reduce the risk of fracture although its combination with vitamin D may be useful. However, although the clinical evidence suggests that calcium supplements do not reduce osteoporotic fracture risk, the data from the meta-analysis of Tang et al.[11], indicate that the administration of calcium supplements alone or in combination with vitamin D is effective in the prevention of osteoporotic fractures (relative risk 0.90, 95% CI 0.80-0.100). The inconsistencies between the studies in favor and against cal-

cium efficacy can be explained by the discrete effect (~ 15%) and variability with respect to the baseline calcium intake (threshold effect)[32], as well as by the coexistence or not of other factors such as the addition of vitamin D[33], intestinal absorption capacity[34] or treatment compliance[21].

Despite the doubts regarding the efficacy of calcium, the clinical trials involving drugs that reduce fracture risk have been carried out contemplating the administration of calcium (plus vitamin D). As a result, the combination of calcium and vitamin D seems adequate as a measure accompanying specific treatment for osteoporosis, and is moreover necessary in individuals with a low dietary consumption (e.g., elderly people, subjects in nursing homes).

Dairy food and calcium intakes have been hypothesized to play roles in cancer. Recently, dairy food and calcium intakes in relation to total cancer as well as cancer at individual sites were examined in the National Institutes of Health (NIH)-AARP Diet and Health Study[35]. During an average of 7 years of follow-up, 36,965 cancer cases in men and 16,605 cancer cases in women were identified. In both men and women, dairy food and calcium intakes up to 1,300 mg/day were associated with a decreased risk of cancers of the digestive system, particularly with colorectal cancer.

Accepting that calcium administration is necessary in an adult with osteoporosis, what amount should be administered? Historically there has been a lack of agreement on this point, due among other reasons to the application of different criteria for assessing the required amount (calcium balance, bone mass and/or PTH levels), and to the fact that the precise amount to be administered depends on the vitamin D status, the degree of absorption (which varies with age), the form of administration, etc. Likewise, in deciding the amount of calcium to be administered, the possible side effects of doses in the upper range must be taken into account (> 1,500 mg/day), including (surprisingly) hip fracture[26,36], as well as myocardial infarction10, renal lithiasis 21 and prostate cancer[37].

In relation to the amount of calcium ingested by the Spanish population, different studies[38,39] indicate that the mean intake in the form of dairy products is in the range of 600 mg. Assuming the additional ingestion of about 300 mg of calcium in the form of non-dairy products (i.e., the rest of the diet), this means that the total daily calcium intake is approximately 900 mg. Accordingly, usually about two-thirds of the daily calcium intake corresponds to dairy products. In any case, some studies have reported lower daily calcium intakes[40,41], while others have reported higher intakes[42,43]. In summary, the mean calcium intake in our country is about 900 mg/day, of which two-thirds correspond to dairy products and the rest to non-dairy products. However, there are important variations, so that each patient requires an individualized evaluation.

As regards the question as to whether calcium administration should be in the form of drug supplements or from food sources, calcium contained in food offers the following advantages: the gastric pH does not interfere with absorption as in the case of the drug supplement; the patient does not have the impression of being medicated, which means a benefit on quality of life; adherence to therapy is probably favored; some nutrients favor its absorption (carbohydrates); and some studies indicate that calcium contained in food exerts a greater effect upon bone mineral density than calcium supplied as drug supplements[44]. In turn, the administration of calcium as drug supplements has other advantages: it is easier to know the precise amount ingested; dose distribution over the course of the day is easier; reaching the required daily amount is also easier; and a lesser ingestion of proteins (milk proteins) is involved. This latter point is important, since excessive proteins increase calcium losses in urine. In this respect, the results of different studies[44,45] appear to exclude the possibility that milk proteins may be deleterious for bone metabolism.

With a view to affording a guiding regimen, the usual intake of a given patient is easy to calculate, and thus it is not difficult to know the calcium increments needed to secure an adequate provision of the element – taking into account that the "basal" diet (without any dairy product consumption) affords an amount of calcium that varies according to the amount of food ingested (in sum, the caloric content), which in turn is very often dependent upon the age and physical activity of the patient. In principle, a daily amount of 300 mg can be estimated for elderly individuals, versus 400 mg for younger people. On the other hand, it can be calculated that a glass of milk without calcium enrichment (skimmed or otherwise) contains about 250 mg of calcium. In comparison, a glass of calcium-enriched milk may contain about 350 mg, versus 125-150 mg in the case of yogurt. Once the amount of calcium ingested by a given patient has been estimated from the corresponding dietary history, the amount to be added as either milk or as a drug supplement can be determined.

A number of practical aspects should be taken into account: a) Since the intestine reduces the percentage of calcium absorbed as the total ingested amount of the element increases, the body is better able to assimilate small calcium doses distributed over the course of the day (e.g., 500 mg every 12 hours) than high doses in the form of a single dose (e.g., 1000 mg once a day); b) If the patient prefers calcium supplements instead of milk, they should be taken with meals (dinner, or lunch and dinner), unless the diet is rich in phytic acid; c) It has been considered (but not demonstrated) that in order to avoid the nocturnal PTH peak, one of the calcium doses should be administered with dinner; and d) If the patient is being treated with proton pump inhibitors and for some reason takes calcium on an empty stomach, the dose preferably should consist of calcium citrate, which requires no acid pH for absorption.

Vitamin D, muscle function and reduction of the risk of falls

Hypovitaminosis D is very common in the general population, particularly among elderly people and subjects with osteoporosis. The postulated underlying causes include a low dietary vitamin D intake, limited exposure to sunlight, reduced cutaneous efficacy in the production of vitamin D, a reduction in kidney active metabolite $1,25(OH)_2D_3$ or calcitriol) conversion capacity, and a certain resistance among elderly osteoporotic individuals to the effects of active vitamin D[46]. The prevalence of low vitamin D levels increases with age, particularly in elderly people confined to their homes or living in nursing homes. This reduction in turn is associated to muscular weakness, loss of bone mass due to secondary hyperparathyroidism, and an increased risk of falls and hip fractures, which are responsible for an elevated morbidity and mortality[47,48]. Many studies have demonstrated an increase in the prevalence of fractures with age, and this tendency can be expected to increase with the gradual aging of the population in the industrialized world[49]. As an example, among the women with the least functional deficit in the Women's Health and Ageing Study, severe hypovitaminosis D increased significantly from 8.3% in the 65-74 years age range to 14% in the 75-84 years interval, and 17.4% for those women aged 85 years or older[50]. The existing scientific evidence supports the importance of correcting vitamin D deficiency by means of a supplement (800 IU/day as a minimum dose) as a strategy to reduce the risk of falls[7,51].

Numerous studies in recent years support the hypothesis that vitamin D deficiency alters muscular function and, therefore, increases the risk of falls, which is particularly relevant in the elderly population. Muscular weakness is a prominent sign of hypovitaminosis D and an important muscular compromise may be present before the appearance of biochemical evidence of bone alterations49. Clinically, muscular weakness associated to hypovitaminosis D is predominantly proximal with loss of muscular mass, hypotonia and pain in response to movements. Histologically, type II muscular fiber atrophy is observed. Such fibers are needed for intense, rapid and short-lasting motor activities, i.e., their correct function is essential for sudden muscle effort such as that in preventing falls. A lack of vitamin D is associated to muscular weakness in a way similar to the situation seen in patients with osteomalacia.

The effects of vitamin D upon skeletal muscle appear to be more related to $1,25(OH)_2D_3$ than to calcitriol or $1,25(OH)_2D_3$. Vitamin D exerts direct action upon skeletal muscle through three different mechanisms: classical genomic action resulting from the binding of $1,25(OH)_2D_3$ to its nuclear receptor, and actions that are non-genomic (rapid) and mediated by a vitamin D receptor at muscle cell membrane level and by allelic variants of the vitamin D receptor (VDR)[46,52]. In this context, vitamin D polymorphisms can affect muscular function, with a difference of 23% in quadriceps strength between VDR genotypes bb and BB in non-obese women over 70 years of age[53].

In a study of the risk factors related to bone health and falls, all patients of both sex and aged over 50 years with clinical fracture seen in the Emergency Service or admitted to Maastricht University Hospital due to clinical fracture in the course of a year were contacted to participate in a systematic risk factors screening program[54]. Bone densitometry was performed in all patients. The study population consisted of 354 females and 101 males (median age of 67 years). The women were compared with a control group of postmenopausal women without fractures. Bone-related risk factors included the following: a history of fracture after 50 years of age, maternal history of fracture, body weight under < 60 kg, severe immobility, corticosteroid treatment, vertebral fracture and more than one bone factor. Regarding the risk of falls, the study considered more than one fall in the last year, the use of psychoactive drugs, low daily life activity levels before the fracture, joint symptoms, vision disorders, urinary incontinence, Parkinson's disease, and more than one fall risk factor. The presence of osteoporosis was defined by a T-score ≤ -2.5 in the lumbar spine and/or femoral neck. The prevalence of fall risk factors was found to be 75%, with a prevalence of bone risk factors of 53%, and a prevalence of osteoporosis at the time of fracture of 35%. In 50% of the patients the bone and fall risk factors were found to overlap. After adjusting for age, body weight and height, women with fractures were seen to have been diagnosed with osteoporosis more often than the controls (*odds ratio* 2.9; 95% CI 2.0-4.1), and had a comparatively more extensive history of falls (*odds ratio* 4.0; 95% CI 2.7-5.9). This study led to the conclusion that the risk factors related to falls in patients over 55 years of age and with recent fractures are greater than the risk as predicted on the basis of their osteoporosis. On the other hand, the risk factors were seen to overlap, were heterogeneous, and were present in multiple combinations. However, findings of this study should be interpreted taking into account the limited number of patients, the lack of laboratory testing, the lack of inclusion of certain bone risk factors, the facts that risk factors were documented during the period of fracture treatment, and that the control group was exclusively formed by women.

In a cross-sectional study conducted in Valladolid (Spain) of elderly individuals living at home, in a nursing home, or admitted to hospital, 454 subjects were evaluated with the purpose of establishing the prevalence of vitamin D deficiency and insufficiency in these three groups[55]. Vitamin D deficiency was defined by 25-hydroxycholecalciferol levels below 10 ng/mL, while insufficiency was defined by levels of less than 20 ng/mL. Serum 25 hydroxycholecalciferol concentration is the best indicator of vitamin D status, since it has a half-life longer than three weeks, and is not subjected to enzyme regulation. The

individuals living at home showed a 79% and 31% prevalence of vitamin D insufficiency and deficiency, respectively. In the case of the patients living in nursing homes and admitted to hospital, these figures were 91% and 32%, and 92% and 52%, respectively. Likewise, the mean serum concentrations of $25(OH)_2D_3$ were 14.8 ± 8 ng/mL, 13.2 ± 6.8 ng/mL and 10.8 ± 5.6 ng/mL in each of these respective groups – these values being far below the threshold of 30 ng/mL recommended for adequate bone health and the reduction of fracture risk. Given the high prevalence of vitamin D deficiency, patients over 65 years of age constitute a fall and fracture risk group due to the muscular weakness associated with hypovitaminosis D. Dietary recommendations are therefore needed to increase ingestion and the use of vitamin D supplements, with a view to correcting this deficit.

The effect of vitamin D upon falls has been examined in a meta-analysis analyzing only randomized, double-blind and controlled trials with an explicit definition of falls, in individuals over 60 years of age[56]. Based on the data from 5 trials with 1,237 participants (81% women, with a mean age of 70 years), the administration of vitamin D reduced the risk of falls by 22% compared with calcium supplementing only or placebo – the number needed to treat (NNT) to avoid a single fall being 15 patients. The inclusion of 5 additional studies with 10,001 patients suggests that the size of the effect is independent of calcium supplementing, the type of vitamin D, patient sex, and the duration of treatment. This meta-analysis allowed the conclusion that vitamin D supplementation reduces the risk of falls by more than 20% in both ambulatory and institutionalized patients.

In a secondary analysis of a randomized, double-blind and controlled trial including 64 institutionalized women aged 65-97 years, an evaluation was made to determine whether vitamin D and calcium supplementation avoided the risk of falls through postural or dynamic balance[57]. Both types of balance were shown to be predictors of the risk of falls, and vitamin D and calcium supplementation was seen to reduce the frequency of falls by 60%, with a 22% involvement of the postural balance and 14% of the dynamic balance. In 242 community-dwelling seniors, supplementation 1000 mg of calcium plus vitamin D resulted in a decrease in the number of subjects with first falls of 27% at month 12 and 39% at month 20 as compared with supplementation with calcium only. Combined calcium and vitamin D supplementation proved superior to calcium alone in reducing the number of falls and improving muscle function in community-dwelling older individuals[58].

Likewise, an analysis has been made of the differences in cost-effectiveness of combined treatment with alendronate 70 mg and vitamin D_3 5,600 IU/week versus no treatment and risedronate 35 mg/week, in the prevention of fractures among postmenopausal women over age 60 years, with a history of vertebral fractures[59]. For this study recently conducted in the Netherlands, data were used from a previous meta-analysis of randomized trials that included vitamin D_3 800 IU/day, alendronate and risedronate, incorporated to a Markov model to evaluate cost-effectiveness in terms of cost per QALY (quality-adjusted life years) gained as a result of the different options. For a 10-year horizon, in comparison with no treatment, combined alendronate and vitamin D treatmentavoided between 13.2 fractures per 100 treated women for the 60 years age segment, and 22.5 fractures for the 80 years age segment. On the other hand, combined treatment with alendronate and vitamin D avoided between 0.6 and 2.6 additional fractures compared with risedronate. It was thus concluded that treatment with alendronate and vitamin D is the economically dominant treatment option versus risedronate in postmenopausal women over 60 years of age with a history of vertebral fracture.

Additional benefits of vitamin D in other disorders

Vitamin D is implicated in a broad range of endocrine and metabolic processes; of these, the maintenance of calcium homeostasis is one of the most important. The vitamin has a dual origin: exogenous when consumed with the diet, and exogenous when ultraviolet radiation in sunlight converts 7-dehydrocholesterol present in the skin to provitamin D. The latter in turn undergoes thermal isomerization and transforms into biologically inert vitamin D that must undergo two hydroxylations – one in the liver to produce 25-OH-D_3 or calcidiol (the serum concentration of which defines the body vitamin D reservoir), and another in the kidney mediated by the enzyme activity of 1-alpha-hydroxylase (CYP27B1) in order to yield the biologically active hormone, $1,25(OH)_2D_3$ or calcitriol.

The biological actions of calcitriol take place through the nuclear receptor for vitamin D (VDR), which is ubiquitously expressed in a great variety of tissues and cells[60,61]. Calcitriol, transported by the vitamin D binding protein (DBP) and probably introduced within the cells by endocytosis, binds to the nuclear VDR and heterodimerizes with other hormone receptors – particularly with the family of retinoid X receptors. This complex binds to DNA sequences known as vitamin D response elements (VDREs) in the promoter regions of the regulated genes. The activated VDR/RXR heterodimers form complexes with an additional series of proteins known as coactivators, to form a bridge in the VDR/RXR complex that joins the VDREs to the proteins responsible for transcription – causing the cellular machinery to start transcription of the respective RNA, and culminating in translation of the protein specifically coded for by it. Thus, VDR acts as a transcription factor which when activated by its ligand (calcitriol) induces a protein synthesis response on the part of genes that are regulated by vitamin D.

VDRs are not restricted to the classical target tissues of vitamin D, such as the intestine, bone, kidneys and parathyroid glands related to calcium

and phosphorus homeostasis, but are found in almost all cells of normal and neoplastic tissues, which explains the great variety of endocrine, paracrine, and autocrine functions of calcitriol within the body. The broad distribution of VDR and of α–1-alpha-hydroxylase (CYP27B1), the enzyme required to convert circulating calcidiol into calcitriol, allows many cell types to form their own calcitriol, provided that an adequate supply of circulating serum calcidiol is available[62].

The effects of calcitriol upon the tissues that contain VDR are pleiotropic and focus much of the current expectations regarding the use of vitamin D and its analogs. Improved knowledge of the different mechanisms of action of vitamin D and the underlying molecular bases in relation to autocrine/paracrine activities, the capacity to control genes associated to innate or acquired immune response, cell growth proliferation and differentiation, the inhibition of angiogenesis and the regulation of apoptosis, as well as the secretion of different hormones, has been crucial for estimating the importance of procuring and maintaining adequate levels of $25(OH)_2D_3$ for the optimal function of many biological processes[63-68].

Approximately 75% of the world population presents low vitamin D levels. This is alarming, particularly when considering the many functions and physiological properties of vitamin D, beyond the acknowledged benefits in relation to bone and mineral metabolism[69-71]. In Spain there is a high prevalence of vitamin D insufficiency in both males and females, regardless of the season of year or the geographical setting – reaching up to 50% for the serum concentration threshold of < 20 ng/mL, and up to 70% for the threshold < 30 ng/mL[1]. Although vitamin D deficiency is important in all stages of life, this high prevalence is particularly relevant in patients with osteoporosis, postmenopausal women, and elderly people. This situation requires the urgent adoption of measures to increase the intake and to correct vitamin D deficiency. The importance of securing a vitamin D supplement is reflected by the results of a meta-analysis of 18 controlled clinical trials involving a total of 57,311 subjects[72]. In these studies, the daily vitamin D dose ranged from 300-2000 IU/day (mean 528 IU/day) and the mean duration of follow-up was 5.7 years. Compared with the control group, the interventional group showed a relative risk of death due to any cause of 0.93 (95% CI 0.87-0.99), although this decrease in risk did not vary according to the concomitant administration of calcium supplements in the interventional group. These results suggest that the ingestion of vitamin D supplements seems to be associated with a reduction in the overall mortality rates.

The implications of the non-classical actions of vitamin D associated to the presence of VDR throughout the body, and to the expression of 1-alpha-hydroxylase in immune cells such as dendritic cells, macrophages, B cells, certain T cell subpopulations and other cell types, particularly through interaction with TLRs (toll-like receptors)[73-75], have broad clinical repercussions regarding the participation of vitamin D in aspects such as innate immunity against infection (e.g., Mycobacterium tuberculosis)[76], immune modulation, reduction in the risk of autoimmune diseases, certain cancers and cardiovascular risk, as well as increased sensitivity and secretion of insulin.

In reference to the therapeutic considerations of vitamin D in autoimmune diseases, studies in different animal models have demonstrated the beneficial effects of vitamin D supplementation in relation to disorders including autoimmune encephalomyelitis, collagen-induced arthritis, type 1 diabetes mellitus, inflammatory bowel disease, and systemic lupus erythematosus[77]. The prevalence of these latter two pathologies is moreover related to solar exposure, and thus to low serum concentrations of vitamin D[77]. In a prospective, nested case-control study of 257 patients with multiple sclerosis matched for age, sex, race and dates of blood sampling for the determination of vitamin D concentrations with two controls, the risk of multiple sclerosis was seen to decrease significantly with increasing vitamin D levels, suggesting that high serum vitamin D levels could be associated to a lesser risk of multiple sclerosis[78]. Recently, a systematic review and meta-analysis of observational studies and case-control studies evaluating the effect of vitamin D supplementation on the risk of developing type 1 diabetes mellitus has shown a risk reduction (*odds ratio* 0.71, 95% CI 0.60-0.84) in the vitamin D-supplemented group versus the group not administered the vitamin. Likewise, a dose-dependent effect was recorded, with greater risk reduction in association with higher vitamin D doses[79]. As regards to type 2 diabetes, hypovitaminosis D is associated with insulin resistance and alpha cell dysfunction[80].

There is also evidence that inadequate vitamin D photosynthesis or an insufficient dietary intake of the vitamin is related to an increased incidence of colon[81,82], breast[83] and prostate cancer[84]. The analysis of the different dose-response gradients derived from a number of observational studies indicates that a vitamin D dose of 1,000 IU/day is associated with a 50% reduction in colorectal cancer compared with a reference dose of 100 IU/day[85]. In the case of breast cancer, it has been reported that a daily intake of 4,000 IU of vitamin D may increase the serum concentrations to 52 ng/mL – this being the threshold associated to a 50% reduction in the incidence of breast cancer (a daily dose of 2,000 IU in turn eliciting a 30% reduction in breast cancer incidence)[82]. Moreover, different studies have reported an increase in cardiovascular risk in situations of moderate or severe hypovitaminosis D[86-88]. This could have important public health implications, given the high prevalence of hypovitaminosis D in industrialized countries, the contribution of life style and geographical setting to vitamin D status, and the safety, simplicity and low cost of treating vitamin D deficiency.

Finally, the association of low 25-hydroxy vitamin D levels with all-cause, cancer, and cardiovas-

cular disease mortality in 13,331 nationally representative adults 20 years or older from the Third National Health and Nutrition Examination Survey (NHANES III) linked mortality files has been recently examined[89]. Participant vitamin D levels were collected from 1988 through 1994, and individuals were passively followed for mortality through 2000. Compared with the highest quartile, being in the lowest quartile (vitamin D levels < 17.8 ng/mL) was associated with a 26% increased rate of all-cause mortality (mortality rate ratio, 1.26; 95% CI, 1.08-1.46). In a prospective cohort study of 3258 consecutive male and female patients[90], quartiles according to 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels were performed. Multivariate-adjusted hazard ratios (HRs) for patients in the lower two 25-hydroxyvitamin D quartiles (median, 7.6 and 13.3 ng/mL) were higher for all-cause mortality (HR = 2.08, 95% CI 1.60-2.70 and HR = 1.53; 95% CI 1.17-2.01, respectively) and for cardiovascular mortality (HR = 2.22, 95% CI 1.57–3.13 and HR = 1.82, 95% CI 1.29-2.58; respectively) compared with patients in the highest 25-hydroxyvitamin D quartile (median 28.4 ng/mL). It is concluded that low 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels are independently associated with all-cause and cardiovascular mortality.

Conclusions

Optimization of the nutritional supply of vitamin D and calcium is the first step in the care of the patient with osteoporosis. Although guidelines for the prevention and treatment of osteoporosis include recommendations regarding calcium and vitamin D intake, the use of an adequate supplementation is frequently deficient in daily practice. In this respect, it is essential to increase awareness among physicians and educational measures for patients regarding the important role of vitamin D and calcium in terms of bone health[91,92]. Based on a review of the current evidence-based literature, the experts participating in the round table discussions in Madrid reached consensus on the following points:

- Oral aminobisphosphonates, alendronate and risedronate, are the treatment of choice for osteoporosis according to the proven efficacy of these agents in vertebral, nonvertebral, and hip fractures.
- The scientific evidence regarding the impact of vitamin D deficiency upon vertebral and nonvertebral fracture risk is of lesser quality and less conclusive than the evidence related to treatment with alendronate. The adequate vitamin D dose could be defined as 800 IU/day for healthy adults and as 1000 IU/day for osteoporotic patients, in order to secure the optimum cut-off serum concentration of 20–30 ng/mL.
- Despite uncertainty regarding the efficacy of calcium *per se* for the reduction of the risk of fracture, specific treatment of osteoporosis should be accompanied by the administration of calcium and vitamin D.
- The mean calcium ingestion in Spain is about 900 mg/day, of which two-thirds correspond to dairy products and the rest to non-dairy products. Since there are important differences, each patient must be analyzed individually.
- In principle, it appears preferable to ingest the calcium in the form of food (mainly milk). However, in the event of difficulty in securing the required intake, drug supplementation should be used.
- The amount of calcium intake is 1,000-12,00 mg/day. This means two glasses of milk a day and some yogurt, although if only part of this amount of dairy products is ingested, a calcium tablet should be taken. If the patient does not consume milk, two calcium tablets should be administered (one dose being required at bedtime). The calcium should be taken with food, except when the latter is rich in oxalate or phytic acid, in which case it is advisable for calcium administration to be independent of food.
- The etiology of osteoporotic fractures is multifactorial, and risk factors for bone fragility and falls are equally relevant as causative factors as the reduction in bone mineral density. People over 65 years of age show a high prevalence of vitamin D deficiency. Hypovitaminosis D is associated with muscular weakness, and correction of the deficiency state improves muscular muscle strength. The correction of vitamin D deficiency reduces the risk of falls (minimum dose 800 IU/day). Although calcium supplementation could be secured through the diet, drug administration is required in the case of vitamin D supplementation. Vitamin D supplementation does not exclude the intervention on other factors that may influence the risk of falls.
- In reference to good bone health, the required daily dose of vitamin D is at least 800-1,200 IU in order to ensure adequate $25(OH)_2D_3$ levels. However, the dose required to maintain an optimum $25(OH)_2D_3$ concentration to allow the correct function of the remaining extraskeletal actions of the vitamin is unknown, but the current evidence suggests that the doses required may be higher. Multidiscipline efforts are needed to define the doses and levels of vitamin D necessary to reduce the risk of diseases linked to the noncalcemic actions of the vitamin.

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In the name of the components of the 1st Multidisciplinary Forum on the Management of Patients with High Risk of Fracture*

Consensual conclusions from the 1st Multidisciplinary Forum on the Management of Patients with High Risk of Fracture (HRF) due to Osteoporosis

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Summary
On the 12th and 13th of February this year the first Forum on High Risk of Fracture was held in Madrid, coordinated by Prof. Díaz Curiel under the auspices of SEIOMM, and with the sponsorship of Nycomed. Around 100 specialists in rheumatology, traumatology, rehabilitation, geriatrics, units of bone metabolism, internal medicine and endocrinology discussed, from a multidisciplinary perspective, the presentations prepared by the group coordinators based on the review of data published and having been previously discussed in two meetings by the members of the scientific committee.
With the difficulties consequent to tackling such a complex theme, a consensus document was developed to reflect the clinical and multidisciplinary reality of the concept of "high risk of osteoporotic fracture". An extract of this document is presented here in summary, with the aim of bringing together the views from the different specialisms involved in the management of disease in this type of at risk patient in our country.

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Introduction

In recent decades there has been a significant advance in knowledge related to osteoporosis, the fruits of which are, amongst other things, the definition operative since the year 2000 of the Consensus Conference of the National Institute of Health in the United States[1], which defines osteoporosis as a skeletal disease characterised by reduced bone resistance which provokes an increase in the risk of fracture. This definition already makes it clear that that low bone density, on the basis of which the disease has come to be diagnosed, is only one of multiple risk factors which are associated with the development of osteoporotic fractures. This allows us to explain that fractures may appear in subjects without densitometric criteria for osteoporosis and, inversely, that many patients with densitometric criteria do not suffer fractures.

Among these risk factors for fracture, female sex, old age, thinness, the presence of previous vertebral or non-vertebral fractures, low bone mass or the presence of diseases or treatments adverse to bone (rheumatoid arthritis or treatment with corticoids for example) have consistently been identified in different studies, systematic reviews and guides to clinical practice[1-11]. This has allowed the development of models which integrate information relating to different independent risk factors for the development of osteoporotic fractures with those which calculate the absolute risk of fracture in the following few years[10,11]. This information on the absolute risk of fracture in the following 5 or 10 years has received significant criticism due to its imprecision in some populations but, in any case it implies an advance at a time when giving an absolute value is much more informative for patients and doctors who are not experts in osteoporosis than concepts such as the T-score, the risk gradient or relative risk. These formulae also allow the calculation of thresholds for specific diagnostic interventions – for example a request for densitometry – or therapies – to start a specific treatment – which make them cost-effective.

According to recent studies, it is estimated that in our country there are at present 2,500,000 osteoporotic women and 500,000 osteoporotic men, accounting for 90,000 hip fractures, 500,000 vertebral fractures and 150,000 Colles fractures annually. The estimated costs, solely in the hospital environment, exceeds 120 million euros. What is particularly notable is the fact that only 15% of women who have osteoporosis in Spain are being treated, or that almost 50% of people who suffered hip or wrist fracture did not receive any anti-osteoporotic treatment after that fracture. It is necessary therefore to define some risk profiles with which are associated a higher possibility of presenting osteoporotic fractures.

Material and method

On the 12th and 13th of February of this year the first Forum on High Risk of Fracture was held in Madrid under the auspices of SEOIMM, and sponsored by Nycomed. Some 100 experts in rheumatology, traumatology, rehabilitation, geriatrics, bone metabolism units, internal medicine and endocrinology, discussed, from a multidisciplinary perspective, the risk profiles according to each of their specialisms. The members of the scientific committee, in two earlier meetings in previous months, had discussed both the objectives of the Forum – the identification of profiles for high risk of fracture (HRF) – the methodology to be used – review of published evidence – and the introductory presentations which were made in the first part of the Forum.

After the presentations from the group co-ordinators those attending the forum were grouped according to their areas of work: traumatology, rheumatology, rehabilitation and geriatrics and bone metabolism units, where they discussed the evidence presented and reached a consensus within each group. Finally, back in a general session, the agreements reached in each of the groups were discussed and a general consensus reached, which is presented in this article.

Profiles for high risk from the working groups

Traumatology and orthopaedic surgery

The group of experts in orthopaedic surgery and traumatology indicated that although a clear definition of the exact profile and standard for an HRF patient, it would be possible to identify these people satisfactorily in daily clinical practice. It was proposed that the risk factors be stratified into two main groups: key factors (age, life experience, previous osteoporotic fracture and bone mass) and significant factors (independent of bone mass, concentration of vitamin D and falls). An age of 70 years was taken as the cut-off point for which the risk of fracture in the population was clearly raised in the cohorts evaluated. The presence of an earlier fracture is, evidently, one of the factors with most weight in the daily activity of this specialism. What happens also is that the risk of re-fracture after an osteoporotic fracture is not only raised but that this risk almost immediate with subsequent fractures happening in the first few months after the aforementioned fracture. The members of the group established that the presence of ≥ 2 vertebral fractures, or ≥ 2 non-vertebral fractures would be supposed to be HRF. Also considered to be HRF are those subjects with fracture of the hip due to their raised risk of re-fracture. In terms of bone mass, it is estimated that a bone mineral density (BMD) expressed as a T-score lower than -3 in the hip also indicates HRF. Another factor noted by this group was vitamin D deficiency, with those patients with insufficient vitamin D (25(OH) < 30 ng/ml) older than 70 years of age with more than one vertebral facture and/or more than one non-vertebral fracture also considered to have HRF. Lastly, the importance of falls (which trigger 90% of fractures of the hip) was also noted. The profiles from this group of HRF patients are shown in Table 1.

Table 1. Profile of High Risk of Fracture (HRF). Traumatology and Orthopaedic Surgery Group

In patients with previous fracture. One or more of:
• Presence of ≥ 2 vertebral fractures • Presence of ≥ 2 non-vertebral fractures which increase the risk of subsequent osteoporotic fractures • Presence of one vertebral fracture and one non-vertebral fracture which increases the risk of subsequent osteoporotic fractures • Existence of a hip fracture • Presence of one vertebral fracture or one non-vertebral fracture which increases the risk of subsequent osteoporotic fractures subsequently associated with reduction in the standard deviation in the T-score for the hip with respect to controls of the same age and sex in patients >70 years. • A patient with fragility fracture and a BMD in the hip < -3 SD should be considered to be HRF.
In patients with rheumatoid arthritis. One of more of:
• Postmenopausal women (especially those over 65 years of age) • BMD similar to that for risk of postmenopausal osteoporosis • Treated with corticoids at doses higher than 15 mg/day • High disability index • Extended illness • Little physical activity
In patients without a previous fracture. Two of more of:
• Age > 70 years • BMD in hip < -3 SD • There is more than one major risk factor (parental history of osteoporotic fracture, rheumatoid arthritis, consumption of corticoids at doses > 7.5 mg/day for more than 3 months, early menopause) • Vitamin D deficiency (< 30 ng/ml) • Falls: should only be taken into account as a factor triggering an osteoporotic fracture, and not as an intrinsic defining factor for HRF.

They also gave a positive evaluation to the new tools or systems of evaluation (FRAX®, Fracture Index®, FRAMO® and Q-Fracture®) which help the clinician to combine, qualify and quantify this risk factors.

On the management of patients with high risk of fracture, four types of measures to be taken were suggested:

1. Correction of modifiable risk factors.
2. Establishment of non-pharmacological measures (ensuring a sufficient protein-calorific supply, supply of calcium, repletion of levels of vitamin D).
3. Take pharmacological measures (with biphosphonates and/or PTH or its anabolic fragment with non-vertebral anti-fractural efficacy; sequential treatment with PTH or its anabolic fragment + maintenance of antiresorptive drug with non-vertebral anti-fractural efficacy .
4. Adoption of measures to prevent falls.

Rheumatology

This group of experts has classified the risk factors which are associated in a way that is most consistent with an increase in fractures in their patients into three groups: key (over 70 years of age; previous history of fragility fractures of the hip or vertebrae; intake of glucocorticoids ≥ 7.5 mg/day for three or more months and BMD (T-score) < -3; significant (maternal history of hip fracture; low body mass index (BMI < 20kg/m^2), frequent falls in elderly people, low measures of physical activity and functions); and moderate (levels of 25 (OH) vitamin D < 30 ng/ml, some harmful factors related to lifestyle (smoking, excessive consumption of alcohol, sedentary lifestyle or excessive consumption of coffee)). Their consensual proposals for the evaluation of HRF on the basis of age and clinical risk factors are listed in Table 2.

The group indicated also the importance of taking a clinical history , carrying out a complete

physical examination and a basic study, to discount secondary causes of osteoporosis (haematochemistry with creatinine, calcium, phosphorus, alkaline phosphatase, PTH, TSH, free T4, 25(OH) vitamin D and urinary calcium), in addition to the measurement of BMD and a lateral spinal X-ray. The members of the group also stressed the usefulness of algorithms which estimate the individual risk of osteoporotic fracture such as the aforementioned FRAX® or QFracture®.

One of the most significant contributions of this group has been the identification of sub-groups of rheumatology patients with HRF due to the presence of diseases of their specialism which result in osteoporotic HRF, of which rheumatoid arthritis is the most significant. Finally, the members of this group propose the establishment of recommendations regarding indications for pharmacological treatment in accord with the evaluation of risk; in addition, they indicate that in patients at high risk of vertebral or hip fracture it is necessary to start drug treatment straight away with no delay being justified. In each patient it is necessary to identify and try to correct, if possible, risk and co-morbidity factors involved in osteoporosis, and to give general health promotion advice, to recommend supplements of calcium (1,500 mg/day) and vitamin D (800 UI/day), in addition to the specific treatment: in patients without fractures biphosphonates or anabolics (PTH or teriparatide (TRPT)) may be considered; in patients with anabolic fractures, PTH/TRPT preferably followed by biphosphonates.

Rehabilitation and Geriatrics

The members of this group stressed the absence of a national or international consensus which would allow a current definition of the concept of HRF. However, they indicate that the criteria set by Hamman and Lane, which identify as patients with HRF those which meet at least one of the following conditions: presence of previous osteoporotic fractures, accumulation of multiple risk factors for fractures (chronic secondary causes of osteoporosis, general fragility, history of osteoporotic fractures or high risk of falls due to physical limitations), failure (measured by means of two criteria: appearance of fractures during treatment or loss of BMD) or intolerance to earlier treatment, notable on the part of these specialisms being advanced age, skeletal factors, previous fragility fracture, falls, low body mass index, treatment corticoids, and physical inactivity.

The members of this group also identified, within their usual clinical practice, the presence of some diseases or conditions in which patients with HRF can be indentified. Notable among these are subjects with neurogenic osteoporosis: medullar lesion, ictus, multiple sclerosis, cranial-encephalic trauma and Parkinson's disease. They also suggested that subjects with three or more falls in the last year be subsidiaries to be studied in order to discount the presence of osteoporosis. It was also highlighted by members of this group that a significant portion of their patients were found already to be in secondary or tertiary prevention due to having suffered fractures and their side effects; However, this group also recognised the advantage which the use of some scales for the calculation of risk of osteoporotic fractures, such as FRAX® and OST-T, even with their limitations, could bring.

Bone Metabolism Units

The Bone Metabolism Units are characterised by their specialisation and their multidisciplinary nature, in dealing with osteoporosis and the prevention of fractures. This group showed that nowadays it is not possible to define unequivocally, unarguably and reproducibly what is high risk of fracture, because it is difficult to establish a hierarchy and specific weight for the very diverse known determinants of the risk of fracture (Figure 1).

The main limitation to defining HRF arises from the difficulty of establishing what is high risk of fracture. Even the different reference guides (such as those of SEIOMM, NICE or NOF) do not manage to fix on one definition of HRF. And it is the fact that the mechanism of producing fractures is multifactorial which reduces considerably the possibilities of defining HRF, given that it is not known how many of these risk factors, and to what degree, are necessary to determine high risk of fracture.

This group cited various scales of transversal risk most commonly used such as Fracture Index®, OST®, FRAMO®, NOF®, ORAI®, SCORE®, ABONE® and more recently, FRAX® or Q-fracture®. Regarding FRAX®, it is indicated that while it presents a promising and sensible approach to dealing with the problem, it is inexact, with clear methodological limitations (not recording falls, and factors such as the degree of consumption of tobacco or alcohol); in addition, the cut-off point is arbitrary and is not based on scientific evidence, is independent in each country, and establishes an inevitable cost/benefit association. In fact in daily practice, the FRAX® scale is used frequently to suspend treatment in patients with low risk. In relation to QFracture®, it is suggested this scale also has inexactitudes and limitations, given that it does not record previous fractures, nor does it take into account family history; in the same way, an absence of a cut-off point is detected, which is also arbitrary and different for each country.

The Bone Metabolism and Internal Medicine Group also said that the only objective estimation of high risk which has been published is for a specific database (that used for QFracture®), which situates the 90th percentile of absolute risk of fracture of this cohort at 8.75% at 10 years in women and 2.11% in men, but there are no data which guarantee the applicability of these findings to Spain or to other countries. However, it was recognised that we all have a subjective perception of what it is to be "high risk", and it is the case that the risk factors which lead to this concept are perfectly well established, their relative risk values being well known. What is more, it is assumed that the existing risk scales, especially FRAX® and Qfracture®, are useful tools to be used in a contexts

Table 2. Profile of High Risk of Fracture (HRF). Rheumatology Group

Factors associated with HRF:
• Advanced age > 70 years • Previous fragility fracture (symptomatic or asymptomatic) • Low BMD < 3 SD • Maternal history of hip fracture • Taking of corticoids (≥ 7.5 mg/day for more than 3 months) • Low weight (BMI < 19 kg/m^2)
Special and common situations in rheumatology patients
• Chronic inflammatory diseases with persistent activity • Rheumatic polymyalgia and/or giant cell arterisis • Transplant (distinguishing between pretransplant and postransplant) • Frequent use of treatments which induce osteoporosis
HRF in patients with rheumatological diseases
Steroidal osteoporosis: • Daily dose of corticoids higher than 15 mg • Period of treatment longer than one year **Rheumatoid arthritis:** • Postmenopausal women (especially those over 65 years of age) • BMD similar to that for risk postmenopausal osteoporosis • Treated with corticoids at doses higher than 15 mg/day • High disability index • Extended disease • Little physical activity **Ankylosing spondylitis** • Patient with a disease of more than10 years standing • Male > 30 years, treated with corticoids • Acute loss of BMD in the first 5 years, with an extended disease • Episodes of lumbago in the last 6 months • Associated inflammatory intestinal disease **Systemic erimatose lupus:** • Postmenopausal woman, with a longstanding disease • Started after the age of 30 • Little exposure to the sun • Use of sun filter, • Low BMD in hip **Systemic schlerosis:** • Age > 50 years • Woman • Early menopause • Body mass index < 25 • Use of systemic corticoids **Rheumatic polymyalgia/Giant cell arterisis** • Age > 60 years • Functional limitation (little physical activity) • Use of systemic corticoids (high accumulated dose) • Loss of strength • Reduced BMD

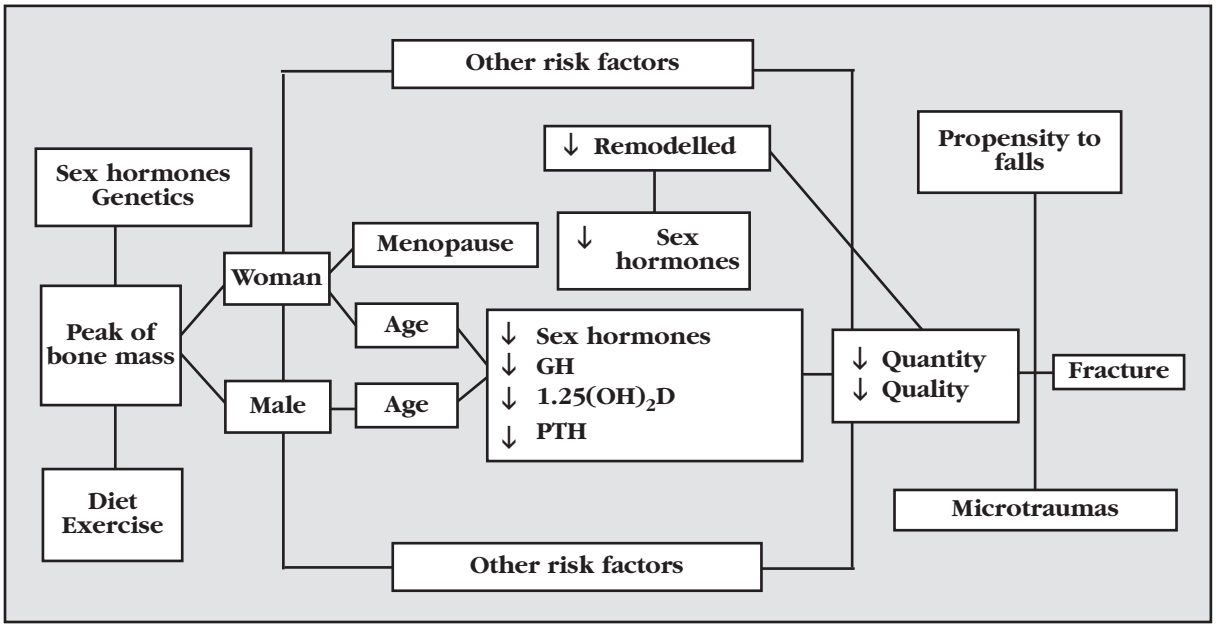
Table 3. Profile for High Risk of Fracture (HRF). Geriatrics Group

Factors associated with HRF: The greater the number of factors the higher the risk:
1. Age: given that the patients are older than 75 years of age by definition, and in practice, almost all are older than 80 years of age, this is a risk factor which is always present in our daily clinical practice, which means that any other factors which we indicate are associated with this one. 2. Low bone mineral density (BMD): given point 1, a T-score < -2,5 in the hip is an indicator of a patient with HRF. 3. Previous fragility fracture, vertebral (clinical or radiological) or non-vertebral (above all, hip). 4. Treatment with steroids at doses higher than 7.5 mg, for more than 3 months. 5. Older people with falls: numerous factors related to falls are associated with risk of fracture: repeat falls, changes in the neuromuscular function of lower limbs (MMII) (an Up and go test and a test of walking speed are proposed for its evaluation), changes in balance and poor vision. In these patients the presence of bone fragility should be assessed, since the risk of fracture is much greater when falls are associated with osteoporosis (carry out a lateral dorsolumbar spinal X-ray, and densitometry, if necessary). 6. Fragile older people: These are older people at risk from falls and osteoporosis. They are very frequently seen in the daily practice of geriatrics. They are patients with low weight, malnutrition, sarcopenia, instability, easily fatigued, inactive. Patients with these characteristics should be evaluated in relation to the risk of fracture and taking into account the general situation of the patient, in order to propose which diagnostic tests and which therapeutic approach is most appropriate. 7. Other risk factors: frequent pathologies in geriatrics which are also associated with HRF: • Dementia, above all, moderate stages with wandering and other conduct disorders. • Longstanding Parkinson's disease • Patients with ictus There are also drug treatments which appear to be associated with falls or which promote bone fragility: • Anticomicials, above all phenytoin, primidone • Long-acting benzodiazepines • Neuroleptics
Special and common situations in rehabilitation and geriatry patients
1. Medular lesion: (above all in the first two years). 2. Cerebrovascular accident: shows a specific osteoporotic pattern, predominantly on the hemiplegic side and more intense in higher limbs. Many risk factors for falls concur (73% suffer at least one fall in the first 6 months after an ictus) and are subject to treatment which could lead to an increased risk of fractures (anticoagulants, anticomicial drugs). Early treatment is advised. 3. Multiple sclerosis: it should be considered in itself as an independent risk factor for the development of osteoporosis, in addition to being associated with other risk factors such as immobility, an increase in number of falls, the continual use of corticoids and vitamin D deficiency. 4. Parkinson's disease: The risk of hip fracture is 5 to 10 times greater (this being more significant from the fifth year after diagnosis). Patients with osteoporosis and Parkinson of ≥ 6 years standing with high risk of falls and low BMI may be considered as having HRF. 5. Patients with cranial-encephalic trauma: they show a high risk of fracture, usually accumulating numerous factors which predispose them to loss of bone mass and falls. 6. Patients with amputation of lower limbs: with many factors which facilitate loss of bone mass and lead to falls, although it has not yet been possible to define what is their HRF profile. 7. Older people: in general they show a higher incidence and concurrence of risk factors for low BMD and falls, associated with an increase in the rate of hip fractures, such as advanced age, previous fragility fractures, radiological evidence of vertebral deformity, reduced height or thoracic kyphosis, low BMI, falls, pluripathology (including disease with high risk of fractures, like ictus), the use of drug treatments which carry risk (steroids, anticomicials), insufficient levels of vitamin D, insufficient intake calcium, fragility and reduction in physical activity. 8. Fragile older people: the presence of at least 3 out of 5 of the following factors: weight loss (> 4.5 kg in one year), subjective tiredness, weakness, low walking speed and low physical activity. They have higher risk of hip fractures and of falls. If there is osteoporosis confirmed by densitometry, drug treatment should be initiated.

Table 4. Profile of High Risk of Fracture (HRF). Bone Metabolism Units Group

Highly relevant, first level:
• Age > 70 years • BMD (T-score) in femoral neck < -3 • Previous existence of at least 2 vertebral fractures or 1 hip fracture
Relevant, second level:
• More than 2-3 falls a year • Use of oral corticoids at doses of 7.5 mg/day, for at least 3 months • BMI < 19 kg/m^2 • Family history of hip fractures • Consumption of tobacco > 10 cigarettes/day

Figure 1. Factors involved in the development of osteoporotic fractures



of global and individual evaluation of each patient. In any case, a very low BMD (T-score < -3) can help determine a high risk of fracture, but only provided it is associated with other variables of increased risk such as age > 70 years, intake of corticoids at a dose > 7.5 mg/day, early menopause, frequent falls, previous existence of at least one vertebral fracture, family history of fractures,...

Conclusions

For doctors involved in the diagnosis and treatment of osteoporosis it is absolutely necessary, and a priority, to determine those patients who have the highest risk of fractures, given that such an occurrence has serious clinical and socioeconomic repercussions. Starting from this reality, this forum has attempted to make an approach to the problem of defining HRF, with the intention of marking a starting point which allows, at least, the definition of the essentials which this area of research should cover in the next few years. In any case, to be able to count in this forum on rheumatologists, traumatologists, doctors of internal medicine, rehabilitators, geriatricians and bone specialists to share their perceptions is already in itself a success. In conclusion, although with the data available it is not possible to define from risk factors a profile for high risk of fracture, advanced age, personal and family history of fractures, as well as very low bone mass, among others, contribute significantly to this increase risk, although each specialism involved in the management of the pathology has a different view as a function of the characteristics of the patients who visit them

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Codification of hip fractures

To the Director:

The fracture of the proximal extremity of the femur, known also as a hip fracture, constitutes the most serious clinical complication of osteoporosis[1], leading to an increase both in the morbidity and the mortality of the patients who suffer it[2-4]. Practically all hip fractures are admitted to hospital, where they are mostly dealt with by surgical intervention[5]. For many years, in all the hospitals in our country, a system of coding for diseases is applied, based on the international classification of diseases, or ICD • 9 •[6]. These codes are applied both in the clinical history and in the databases of hospital archives

It might be thought that the collection of epidemiological data on hip fractures is simple, since by practically all cases being admitted to hospitals, they would be easily identifiable[7]. However, we believe that in reality this is not the case, and that it is possible that we are losing information on the true prevalence both of fractures of the hip and on osteoporosis and vertebral fractures, because the current coding allows many options.

Thus, for example, with a fracture of the femoral neck in an older woman of 80 years who slips and falls on the floor, if the slip is recorded as the cause of the fall, it is coded as E885.9, and if it is not specified how the fracture was produced, the code E887, "fracture with no specified cause", is used. However, if the practitioner considers that the fracture is the consequence of osteoporosis, the same fracture could be coded as 733.14, "pathological fracture of the femoral neck", having to be preceded by the code 733.00, which corresponds to "non-specific osteoporosis". We see, therefore, that the same fracture can be coded in three different ways, all correct.

The same confusion can be observed in the case of vertebral fracture, which again can be coded as 733.00 ("osteoporosis") and then as 733.13, "pathological fracture of vertebrae". However, if the clinician indicates only a vertebral fracture and does not specify the existence of osteoporosis, the corresponding code is 805.8 ("non-specified vertebral fracture, closed"). To complete the confusion, it can also be coded as 733.00, and, therefore be considered as "osteoporosis" when the clinical report has the terms "thinned vertebra", "cuneiform degeneration of the vertebra", or "cuneiform vertebra", which would possibly be better termed as vertebral fractures. Finally, if the diagnosis is given as "osteoporosis" it can be coded as 733.00 ("non-specified osteoporosis"), 733.01 ("senile osteoporosis"), 733.02 ("idiopathic osteoporosis"), 733.03 ("osteoporosis due to disuse") and 733.09 ("other").

In this letter to the Director, we would like to bring attention to the fact that there probably exist very many ways of coding both osteoporosis and fragility fractures. And this makes us reflect that, at the time of carrying out an epidemiological study on any of these processes in a hospital environment, it is necessary to take into account each and every one of the existing possibilities for coding these processes, because it is certain that, with any other approach, cases would be lost.

Finally, as a result of these comments, we put a proposal to try to unite clinicians in ensuring that the diagnostic expression used reaches the highest possible level of specificity, so that, in coding the clinical histories for both hip and vertebral fractures, we can achieve the single, most precise and specific code, from among those that exist.

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Canarian Working Group on Osteoporosis

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Revista de Osteoporosis y Metabolismo Mineral is the official scientific organ of the Spanish Society for Bone and Mineral Metabolism Research (SEIOMM). It will publish scientific articles in this field in two languages, Spanish and English, every four months, with the third issue each year consisting of a monographic edition bringing together the material presented at the annual conference of SEIOMM. In addition, supplements of a monographic nature may also be published.

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-All works to be submitted in A4 format
-Font: Arial
-Font size: 12 point
-30 lines per page (1.5 spaced)
-The first page should consist of: title of work; names and surname(s) of author or authors, and the work place of each of them; contact details of the author responsible for correspondence: complete address with post code, telephone number, e-mail address and fax.

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1. Original articles: These should be works of research on themes related to bone mineral metabolism in whatever form: basic research, epidemiological studies, clinical studies… etc. On the first page should be shown the authors' names and surname(s) and the place of work of each of them, and name and contact details of the author who is responsible for correspondence: complete address with post code, telephone number, e-mail address and fax. It is advisable that the number of authors should not be greater than 6. Next should be presented a summary, which should occupy a maximum of one page and be structured in the following sections: Background, Material and Method, Results, and Conclusions. A list of key words should follow. The number of tables and figures, combined, should be fewer than 6. It is not necessary to present the summary in English as the Journal has a translation service. The maximum number of pages may not exceed 20, including the bibliography, tables and figures. It is advisable that the number of bibliographical citations not exceed 30.

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