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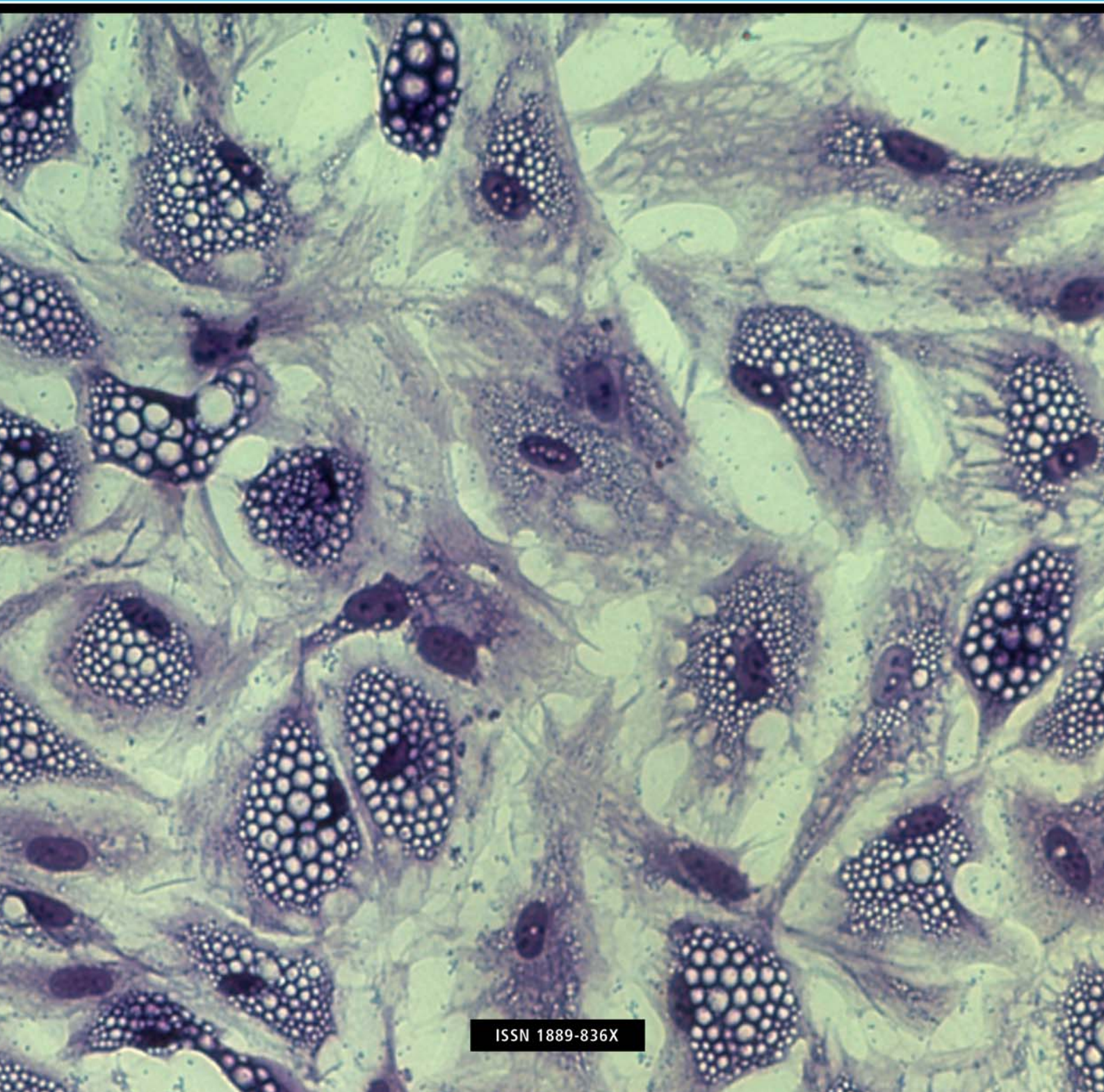

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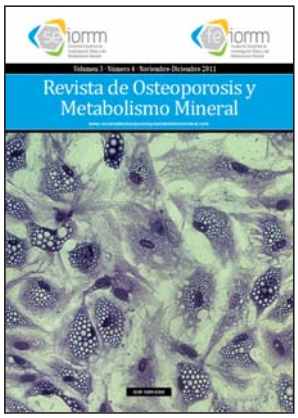

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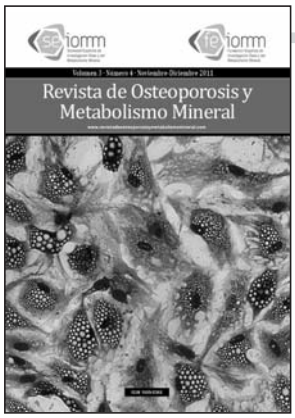

Our cover

Adipocyte differentiation from mesenchymal stem cells isolated from human bone marrow

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Isoflavones and bone

Cano A

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The appearance of scales for the prediction of the absolute risk of fragility fracture and the consequent definition of thresholds for pharmacological intervention has significantly limited the number of women eligible for treatment among those who are in their first years of the menopause. What is certain is the deterioration that many of them suffer in terms of bone metabolism as a consequence of rapid hypogonadism, but there are no defined strategies for the use of drugs to limit this phenomenon. In its day, hormone therapy solved this problem, but its limitations to use in women with symptoms sufficient to affect quality of life has left many users without an efficacious option. It is true that life style changes, especially diet and exercise, alleviate the problem, but they are not an entirely satisfactory solution. The advances which are being made in the action mechanism of plant extracts, both in the form of pure molecules prepared to the equal quality of medicines, or foods in which they are found in sufficient concentrations (functional foods), are raising new expectations. There has been significant progress in the knowledge of the molecular mechanisms of many of these substances, especially the isoflavones. Although there are differences between their components, we know that they are capable of activating estrogen receptors, particularly isoform β, and that this is followed by the activation of different signalling pathways in various experimental models, essentially cellular. The fundamental question, however, is what is their true clinical significance.

On this point the evidence is more limited and to date, still confusing. On the one hand is the unfinished business of the symptoms, where there are few clinical studies of quality, and those that there are present difficulties derived from their inclusion of groups with low numbers of participants or of other methodological drawbacks. On the other, there is the question of their eventual efficacy in limiting chronic diseases which more or less clearly have their roots in hypogonadism, such as cardiovascular disease or osteoporosis. The questions in relation to the former have recently been reviewed[1], and with respect to the latter, particularly welcome is the article by García-Martín et al. in this issue[2]. With a control group and with a randomised double blind design, the authors conclude that after a year of follow up the supplementation with 50 mg/day of isoflavones improves the bone parameters evaluated by ultrasound. There are favourable changes overall in some of the markers for bone metabolism evaluated, although without differences between the two groups. Perhaps the inclusion of a high number of cases would have revealed the suggested advantage of the isoflavones. It is curious that the intervention is associated with a decrease in osteoprotegerin (OPG). This finding is contrary to that published by other groups[3], and *a priori*, is in opposition to the protection seen in the ultrasound parameters. Therefore, what cannot be discounted is that this data has even greater value, given the highly varied provenance of the OPG, and of its growing value as biomarker for cardiovascular disease, as the same group has just well reviewed[4].

A literature review regarding the actions of the isoflavones in bone, however, shows that were are dealing with an area in which there are significant discrepancies. For example, a recent clinical trial

did not find a protective effect on the bone in women who took tablets containing 200 mg of isoflavones for two years[5], and a meta-analysis which examined the action on bone mineral density came to similar conclusions[6]. However, another meta-analysis found there to be protection, albeit reduced[7]. Also, with regard to biochemical markers for bone metabolism, a recent meta-analysis found a slight protective action in relation to resorption[8]. Finally, there is very little information on the effect on ultrasound parameters, and again, in this, the value of the García-Martín study should be highlighted.

How to cast some light on this apparently tricky matter? Evidently, more clinical research is required, but this does not seem to be a simple task due to a series of conditions particular to these types of preparations.

On the one hand, is the great variety of molecules and the differences between their effects, including the metabolic capacities of the individual, which is not the same between, for example, the isoflavones genistein and biochanin A. And in terms of individual metabolisation, it is also important to note that equol, a metabolite of daidzein, is generated by the action of intestinal flora, but only in certain individuals. There are no exact figures, but it is calculated that between 35% and 50% of individuals are capable of producing it. This adds an important factor to the variability of the results of therapeutic actions, given that equol is considered to be one of the most powerful isoflavones. In this area, it would have been useful if the García-Martín[2] study had included details of the mixture of isoflavones used.

But on the other hand, there is the response threshold. A meta-analysis which examined the action on vasomotor symptoms found a clear dose-dependent action in a period which reached up to 160 mg/day of isoflavones, with a threshold of acceptability of approximately 80 mg/day[9]. There are also differences between purified isoflavones and soya protein, at least in the matter of cardiovascular protection, as has been demonstrated in the analysis of the American Heart Association[10].

In conclusion, therefore, this is a promising field, but one in which order needs to be imposed. What needs to be clarified is what isoflavones should be used, purified or not, at what dose and, probably, what type of user will obtain, or not, some protective effect. Studies such as that of García Martín are particularly welcome, given their good design which contributes to the accumulation of more evidence.

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Changes in bone metabolism markers and ultrasound parameters in postmenopausal women induced by soy isoflavones

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Summary

Introduction: the results of the works published on the role of isoflavones in the prevention of postmenopausal osteoporosis are contradictory. The objective of our study is to evaluate the effects of nutritional intervention with a milk product enriched with soy isoflavones on bone metabolism in Spanish postmenopausal women.

Subjects and methods: a randomised controlled double blind trial was carried out in 99 postmenopausal women who were allocated to two groups: group S (n=48), with a consumption of a milk product enriched with soy isoflavones (50mg/day), and group C (n=51), with a consumption of a control milk product over 12 months. Hormone parameters and markers for bone metabolism were assessed at the baseline and at one year. Ultrasound of the calcaneum (QUS, Hologic Sahara®, North Carolina, US.) was used as the evaluation tool for bone mass.

Results: at 12 months, a decrease in blood levels of tartrate-resistant acid phosphatase and osteoprotegerin occurred (2.18 ± 0.8 vs 1.76 ± 0.54 U/l, p<0.001, and 5.21 ± 3.36 vs 3.89 ± 1.47 pmol/L, p=0.007, respectively), as well as an increase in 25-OH-vitamin D (24.48 ± 9.85 vs 28.18 ± 10.45 ng/ml, p<0.001) with no differences between the groups. There were no significant changes in hormone parameters and the rest of the bone markers. In terms of the QUS, in the total sample there was an increase in the sound velocity [SOS] (1517.86 ± 38.13 vs 1525.11 ± 35.6 m/s, p=0.036), QUI (76.37 ±19.87 vs 80.82 ± 18.26, p=0.012), estimated bone mineral density [Est. BMD] (0.408 ± 0.13 vs 0.435 ± 0.12 g/cm², p=0.013) and T-score (-1.55 ± 1.12 vs -1.31 ± 1.03, p=0.019). In group S, positive changes occurred in QUI (74.37 ± 18.87 vs 78.83 ± 13.68, p=0.032) and Est. BMD (0.397 ± 0.12 vs 0.423 ± 0.09 g/cm², p=0.04), whilst in group C there were no significant differences.

Conclusions: the daily consumption of these milk products increases levels of 25-OH-vitamin D and results in a decrease in markers for bone metabolism. A diet rich in soy isoflavones may be an option as a preventative measure against the effects of the menopause on bone.

Key words: *soy isoflavones, bone metabolism, postmenopausal.*

Introduction

The post- and peri-menopausal periods are a physiological state characterised by the cessation of ovarian hormonal secretion, leading to significant physiological and psychosocial changes in the lives of women[1].

In the light of the adverse effects of hormone replacement therapy, there has been increased interest in alternatives to improve menopausal symptoms and their long-term complications. The phytoestrogens are non-steroidal compounds which are structurally and/or functionally related to the placental or ovarian estrogens, and which may have antagonistic, agonistic or partial effects on the estrogen receptor. The isoflavones are the most active phytoestrogens, the most notable being those found in soya.

Due to this similarity with estradiol, the action of the phytoestrogens is mediated by the estrogenic receptors (ER) α and β. Their tissue distribution is different, the action of their natural or synthetic ligands having specific effects in each tissue. The isoflavones have greater affinity for ERβ. This finding has been put forward to explain the low incidence of clinical effects associated with the menopause in countries with a high consumption of phytoestrogens. Also, lower stimulatory effects are obtained in the breast and endometrium compared with 17β-estradiol, which triggers the transcriptional pathway of ERα[2].

Taking into account these data, foods enriched with soya isoflavones could be considered as "functional foods" – those which include a component which provides a specific beneficial physiological, in addition to a purely nutritional, effect, and which results in an improvement in the state of health and contribute to the risk of developing diseases[3].

The aim of our study is to evaluate the effects of nutritional intervention with a milk product enriched with soya isoflavones on the bone metabolism of Spanish postmenopausal women.

Subjects and methods

This nutritional study was carried out with a randomised, controlled double blind design. The participants were recruited from the Endocrinology Clinic at the Centre for Specialisation in the University Hospital of San Cecilio, Granada. They all gave their signed informed consent to be included. The study was carried out with the approval of the Ethics Committee of the hospital, and was adjusted to meet the relevant directives for research in humans.

99 postmenopausal women between 45 and 65 years of age with physiological amenorrhea of at least one year's development, were selected. The study excluded patients with: serious cardiorespiratory, renal, hepatic or gastrointestinal disease; any hormonal drug treatment or any treatment affecting bone mass or vitamin D metabolism, including calcium and vitamin D supplements. The participants were distributed by random sampling into two groups: Group S, with 48 women, who consumed the milk product enriched with isoflavones, and Group C, of 51 women, who consumed a control milk product. The daily amount of both products consumed was 500 ml over 12 months. In Group S, the daily quantity of isoflavones administered was 50 mg (Table 1).

At the start of the study epidemiological data was collected regarding age, time of development of the menopause, smoking habits and consumption of alcohol, and a basic physical examination was carried out to determine the body mass index (BMI) and the systolic (SPL) and diastolic (DPL) pressure levels.

Measurements were taken at the baseline and at 12 months for hormones, biochemistry and markers for remodelled bone. The hormonal data analysed were: follicle-stimulating hormone (FSH), leutinising hormone (LH) and 17β-estradiol. In addition, blood levels of calcium, phosphorus, parathormone, 25-OH-vitamin D and osteoprotegerin (OPG, ELISA BI-20402, BIO-MEDICA-GRUPPE. Wien, Austria) were measured. The markers for remodelled bone for formation measured were osteocalcin (OC, electrochemiluminescence immunoassay, analyser Elecsys, Roche Diagnostics, IN) and bone alkaline phosphatase (FAO, ELISA, Tandem-R Ostase TM, Hybritech Europe, Liege, Belgium). The markers for resorption included were tartrate-resistant acid phosphatase 5β (TRAP5β, colourimetry, Hitachi 704 Boehringer Manheim GmbH) and carboxy-terminal telopeptide of type I collagen (CTX, enzymatic immunoassay, analyser Elecsys CrossLaps, Roche Diagnostics SL, Barcelona, Spain).

At the start of the study and at 12 months bone mass was estimated using ultrasound of the calcaneum (QUS, Hologic® Sahara® Waltham, NC, USA). The parameters provided were: speed of sound (SOS), attenuation coefficient (BUA, broadband ultrasound attenuation), QUI [QUI = 0.41(SOS) + 0.41(BUA) – 571)], and estimated bone mineral density [Est. BMD = 0.002592 × (BUA+SOS) – 3.687 g/cm^2]. The measurements were carried out in the dominant foot in the manufacturers' standard conditions[4,5].

The statistical programme used was SPSS version 15.0. The quantitative variables were expressed as averages and standard deviations (SD) and the dichotomous variables as a percentage. The normality of the variables was analysed using the Kolmogorov-Smirnov test. A value of $p<0.05$ was considered to be statistically significant. For the comparison of the qualitative variables the chi-squared test was used. In the quantitative variables the t-student average comparison test for independent samples (intergroup differences) and paired samples (intragroup differences) was used.

Results

Epidemiological characteristics

The average age was 55.8 years (SD=6.9) with an average menopausal development time of 3.9 years (SD=4.1). 76.8% did not consume alcohol and 79.8% did not smoke. The average BMI was

28.35 kg/m² (SD=4.67); the average SPL, was 126 mmHg (SD=18) and DPL, 79 mmHg (SD=11). Statistically significant differences were found in the two groups (Group C compared with Group S) in the period of development of the menopause: 5.8 years (SD=3.7) as opposed to 7.9 years (SD=4.2), p=0.008.

Development of markers for bone metabolism

Table 2 specifies the markers for bone metabolism in the population studied during the follow up period.

In the total sample there was an increase in blood concentration of 25-OH-vitamin D (p<0.001). In addition, the OPG (p=0.007) and the TRAP (p<0.001) diminished. Notable in Group C was the increase in the blood concentration of 25-OH-vitamin D (p=0.023). There was a decrease in OPG (p=0.05) and TRAP (p=0.001). In Group S there was also an increase in blood concentration of 25-OH-vitamin D (p=0.001) and a decrease in OPG (p=0.037) and TRAP (p<0.001). No statistically significant differences were found between the two groups in the rest of the measurements.

Development of bone mass estimated by ultrasound of the calcaneum

The parameters measured by QUS are shown In Table 3 and Figure 1.

In the total sample there was a significant increase in SOS (p=0.036), QUI (p=0.012), and estimated BMD (p=0.013) and T-score (p=0.019) between the start and after 12 months of the study. In Group C these changes were not significant, while in Group S there were favourable changes in QUI (p=0.032) and estimated BMD (p=0.04). There were no statistically significant differences found between the two groups.

Table 1. Nutritional content of milk products used in the study

Composition 500 ml	Group C	Group S
Calorific value (Kcal)	232	266
Proteins (g)	15.4	19.7
Carbohydrates (G)	23.6	29
Fats (g)	8.6	8
Vitamin A (UI)	3,000	3,000
Vitamin D (UI)	152	148.8
Vitamin B12 (µg)	1.9	2.1
Calcium (ng)	600	800
Phosphorus (ng)	600	630
Soya isoflavones (mg)	---	50

Discussion

One of the central problems in relation to functional foods is to establish a scientific basis on which to support the beneficial properties which are attributed to their components. The epidemiological evidence suggests that the consumption of soya products is beneficial in relation to problems associated with the menopause. In this context we proposed to evaluate the effects of nutritional intervention with a milk product enriched with soya isoflavones on the bone metabolism in a group of Spanish postmenopausal women. In our study, the consumption of soya isoflavones resulted in favourable changes in bone mass.

Postmenopausal osteoporosis translates clinically into an increase in the risk of fracture and is a public health problem[6]. The observation that women from southeast Asia show a lower incidence of osteoporosis led to the hypotheses that the phytoestrogens from soya could be an alternative for the prevention of loss of bone mass associated with the menopause.

The role of the estrogens *in vitro* is to inhibit the development of the osteoclasts, favouring their apoptosis by stimulating the production of growth transformation factor beta (TGF-β) by the osteoblasts, in addition to inhibiting the production of interleukin 6 (IL-6), the principal stimulant for resorption. They also prevent osteoblast apoptosis. Deficiency estrogen also increases the apoptosis of the osteocytes, which alters the mechano-sensory function of the canalicular system for repairing microdamage, contributing to bone fragility[7]. The action mechanism by which the isoflavones protect against bone loss is not completely known, it being suggested that they modulate the receptor activator osteoprotegerin/ligand system for nuclear factor κB (OPG/RANKL). With estrogen deficiency the production of OPG reduces and there is a strong response by the osteoclast precursors to RANKL[8]. The isoflavones, and specifically the genisteins, stimulate the activity of the osteoprotegerin. Moderate activity is sufficient to stimulate bone formation[9,10].

The clinical studies carried out are highly variable in terms of their design, taking into account the duration of the supplementation, the dose prescribed and taken, the source of soya used, or the epidemiological characteristics of the population. A meta-analysis which reviewed ten clinical trials concluded that nutritional intervention with isoflavones could attenuate bone loss in the spines of postmenopausal women[11], coinciding with the findings of Marini et al. who confirmed how treatment over two years with genistein had positive effects in the BMD of postmenopausal women with osteopenia[12]. A study of the effect on ultrasound of the calcaneum obtained similar results[13].

Table 2. Change in markers for bone metabolism

		0 months average (SD)	12 months average (SD)	p
Calcium (mg/dl)	Total	9.25 (0.33)	9.17 (0.33)	0.388
	Group C	9.22 (0.32)	9.14 (0.35)	0.095
	Group S	9.29 (0.34)	9.37 (0.43)	0.336
Phosphorus (mg/dl)	Total	3.37 (0.45)	3.60 (0.43)	0.219
	Group C	3.4 (0.39)	3.6 (0.44)	0.776
	Group S	3.35 (0.5)	3.61 (0.97)	0.098
PTH intact (pg/ml)	Total	47.22 (16.84)	45.91 (16.51)	0.16
	Group C	47.83 (15.98)	47.27 (15.71)	0.582
	Group S	46.58 (17.86)	44.45 (17.39)	0.118
25-OH-vitamin D (ng/ml)	Total	24.48 (9.85)	28.18 (10.45)	<0.001*
	Group C	23.56 (10.16)	26.48 (10.69)	0.023*
	Group S	25.46 (9.51)	29.91 (10.02)	0.001*
OPG (pmol/L)	Total	5.21 (3.36)	3.89 (1.47)	0.007*
	Group C	5.68 (4.05)	4.1 (1.83)	0.05*
	Group S	4.72 (2.35)	3.69 (0.95)	0.037*
OC (ng/ml)	Total	15.46 (7.1)	17.13 (7.36)	0.096
	Group C	14.46 (7.15)	16.21 (6.84)	0.803
	Group S	16.31 (7.02)	18.1 (7.82)	0.083
FAO (µg/ml)	Total	15.47 (9.25)	16.03 (6.43)	0.068
	Group C	15.52 (11.63)	15.51 (7.01)	0.946
	Group S	15.42 (5.86)	16.59 (5.76)	0.092
TRAP5β (U/l)	Total	2.18 (0.8)	1.76 (0.54)	<0.001*
	Group C	2.15 (0,81)	1.74 (0,5)	0.001*
	Group S	2.21 (0.79)	1.78 (0.59)	<0.001*
CTX (ng/ml)	Total	0.47 (0.21)	0.42 (0.2)	0.064
	Group C	0.44 (0.19)	0.41 (0.19)	0.122
	Group S	0.52 (0.22)	0.42 (0.23)	0.335

PTH intact: parathormone intact; OPG: osteoprotegerin; OC: osteocalcin; FAO: bone alkaline phosphatase; TRAP5β: tartrate-resistant acid phosphatase 5β; CTX: carboxy-terminal telopeptide of type I collagen.
*p: statistically significant intragroup differences (p<0,05)

Table 3. Changes in bone mass estimated by QUS

		0 months average (DE)	12 months average (DE)	p
SOS (m/s)	Total	1517.86 (38.13)	1525.11 (35.6)	0.036*
	Group C	1520.2 (40.9)	1527.72 (42.51)	0.161
	Group S	1515.66 (35.59)	1522.66 (27.85)	0.120
BUA (dB/MHZ)	Total	61.6 (15.71)	64.38 (14.99)	0.057
	Group C	63.29 (15.73)	67.21 (16.89)	0.180
	Group S	60.18 (15.68)	61.72 (12.58)	0.182
QUI	Total	76.37 (19.87)	80.82 (18.26)	0.012*
	Group C	78.5 (20.87)	82.94 (22.1)	0.143
	Group S	74.37 (18.87)	78.84 (13.68)	0.032*
DMO (g/cm^2)	Total	0.407 (0.13)	0.435 (0.12)	0.013*
	Group C	0.419 (0.13)	0.449 (0.14)	0.135
	Group S	0.397 (0.12)	0.423 (0.09)	0.040*
T-score	Total	-1.55 (1.12)	-1.31 (1.03)	0.019*
	Group C	-1.44 (1.17)	-1.19 (1,25)	0.144
	Group S	-1.64 (1.07)	-1.43 (0.79)	0.056

QUS: quantitative ultrasound in the calcaneum; SOS: speed of sound; BUA: broadband ultrasound attenuation, coefficient of attenuation; QUI= 0.41 (SOS) + 0.41 (BUA) – 571); BMD: estimated bone mineral density [Est. BMD=0.002592 × (BUA+SOS)-3.687, g/cm^2].
* p: statistically significant intragroup differences ($p<0.05$)

However, in spite of these favourable results there are also works which do not give evidence of change[14]. A recent intervention study in premenopausal women which evaluated the status of various ions, markers for bone metabolism and thyroid function found no differences in these parameters after the incorporation in the diet over ten weeks of soya isoflavones[15].

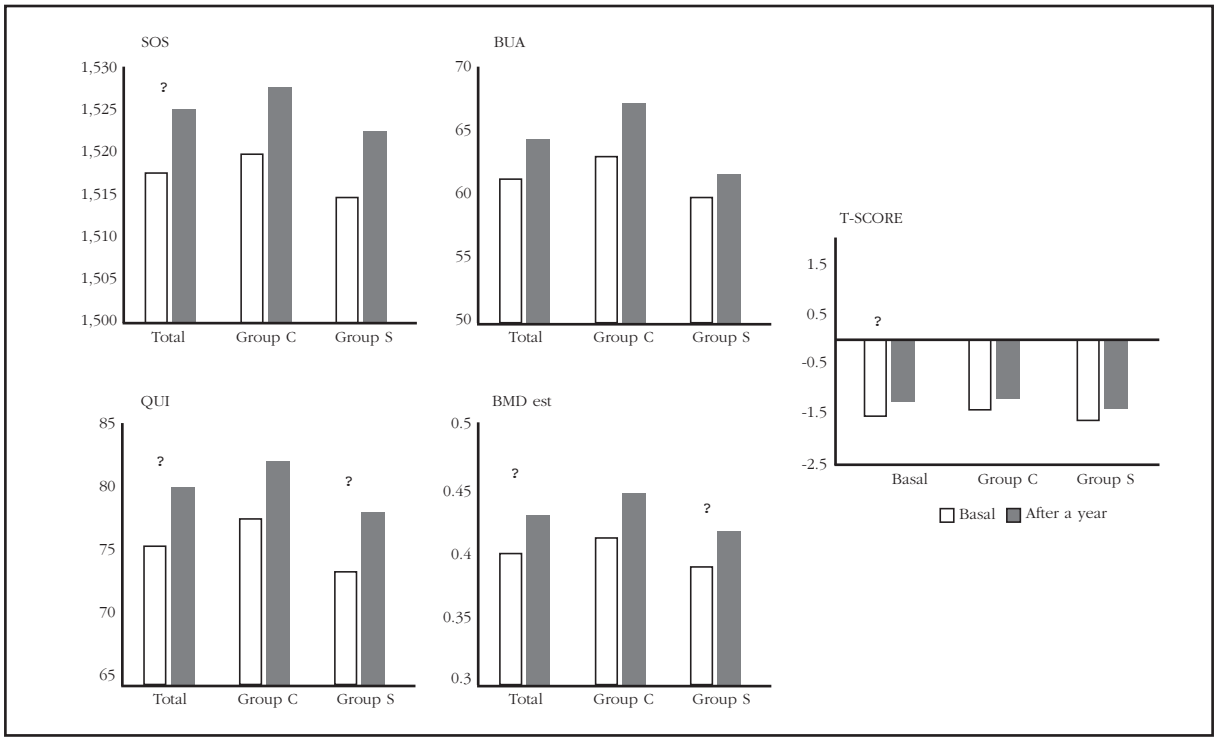
It can be said that, although there is some experimental evidence which suggests a relationship between the consumption of isoflavones and an improvement in bone condition, these are not considered to be conclusive[16].

In relation to our results, there was a decrease in blood concentrations of TRAP and OPG and an increase in levels of vitamin D without differences between groups, which may be explained by the calcium and 25-OH-vitamin D contained in the milk preparations used. With respect to the evaluation of bone mass through ultrasound in the calcaneum, a global increase was observed in all the parameters after a year of follow up, although the changes in QUI and estimated BMD in the group which consumed soya isoflavones were significant.

Our work suffers from some methodological limitations which do not make it possible to be certain whether the differences encountered were solely due to the supplementation with soya isoflavones. One way, hypothesis contrast models used are valid as a statistical method for comparison between groups. In conclusion, the daily consumption of these milk products increases levels of 25-OH-vitamin D and results in a decrease in markers for bone remodelling. A diet rich in soya isoflavones may be an option as a preventative measure against the effects of the menopause on the bone.

Conflict of interest: JFC is a member of the Research Department of Puleva Biotech, Granada, Spain.

Figure 1. Changes in the parameters of ultrasound in the calcaneum (QUS)



QUS: quantitative ultrasound in the calcaneum; SOS: speed of sound; BUA: broadband ultrasound attenuation, coefficient of attenuation; QUI= 0.41 (SOS) + 0.41 (BUA) – 571); BMD: estimated bone mineral density [Est. BMD=0.002592 × (BUA+SOS)-3.687, g/cm^2] .
* p: statistically significant intragroup differences ($p<0.05$)

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Usefulness of FRAX® in the study of fractures in the alcoholic patient

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Summary

FRAX® index is a prognostic tool to assess the risk of osteoporotic fracture. Although ethanol ingestion, liver disease and body mass index are considered independent prognostic factors in the FRAX® score, we have observed that in chronic alcoholics there are several variables not included in the FRAX® index, which show a relation with prevalent fractures and/or low BMD. Therefore, in this study we compare the relation of FRAX® index with those of other variables, such as lean and fat mass, liver function parameters, and amount of ethanol consumed, with the presence or not of prevalent fractures in 57 chronic alcoholic men, older than 40 years, drinkers of more than 200 g ethanol/day during a long time. We found that FRAX® index was significantly higher among those with any fracture, but the same happened with BMI, total fat amount, and fat amount at arms, as well as total amount of ethanol. The FRAX® index did not show differences among those with or without vertebral fractures, or rib fractures. Patients with rib fractures showed differences in total fat amount and right arm fat amount when compared with patients without rib fractures. Therefore, these results suggest that in the alcoholic, other variables, such as amount of ethanol consumed and fat mass, should be considered, in addition to FRAX®, in the prediction of fractures.

Key words: *FRAX® index, alcoholism, bone alterations, fractures, osteopenia, body composition.*

Introduction

The alcoholic patient is exposed to a higher risk of fractures, due, essentially, to two factors: on the one hand, the reduction in bone mass, a multifactorial phenomenon, influenced by many mechanisms, such as the alcohol itself[1-5], the associated malnutrition[6,7], the eventual hepatopathy[8], the secondary hormonal alterations due both to the alcohol and the hepatopathy, and the possible effect of the pro-inflammatory cytokines; on the other hand, the kind of life the alcoholic has, which exposes these patients to falls and traumas which contribute to these fractures[9]. Today, we have clinical tools which allow us to predict the risk of fracture prospectively. One of these, currently in vogue, is FRAX®, an index which includes variables such as the body mass index (BMI), bone mineral density (BMD), age, history of fracture, family history of fracture, alcohol itself, conditions associated with osteoporosis such as hypogonadism (which also affects alcoholics), corticoids, hepatopathy, and others[10]. However, in previous studies we have seen that bone mass in alcoholics is related to lean mass and fat mass[6,11], and that various cytokines, by acting on the receptor activator for nuclear factor κ B (RANK), and its ligand RANKL[12], may also play a pathogenic role. In addition, other variables such as vitamin D[7], may have an influence on fractures, as well as certain social and personal aspects of the environment of the alcoholic, which impact on their life style and their risk of fracture and trauma. None of these parameters is directly included in FRAX®, which means that it is important to compare the value of this tool with those of the variables cited, and to analyse whether lean mass, fat mass, hepatic function, quantity of alcohol consumed, or FRAX® is associated most closely with the presence of fracture in the alcoholic patient, in a cross section of a population with a certain number previous fractures. This is the objective of this work, part of a wider prospective study designed to analyse the relative value of the aforementioned parameters in the diagnosis of fractures occurring in this group of alcoholics followed in the long term.

Patients and methods

57 male patients over 40 years of age, who had given their informed consent, and who had been consecutively admitted to the internal medicine service of our Centre due to organic problems related to the excessive consumption of alcohol, drinkers of great quantities of alcohol (210 ± 90 g/day) over 31 ± 9 years, were included, adapting the FRAX® criteria, designed for the evaluation of risk of fracture in individuals over the age of 40 years. The patients included in this study had sustained significant after effects as a result of their chronic consumption of alcohol: thirty three were cirrhotic, 8 had neoplasms, and 22 died within a period of 18 months (inter-quartile rate 11-56 months) from their inclusion in the study.

X-rays (Xr) of the post-anterior (PA) and lateral (L) thorax were carried out in order to evaluate the presence of rib fractures, while in the lateral Xr we were looking for dorsal vertebral fractures, applying morphometric criteria[13]. To this we added a detailed anamnesis, to see whether or not they had earlier fractures. In some cases it was not possible to correctly evaluate the Xr in the thorax. We also performed a densitometric study using double energy X-ray absorptiometry (DXA) with a LUNAR densitometer (GE HealthCare), to evaluate bone mass in different parts of the skeleton (bones of upper limbs, lower limbs, ribs, spine, pelvis and total), and the T-score in the spinal column and hip. Using these T-score values we grouped our patients as osteoporotic, osteopenic or normal, according to the criteria currently in use[14].

We carried out a nutrition assessment including, in addition to the aforementioned densitometric parameters, a previously validated subjective scale of nutritional assessment, which is based on the qualitative assessment of the lean mass and fat mass in the abdomen, upper and lower limbs, temporal muscle and Bichat's ball[15]. We calculated FRAX® in all the cases[10].

A routine analysis was carried out in all patients, which included albumen, prothrombin activity and blood bilirubin, as well as determining IGF-1 (chemoluminescence, DPC, Los Angeles, CA, USA), 1-25 dihydroxyvitamin D3 (radioimmunoanalysis, Nichols, San Juan de Capistrano, CA, USA), and parathyroid hormone (PTH, immunochemiluminescence, Siemens, Munich, Germany).

This study had the approval of the Ethics Committee of the University Hospital of the Canary Islands. It forms part of a wider prospective study designed to analyse the relative value of the aforementioned parameters in the diagnosis of fractures occurring in this group of alcoholics followed over the long term.

Statistical method

We calculated the difference existing between patients with and without existing fractures in relation to the FRAX® index, lean mass, fat mass, nutritional assessment, and analytical parameters related to hepatic function. Through the Kolmogorov-Smirnov test we determined whether the variables studied were adjusted or not to a parametric distribution. The tests used to compare differences between two groups were the student's T test, and the Mann-Whitney U test in the case of a non-parametric distribution of the variable analysed. To determine which variables were independently related to the FRAX® index we carried out multivariant analysis, introducing lean mass, fat mass, age, prothrombin, albumin, bilirubin, FRAX® index, BMI and subjective nutritional assessment.

Results

Thirty two of the 57 patients studied had had at least one fracture. In 4 cases this fracture was related to a serious trauma (in general, traffic accidents): 1 fracture of the tibia, another of the tibia and fibula, another of both hips, and the other of lumbar vertebrae and multiple ribs. In the thoracic Xr 24 old rib fractures were identified (as opposed

Table 1. Differences between patients with or without fractures (all types of fractures included)

	With fracture (n=32)	Without fracture (n=24)	T (Z); p
Age (years)	53.94 ± 8.81	54.21 ± 11.03	T=0.10; NS
Body mass index	24.79 ± 3.23	27.05 ± 4.29	T=2.04; p=0.047
FRAX® index	4.14 ± 2.27	2.30 ± 1.28	T=3.7; p<0.001
Daily alcohol consumption (g)	214 ± 88	203 ± 98	T=0.42; NS
Years of consumption	33.03 ± 8.51	28.30 ± 8.01	T=1.98; p=0.053
Vitamin D (pg/ml)	28.00 ± 16.87	31.85 ± 14.23	T=0.79; NS
IGF-1 (ng/ml)	99.7 ± 104.6 47.1 (27.9-183.60)	67.8 ± 44.85 48.3 (32.9-105.0)	Z=0.21; NS
PTH (pg/ml)	90.23 ± 132.01 51.40 (29.83-86.23)	60.62 ± 47.37 49.0 (26.25-82.40)	Z=0.56; NS
Prothrombin (%)	75.46 ± 22.13	68.98 ± 27.90	T=0.92; NS
Albumin (g/dl)	3.29 ± 0.57	3.29 ± 0.82	T=0.03; NS
Bilirubin (mg/dl)	3.61 ± 3.65 2.5 (1.1-6)	4.43 ± 4.60 2.35 (1.2-5)	Z=0.73; NS
Total BMD (g/cm^2)	1.07 ± 0.10	1.08 ± 0.095	T=0.59; NS
T-score total hip	-1.28 ± 1.09	-0.83 ± 1.10	T=1.52; NS
T-score L2-L4	-1.39 ± 1.15	-1.39 ± 1.16	T=0.38; NS
Total lean mass (g)	50.085 ± 5.145	53.052 ± 7.653	T=1.64: NS
Total fat mass (g)	17.704 ± 6.620	22.584 ± 9.656	T=2.12; p=0.039

The data are expressed as the mean ± standard deviation, and were compared using the student's T test (T). After the application of the Kolmogorov-Smirnov test it was observed that some variables did not adjust to a parametric distribution. In these cases, in addition to the mean and standard deviation the median and, in brackets, the interquartile range were also provided, and the two groups (with or without fractures) were compared using the Mann-Whitney U test (Z)

to 20 without fracture) and in the spinal Xr, 13 (as against 25). In Tables 1-3 the data from patients with or without fractures in the different locations analysed is summarised. As we see, the total fat mass was greater in those who did not have fractures (any fracture, not even of the rib), and the same for BMI, and marginally, those patients who had been drinkers for longer also had more fractures.

It is notable that in no case was the total BMD significantly different between patients with or without previous fractures. With the variables already mentioned we carried out a logistic regression study to see which factors could be independently related to fractures. We found that, although, with respect to any fracture, the factors to which they were independently related were, first the FRAX® index, then prothrombin activity and lastly the duration of (alcohol) intake in years (Table 4), in relation to rib fractures the first parameter to which it was independently related was total fat mass (Table 5). It is also worth highlighting the fact that none of the parameters chosen played an independent role in relation to the presence or absence of vertebral fractures.

In Figures 1 and 2 we show the ROC curves which illustrate the global capacity of the fat mass and the FRAX® index to diagnose any fracture (1a and 1b) and rib fracture (2a and 2b). As can be seen, FRAX® is useful in both cases, especially to diagnose any osteoporotic fracture, while in the fat area it is only the rib fracture which is diagnosed.

Discussion

The FRAX® index is a widely used index for the diagnosis of risk of fracture[10]. It is, therefore, a prognostic index, and it is as such that it should

Tabla 2. Patients with or without rib fractures

	With fracture (n=24)	Without fracture (n=20)	T (Z); p
Age (years)	52.96 ± 8.33	54.90 ± 12.15	T=0.63 ; NS
Body mass index	24.78 ± 3.36	27.04 ± 3.97	T=2.05; p=0.047
FRAX® index	3.76 ± 1.93	3.04 ± 2.30	T=1.14; NS
Daily alcohol consumption (g)	217 ± 94	198 ± 96	T=0.68; NS
Years of consumption	31.96 ± 6.57	32.15 ± 11.20	T=0.70; NS
Vitamin D (pg/ml)	26.86 ± 16.07	33.86 ± 15.97	T=1.36; NS
IGF-1 (ng/ml)	108.2 ± 112.5 47.1 (28.4-191.0)	80.6 ± 61.13 53.5 (32.9-118.2)	Z=0.04; NS
PTH (pg/ml)	58.37 ± 44.35 45.60 (28.7-85.4)	82.18 ± 80.93 52.8 (30.55-95.68)	Z=0.85 ; NS
Prothrombin (%)	77.69 ± 22.05	71.03 ± 27.44	T=0.79; NS
Albumin (g/dl)	3.35 ± 0.56	3.28 ± 0.73	T=0.38; NS
Bilirubin (mg/dl)	3.18 ± 2.42 2.25 (1.23-5)	4.33 ± 4.54 3.20 (1.1-5.6)	Z=0.73; NS
Total BMD (g/cm^2)	1.06 ± 0.11	1.07 ± 0.08	T=0.23; NS
T-score total hip	-1.33 ± 1.10	-0.88 ± 0.86	T=1.49; NS
T-score L2-L4	-1.38 ± 1.25	-1.54 ± 0.87	T=0.19; NS
Total lean mass (g)	50.321 ± 5.201	53.063 ± 8.136	T=1.38: NS
Total fat mass (g)	17.015 ± 6.250	21.671 ± 8.827	T=2.00; p=0.052

The data are expressed as the mean ± standard deviation, and were compared using the student's T test (T). After the application of the Kolmogorov-Smirnov test it was observed that some variables did not adjust to a parametric distribution. In these cases, in addition to the mean and standard deviation the median and, in brackets, the interquartile range were also provided, and the two groups were compared using the Mann-Whitney U test (Z)

be considered, although it is obvious that the same factors which allow one to predict a future fracture ought also to be capable of differentiating between patients with or without fractures at any given moment. In this work we have analysed the capacity of this index to detect these differences in alcoholic patients, since in this group there is a series of factors which may distort its value. There is no doubt as to the existence of osteopathy in the chronic alcoholic. Already observed by Saville in the 1960s[16], Oppenheim[9] subsequently applied the term "battered alcoholic syndrome" to those alcoholic patients with more than three fractures in different states of consolidation. Later, the classic works of Israel[1], Diamond[2] and others[17-19], to cite only a few, serve only to confirm that in alcoholics, independently of cirrhosis, there is a metabolic osteopathy characterised by osteopenia, in which malnutrition plays a significant role[6,20]. This is due, above all, to defective bone formation, although there being some controversy with respect to reabsorption, which expresses an imbalance between the formation and destruction of bone. But certain aspects, on which we comment below, make this different. Firstly, age: alcohol reduces life expectancy, and osteoporosis in the alcoholic, although increasingly serious with age, appears much earlier than when associated with the menopause, for example, or with senility. Secondly, the nutritional state. This is often clinically evaluated in a general way, through BMI, or subjectively, but without paying attention to the fat or lean areas of the body which may be altered selectively; it is common for some alcoholics to have a relative increase in fat mass accompanied by a parallel decrease in lean mass, with a

Tabla 3. Patients with or without dorsal fractures

	With fracture (n=13)	Without fracture (n=25)	T (Z); p
Age (years)	56.15 ± 9.67	54.48 ± 10.52	T=0.48 ; NS
Body mass index	27.39 ± 4.09	26.28 ± 3.86	T=0.74; NS
FRAX® index	4.17 ± 2.69	2.96 ± 1.67	T=1.71; NS
Daily alcohol consumption (g)	202 ± 130	223 ± 91	T=0.53; NS
Years of consumption	35.77 ± 10.64	29.33 ± 7.43	T=2.08; p=0.046
Vitamin D (pg/ml)	30.30 ± 18.70	34.14 ± 18.64	T=0.56; NS
IGF-1 (ng/ml)	89.1 ± 74.8 53.5 (33.8-152.1)	81.7 ± 99.0 46.9 (30.4-91.2)	Z=0.53; NS
PTH (pg/ml)	82.45 ± 93.32 55.10 (27.02-93.05)	99.99 ± 143.18 55.0 (42.25-92.20)	Z=0.62; NS
Prothrombin (%)	74.00 ± 24.47	70.91 ± 21.14	T=0.39; NS
Albumin (g/dl)	3.55 ± 0.77	3.17 ± 0.59	T=1.61; NS
Bilirubin (mg/dl)	3.63 ± 3.61 2.2 (1.0-5.3)	4.49 ± 5.09 2.75 (1.15-5.88)	Z=0.30; NS
Total BMD (g/cm^2)	1.07 ± 0.10	1.10 ± 0.10	T=0.69; NS
T-score total hip	-1.09 ± 1.25	-0.98 ± 1.12	T=0.28; NS
T-score L2-L4	-1.79 ± 1.18	-1.14 ± 1.34	T=1.48; NS
Total lean mass (g)	51.271 ± 7.673	51.947 ± 5.149	T=0.30: NS
Total fat mass (g)	23.778 ± 9.270	20.682 ± 7.301	T=1.09; NS

The data are expressed as the mean ± standard deviation, and were compared using the student's T test (T). After the application of the Kolmogorov-Smirnov test it was observed that some variables did not adjust to a parametric distribution. In these cases, in addition to the mean and standard deviation the median and, in brackets, the interquartile range were also provided, and the two groups (with or without fractures) were compared using the Mann-Whitney U test (Z)

normal or even raised BMI (malnourished obesity). This is important since although the decrease in lean mass reduces bone formation[21], the fat may exert opposing effects, since, although contributing to the weight, and thus increasing the bone mass, it may also be the source of cytokines which can cause bone lesions, such as tumour necrosis factor (TNF)[22]. It is also notable that the total fat mass replaces the FRAX® index in its capacity to diagnose existing fractures at any given moment. As we have just indicated, the fat mass, which may be elevated in the alcoholic, contributes significantly to total weight. It is this weight which is opposed to gravity, and which our skeleton has to support, which exerts a stimulating effect on osteoformation. But it is also worth noting that we did not see a relationship between fracture and lean mass. Lean mass determined by densitometry may be misleading in the alcoholic since the presence of ascites or oedemas may falsify the results[23]. In this study we cannot discount the influence of hydrosaline retention, although generally the densitometry was carried out when the patient was ready to be discharged, or, at least, a few days after treatment.

A third factor to consider in the osteopathy of the alcoholic is hormonal alterations. This is due in part to the cirrhosis, although the alcohol in itself, without the need for the coexistence of cirrhosis, provokes hypogonadism, altering the levels of vitamin D and the cortisol metabolism, even though the effects of these hormones are contained, in one way or another, in the FRAX® index.

FRAX® is, without a doubt, a useful tool. In fact, if we consider its value in the diagnosis of

Table 4. Logistic regression in successive steps, which shows that the FRAX® index (FRAXfrac), the activity of prothrombin (ptbna) and the year of consumption of alcohol (tconsumo) are the only parameters which hold an independent relationship with the presence of any fracture

Fracture (total)				
		B	**Wald**	**Sig.**
Step 1(a)	FRAXfrac	-1.398	8.330	0.004
	Constant	3.161	6.275	0.012
Step 2(b)	ptbna	-0.067	5.800	0.016
	FRAXfrac	-2.225	9.257	0.002
	Constant	10.592	7.820	0.005
Step 3(c)	Ptbna	-0.069	5.464	0.019
	tconsumption	-0.136	3.501	0.061
	FRAXfrac	-2.598	7.040	0.008
	Constant	15.688	7.851	0.005

a) Variable(s) introduced in step 1: FRAXfrac. b) Variable(s) introduced in step 2: ptbna.
c) Variable(s) introduced in step 3: tconsumption

Table 5. Logistical regression in successive steps which shows that the only parameter which shows an independent relationship with the presence or absence of costal fractures is the total quantity of fat (totfatab)

Rib Fracture				
		B	**Wald**	**Sig.**
Step 1(a)	totfatab	0.000	4.115	0.042
	Constant	-2.234	5.010	0.025

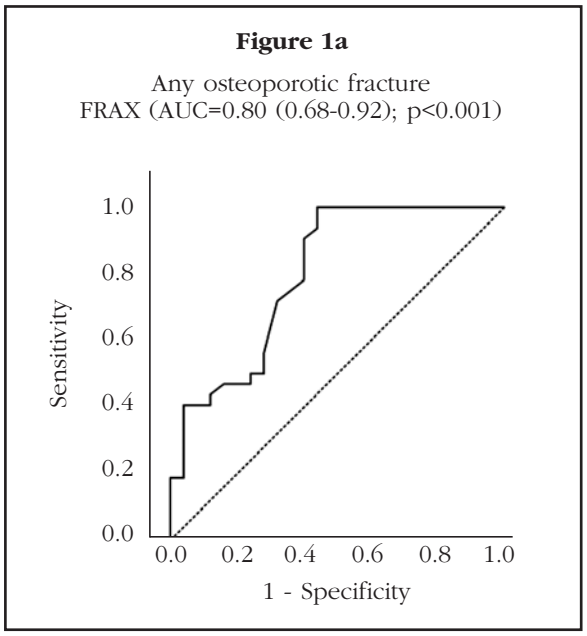
a) Variable(s) introduced in step 1: totfatab

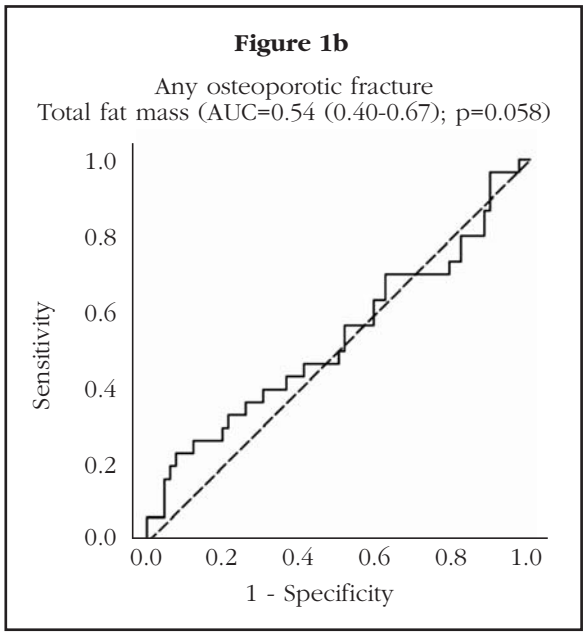
any type of fracture, those for which FRAX® should really be used to make a prognosis, we see that the ROC curve shows an area below the curve of 0.8, which is to say, acceptable enough, and better than that obtained when the diagnostic value of other variables is explored. However, our study, still preliminary, does not allow us to infer conclusions about the prognostic role of FRAX®.

It is notable that, in relation to costal fractures, it is the fat mass which replaces the other variables. In an earlier work we found that what was really associated with costal fractures was irregular eating and disordered life-style[24], in summary, the "marginality" of the inveterate alcoholic, at least in our environment. The finding of a higher number of fractures in widowers and men who are separated, as has been referred to years ago by Keso et al.[25], may be interpreted in the same way. The fact that fat mass is now being related to fracture may also be interpreted in this way, in that marginality and solitude results in a worse nutritional state, with a decrease in fat (and lean) mass and a life-style with a propensity to traumatic fracture.

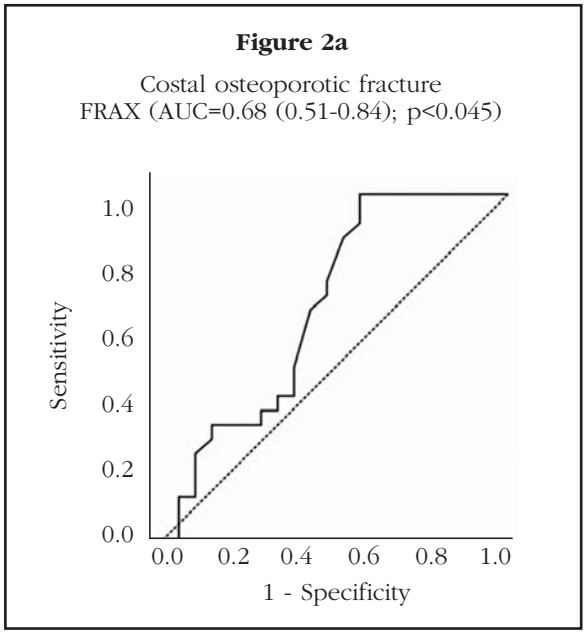
In conclusion, FRAX® also appears to be a useful tool in the prediction of risk of fracture in the alcoholic patient, even though its predictive capacity in these patients is still to be determined. However, the fact that fat mass replaces the FRAX® index in the diagnosis of costal fractures obliges us to take into account that the detailed analysis of the composition of the body, not contemplated in the FRAX® index, may need to be considered in the prognostic evaluation of fractures in these patients.

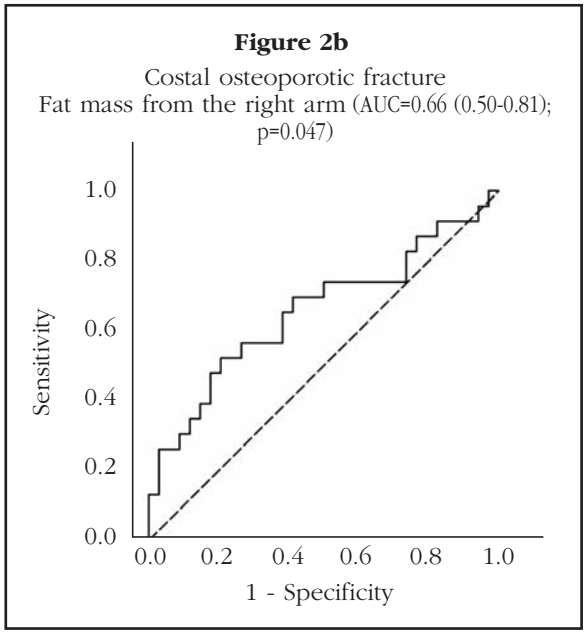
Figured 1a and 2b. ROC curves which illustrate the specificity and sensitivity of FRAX® and fat mass in the diagnosis of any osteoporotic fracture





Figures 2a and 2b. ROC curves which illustrate the specificity and sensitivity of FRAX® and fat mass (from the right arm) in the diagnosis of costal osteoporotic fracture





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Risk of fracture according to FRAX®, hypovitaminosis D, and quality of life in a population with osteoporotic fracture cared for in primary care: baseline description of the VERFOECAP cohort

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Summary

Background: the patient with an osteoporotic fracture cared for in primary care has seldom been studied. The VERFOECAP study has dual objectives: to estimate if the risk of fracture (FRAX®) in fractured patients is different in patients with or without a re-fracture; and to study the prevalence of hypovitaminosis D and the impact of the fracture on quality of life. We present a baseline description.

Material and method: design and ambit: multicentred prospective cohort study in primary care (12 centres in Catalonia). Population: random sample of patients with a history of principal osteoporotic fracture between 2006 and 2008 cared for in primary care. Information gathering: at initial inclusion meetings clinical information was gathered, quality of life questionnaires ECOS16 (specific) and EuroQol-5D (generic) completed, spinal X-ray carried out, and levels of vitamin D in the blood measured. Subjects were followed up for two years. Analysis: comparison between two groups using T-test or chi-squared test. Prevalence of hypovitaminosis D and confidence interval using binomial test.

Results: 194 patients were included. The average risk (standard deviation) of fracture of the hip, according to FRAX® was calculated as: 6.9% (6.4), and of principal osteoporotic fractures: 14.8% (8.6). EuroQol-5D showed frequent limitations to walking (47.6%) and to daily activities (45.5%); 55.0% reported moderate pain, and 41.0% anxiety/depression. The ECOS-16 score was higher in patients with a history of vertebral fracture ($p<0.001$). The prevalence of hypovitaminosis D was 61.4% (CI 95%: 53.6%-68.9%).

Conclusions: the VERFOECAP cohort includes patients with osteoporotic fractures cared for in primary care at high risk of re-fracture with significant deterioration in quality of life. In these patients vitamin D deficiency is highly prevalent.

Key words: *osteoporosis, primary care, vitamin D, osteoporotic fractures, risk factors, FRAX®, therapeutic compliance.*

Introduction

Osteoporosis is a chronic process characterised by low bone mass and alterations in microarchitecture which result in bone fragility and, therefore, a high probability of suffering fractures[1]. It is a silent pathology until the moment a fracture occurs, which means that the evaluation of individual risk of osteoporosis is important for the prevention of their occurrence. It is, in addition, a common disease which mainly affects people in developed countries such as in North America, in Europe and Japan; in general terms it is estimated that 33% of women over 50 years of age will suffer from osteoporosis during their lifetimes[2]. The prevalence of osteoporosis increases with age. In Spain the global prevalence of osteoporosis in the femoral neck is 4.3%, and in the lumbar spine, 11.3%. In the population of Spanish women over 50 years of age, the prevalence of femoral osteoporosis would be around 9% and in the lumbar region, it would exceed 23%[3].

The measurement of bone mineral density by means of densitometry with DXA (dual source X-ray absorptiometry) was previously considered by the WHO[4] as a valid method of diagnosis, capable of predicting the risk of fracture. However, certain limitations have been found, such as the fact that different populational cohorts[5,6] show that 50% of women with fractures are classified, according to the WHO, as normal or osteopenic, which means that the BMD used as the single determinant does not identify well the risk of suffering a fracture[7]. In addition, it is not a method which is available in all geographic areas. In the year 2007, the WHO published a new document[4] in which it recognised the need to include clinical risk factors in the assessment of risk of fracture, with those considered to be the main ones being age, previous personal or family history of fractures, the use of corticoids over long periods, sedentary life-style and active smoking[8].

After the publication in 2008 of the FRAX® scale[9], this has become a good tool for identifying the absolute risk at 10 years of suffering both a fracture of the hip, as well as other principal fractures (clinical vertebral, humerus and forearm fractures). This is based on the aforementioned risk factors, and may include the DXA value, if available. One of the current needs is to confirm the usefulness of this scale in each of the different populations before introducing it into routine practice in primary care.

Hence, the VERFOECAP (Evaluation of the FRAX® risk scale in established osteoporosis in primary care in Catalonia) cohort study was designed with the main aim of confirming if there were differences in FRAX® risk between patients who suffered fractures during the follow up and those who did not. In addition, secondary objectives were set, which were to determine the prevalence of hypovitaminosis D in the population of those with fragility fractures seen in primary care, and to assess the impact of osteoporotic fractures on quality of life. We present here the baseline description of this cohort.

Patients and methods

Design: prospective, multi-centred cohort study: VERFOECAP cohort. We present the baseline description of the patients recruited.

Ambit: carried out in twelve urban primary care centres in Catalonia.

Sample size: accepting an alpha risk of 0.05 and a beta risk of 0.2 in a bilateral contrast, and assuming an annual incidence of fracture of 2% (20% at 10 years on average according to the FRAX® estimate) 190 subjects were required to detect a difference equal to or greater than a standard deviation between groups (patients with incident fractures versus those who did not suffer fracture during the follow up period) in the variable "absolute risk of fracture estimated according to the FRAX® tool". A forecast rate of loss during the follow up period of 15% has been taken into account. With respect to one of the secondary objectives, accepting an alpha risk of 0.95, in a bilateral contrast for the prevalence of vitamin D insufficiency estimated at 7%, according to the literature[10], a randomised sample of 81 subjects would be sufficient to ensure a precision of 10% in our estimate.

Participants: using each centre's computerised clinical history records system (eCAP program), lists of patients were obtained of both sexes, and aged between 40 and 90 years of age, of Spanish nationality, and who had had a principal fragility fracture between January 2006 and December 2008 in the humerus, distal radius, vertebrae, hip or pelvis (see the list of CIE-10 codes used in Appendix 1).

The participant population was selected using simple randomised sampling. Patients whose telephone contact details were not available, those with dementia or serious psychiatric illness, those who were suffering from terminal illness or who were being cared for at home, those who had had in the last year a weight loss of more than 10% or who had a history of any disease which might cause secondary osteoporosis (except for corticotherapy and rheumatoid arthritis, both included in the FRAX® tool) were excluded.

Each patient was contacted by telephone to confirm that the fracture was a fragility fracture, that the location of the fracture was correct, that it had occurred in the period indicated and that they complied with the inclusion criteria. The affirmative cases were invited for an interview with a researcher at which the objectives of the study were explained, and in cases where the subject was interested in participating they were asked to give their informed consent. This study was presented to and approved by the clinical research ethics committee (CEIC) of the Jordi Gol IDIAP.

Information gathering:

By means of a clinical interview, information was gathered on the location of fragility fractures, record of personal history of osteoporosis and previous densitometry, use of anti-osteoporosis treatment or calcium and vitamin D supplements and

compliance with the former (using the Morinsky-Green and Haynes-Sackett tests), number of falls in the last year, and variables necessary for the calculation of the FRAX® risk of fracture (age, sex, weight in kilograms and height in centimetres measured at the inclusion visit, active smoking, consumption of alcohol above 3 standard units a day, paternal/maternal family history of hip fracture, presence of rheumatoid arthritis or corticotherapy above 5 mg/day of prednisone or equivalent for more than 3 months). The probability of having a hip fracture or principal osteoporotic fracture at 10 years was calculated according to the on-line FRAX® tool for the Spanish population [http://www.sheffield.ac.uk/FRAX/tool.jsp?lang=sp]. The impact of the fractures on quality of life was also measured using generic (EuroQol-5D) and specific (ECOS-16) questionnaires[11,12]. The intake of calcium in the diet was assessed using the validated survey INDICAD[13]. Finally, lateral X-rays of dorsal and lumbar spine in lateral projection were requested to discount earlier silent fractures, and an analysis was carried out to discount secondary causes of osteoporosis, measuring the following parameters: 25-hydroxyvitamin D-25(OH)D-, calcium, phosphorus, albumin, alkaline phosphatase, creatinine, glomerular filtration rate estimated by MDRD-4, velocity of sedimentation and thyroid function. In case where a secondary cause appeared the patient was excluded but offered the same follow up as those included.

Table 1. Baseline characteristics of the VERFOECAP cohort

Age (years): mean, SD	74	9.1
Male gender: n, %	26	13.4
Height (m): mean, SD	1.54	0.08
Weight (kg): mean, SD	68	12.4
BMI (kg/m²): mean, SD	28.6	4.8
Low weight (BMI<19 kg/m²): n, %	4	2.1
Normal weight: n, %	37	19.0
Overweight: n, %	88	45.4
Obesity I: n, %	46	23.7
Obesity ≥II: n, %	19	9.8
Patients on antiosteoporotic drugs: n, %	129	66.5
Oral biphosphonates	104	80.6
Iv biphosphonates	4	3.1
Strontium ranelate	15	11.6
PTH	4	3.1
Calcitonin	2	1.6
Years of treatment: mean, SD	2.4	2.8
Calcium supplements: n, %	127	65.5
Vitamin D supplements: n, %	124	63.9

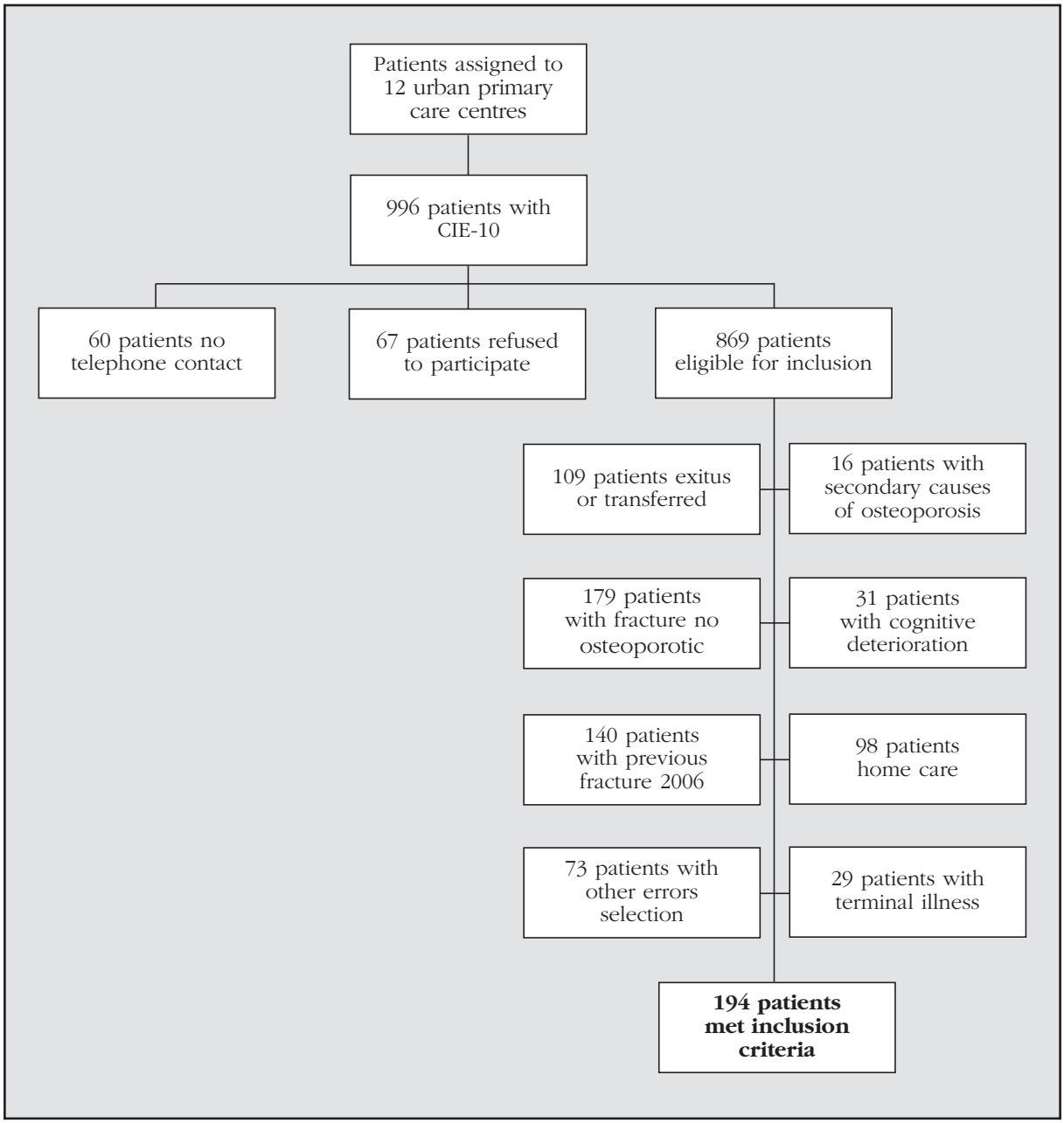
Statistical analysis:
The characteristics of the population studied are described by means of unvivariant descriptive analysis, calculating mean and standard deviation for the continuous variables, and absolute frequency and percentage for the variable categories. To find the distribution of risk factors associated with suffering a fracture, bivariant comparisons were made using the chi squared test between categorical variables and the student's T test between continuous and categorical variables. All the statistical tests were carried out with a confidence of 95% and assuming a bilateral contrast. The SPSS statistical software package was used.

Results

194 patients were included, recruited in twelve primary care centres in Catalonia. The baseline characteristics of the population included are shown in Table 1. The flow diagram of the population included can be seen in Figure 1.
In 143 cases (74.9%) densitometry had been requested, and in 80 (41.2%) the results of the test had been recorded. Only 7 (8.8%) patients had normal BMD, 48 (59.9%) had osteopenia, and the remaining 25 (31.3%) showed values compatible with osteoporosis (Figure 2).

On analysing the risk factors for fracture included in the FRAX® calculation, 157 patients (80.9%) were over the age of 65, 168 (86.6%) were women, 35 (20.8%) had early menopause, 45 (23.2%) had paternal history of hip fracture, 15 (7.7%) were active smokers, 9 (4.6%) consumed more than 3 standard units/day of alcohol, 7 had received corticotherapy, 4 (2.1%) had a BMI ≤ 19 kg/m² and 4 (2.1%) had a history of rheumatoid arthritis. Out of all the cases, 113 (61.4%), 39 (21.2%), 5 (2.7%) and 3 (1.6%) cases had 3, 4, 5

Figure 1. Scheme of the study. Population flow



and 6 accumulated risk factors, respectively. The mean (standard deviation) of the absolute risks of fracture estimated at 10 years according to FRAX® was 6.9% (6.4) and 14.8 (8.6) for hip and principal fracture, respectively. Table 2 shows the median and interquartile range of FRAX® risk in the total population, according to number of prevailing fractures, and by age groups, and the number and percentage of patients with risk higher than the therapeutic threshold proposed by the British NOGG (National Osteoporosis Guidelines Group) guide, and the European guide to osteoporosis. 89.7% of the participants had an estimated risk of fracture of the hip which exceeded the therapeutic threshold proposed in these guides. In addition, of the 194 patients included, all with previous fracture, 59 (20.1%) had two fractures, and 23 (11.9%) three or more fractures. The most prevalent fractures were vertebral, followed by those of the humerus (Table 3). After reviewing the dorsolumbar spinal X-ray from the baseline visit, it was observed that 18.8% of the cases with vertebral fracture had more than one vertebra affected. The absolute risk of fracture estimated according to FRAX® was not significantly different between those patients with one or more fractures (p=0.39 for FRAX® for the hip and p=0.43 for FRAX® for the principal fracture) (Table 2).

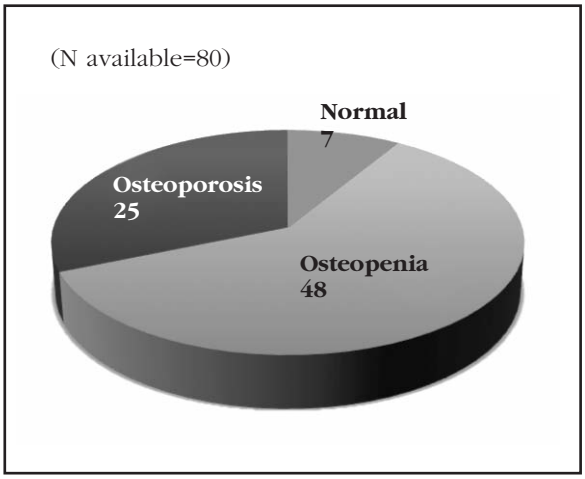
The impact on quality of life in relation to health (ICVRS) of the fractures in our patients measured using the EuroQol-5D questionnaire showed limitations in walking in 47.6%, in personal care in 20.4% and in daily activities in 45.5%; 55.0% reported moderate pain or discomfort, and 13.6% heavy pain or discomfort; 41% reported anxiety or depression.

The mean (standard deviation) of the ECOS-16 score was 2.03 (0.96). In those patients with previous vertebral fracture, the ECOS-16 score was significantly higher than in those patients with fractures in other places (p<0.001, with an average difference of 0.62 [CI 95%: 0.39-0.85]).

In those patients who were already receiving a drug for the treatment of osteoporosis, the declared compliance level was 63.4% with the Morinsky-Green test, and 77.8% with the Haynes-Sackett test (Kappa index 0.54; p<0.001).

Of the 166 patients in whom the levels of 25(OH)D had been determined, the mean value (standard deviation) was 31.3 (23.3) ng/ml. 102 patients (61.4% [CI 95%: 53.6%-68.9%]) had levels compatible with vitamin D insufficiency, in 47 (28.3%) with a deficit (below 20 ng/ml) and in 14 (8.4%) with a severe deficit (below 10 ng/ml). The levels of 25(OH)D were significantly higher in the 106 patients who took calcium and vitamin D supplements (51 patients -48%- took 800 units/day, and 55 patients -52%- took 400), than in those 60 patients who did not take them (p<0.001 with an average difference of 13.5 ng/ml [CI 95%: 6.2 - 20.9]). No significant differences were found in levels of blood vitamin D between those patients with a single fracture and those with two or more (p=0.91) (Figure 3), or between the different places in which the fractures had occurred (p=0.16).

Figure 2. Bone mineral density in the VERFOECAP cohort according to WHO criteria



Discussion

This study shows the clinical characteristics, the risk factors and the impact on the quality of life of osteoporotic fractures in primary health care and in the Spanish population. This consists of patients with a high risk profile for future fractures: 75% had osteoporosis, more than 80% were over 65 years of age and female, and nearly a quarter had paternal/maternal history of hip fracture. In terms of future risk of new fractures estimated by the FRAX® tool, the majority (more than 65%) had at least three of those risk factors included in the tool, and the absolute risk of re-fracture at 10 years, estimated according to the same formula, was around 7% and 15% on average for hip and principal fractures, respectively. Although there are no established risk thresholds for FRAX® in this country, some authors work with the thresholds proposed for the United Kingdom[14]. Almost 90% of patients included had an average risk above that of the treatment threshold according to FRAX® of the hip proposed in the last European guide to osteoporosis[15], and in the British NOGG guide[16]. It is remarkable that despite consisting of patients at high risk and existing fractures, a third of the population recruited did not take any treatment to prevent fractures occurring. In addition, of those patients which did take them, nearly 40% were not compliant (with a moderate agreement between the two tests used).

With respect to the prevalence of fractures in these patients, almost a third of them had at least two fractures. Those patients with two or more fractures did not have a higher FRAX® risk than those with only one fracture, which suggests that this tool does not discriminate well with high risk patients, in addition to the fact that it does not take into account the number of fractures, rather the history of fracture (yes or no) as a risk factor. This has been criticised as one of the limitations of FRAX®[17], since, as the literature shows, the number and severity of existing fractures are related to the risk of future fractures[18].

We also found that there were few patients (scarcely 2%) with low weight, and in contract, a high proportion (more than a third) of obese patients with fractures in this cohort. This is consistent in the work recently published by Premaor et al.[19] which highlights the possibility that the association between the body mass index and fracture is complex, and that obese patients also have a high a risk of fracture, in particular in some specific locations, such as in the upper extremities.

With regard to the prevalence of hypovitaminosis D, we showed that in the VERFOECAP cohort this is higher than 60%, a datum consistent with other studies which analysed levels of vitamin D in the blood in a population with fractures treated in hospital for hip fracture in the same region[10].

Also significant is the high impact observed in this population in terms of quality of life: almost half the patients recruited had limitations in walking and daily activities, pain or discomfort, and anxiety or depression. This has been shown in different studies which included populations treated in hospital[20], and, recently, in a populational study carried out in Valencia[21].

Table 2. Absolute risk of fracture at 10 years according to the FRAX® tool in the population of the VERFOECAP cohort

		Risk FRAX® of principal fracture		Risk FRAX® of hip fracture	
		Median (range inter-quartile)	N (%) nogg above threshold	Median (range inter-quartile)	N (%) nogg above threshold
Total population (N=194)		14.0 (7.9-20.0)	22 (11.3)	5.2 (2.4-9.4)	174 (89.7)
By background fracture (No. of fractures)	1	13.0 (7.1-20.3)	13 (9.8)	4.9 (2.1-9.2)	118 (89.4)
	2	14.0 (8.9-20.0)	4 (10.3)	5.2 (2.7-10)	36 (92.3)
	≥3	19.0 (8.6-22.0)	5 (21.7)	7.4 (2.9-9.3)	20 (87.0)
By age (years)	≥50 a <55	7.0 (4.0-10.0)	1 (50.0)	2.5 (0.7-4.3)	2 (100)
	≥55 a <60	8.2 (6.5-9.4)	2 (22.2)	1.2 (0.8-2.6)	9 (100)
	≥60 a <65	5.3 (3.6-6.6)	21 (100)	1.3 (0.8-1.6)	20 (95.2)
	≥65 a <70	8.1 (6.4-11.0)	2 (10.5)	2.3 (1.7-4.2)	18 (94.7)
	≥70 a <75	12.0 (8.8-16.8)	5 (13.9)	4.7 (3.1-7.7)	35 (97.2)
	≥75 a <80	15.5 (11.0-20.0)	3 (7.1)	6.5 (4.4-9.8)	37 (88.1)
	≥80	21.5 (18.8-25.0)	9 (15.0)	9.4 (7.4-13)	53 (88.3)

In spite of the fact that the FRAX® tool was designed for application in patients not treated with drugs for osteoporosis, our study includes patients treated at the time of inclusion. Although this is a limitation, the non-treatment of patients with established osteoporosis, the patients recruited to the VERFOECAP cohort, is of dubious justification, both ethically and clinically. It will be interesting to see the predictive capacity for new fractures in these patients with previous treatment. The limitations and after effects of the fracture in this type of patient has made their inclusion in this study enormously difficult, since many of the patients with recent history of fracture of the hip were either institutionalised or being cared for at home during the period of recruitment. This may limit the external validity of our results, which will only be valid for those patients with previous fracture who have maintained their autonomy and live in their normal home after having the fracture.

Conclusions

The VERFOECAP cohort consists of a population of patients with fragility fractures seen in primary care, who have a high absolute risk of future fracture, and show a high degree of deterioration of quality of life in relation to health. In these patients, the vitamin D deficit is high. Currently, the cohort continues with prospective visits, and a follow up is planned for at least two years. This will give us information regarding the usefulness of the FRAX® formula in the prediction of risk in these patients within our ambit on the impact of vitamin D deficit on the risk of fracture and on the impact of the incident fracture on the quality of life of those patients who have already suffered a previous fracture.

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Figure 3. Levels of vitamin D and number of fractures

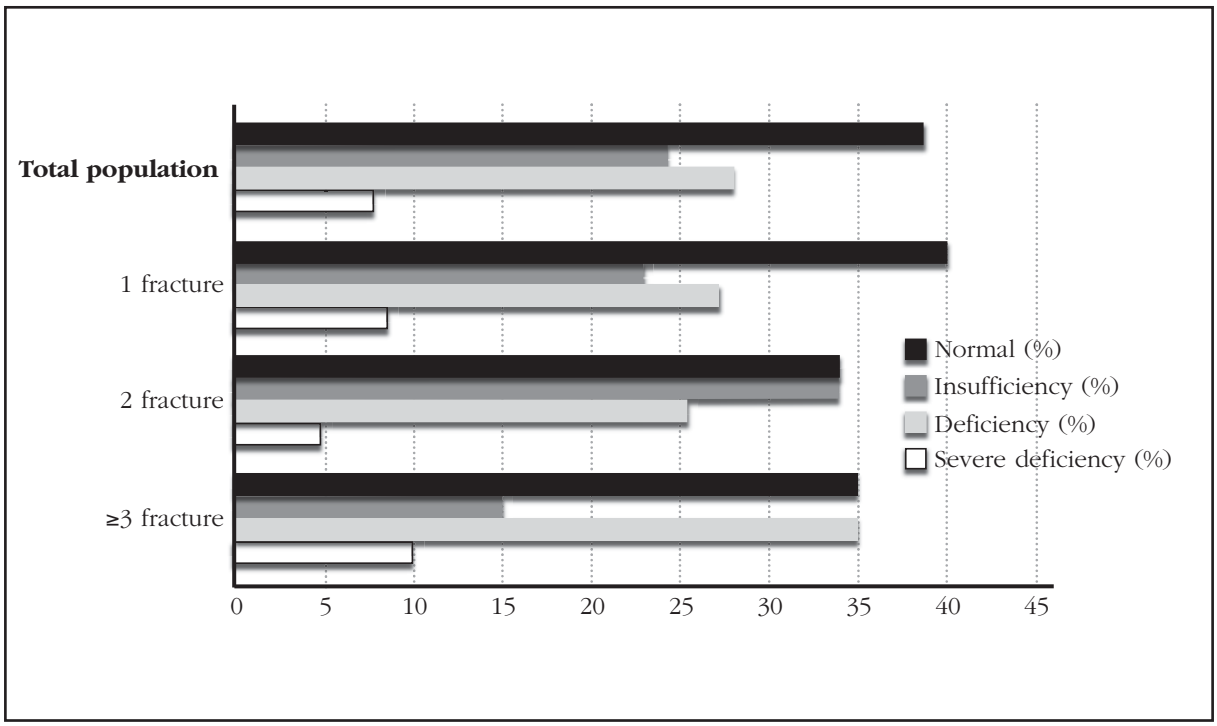

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Table 3. Number and locations of existing fractures in the VERFOECAP cohort

		N	%
Number of fractures	1	132	68.0
	2	39	20.1
	≥3	23	11.9
Location	Vertebral	85	43.8
	Humerus	77	39.4
	Colles	27	13.9
	Hip	36	18.6
	Pelvis	7	3.6

Appendix 1: List of diagnoses

M80	Osteoporosis with pathological fracture.
M80.0	Postmenopausal osteoporosis, with pathological fracture.
M80.1	Postooforectomal osteoporosis, with pathological fracture.
M80.2	Osteoporosis due to disuse, with pathological fracture.
M80.3	Osteoporosis due to postsurgical malabsorption, with pathological fracture.
M80.4	Drug-induced osteoporosis, with pathological fracture.
M80.5	Idiopathic osteoporosis, with pathological fracture.
M80.8	Other types of osteoporosis, with pathological fracture.
M80.9	Non-specified osteoporosis, with pathological fracture.
S22	Fracture of ribs, sternum and thoracic spine (dorsal).
S22.0	Fracture of thoracic vertebra.
S22.1	Multiple fractures of thoracic spine.
S32	Fracture of lumbar spine and pelvis.
S32.0	Fracture of lumbar vertebra.
S32.1	Fracture of sacrum.
S32.2	Fracture of coccyx.
S32.3	Fracture of iliac bone.
S32.4	Fracture of acetabulum.
S32.5	Fracture of pubis.
S32.7	Multiple fractures of the lumbar spine and pelvis.
S32.8	Fracture of other parts and unspecified in the lumbar spine and pelvis.
S52	Fracture of forearm.
S52.0	Fracture of the upper epiphysis of the ulna.
S52.1	Fracture of the upper radial epiphysis.
S52.2	Fracture of the diaphysis of the ulna.
S52.3	Fracture of the radial diaphysis.
S52.4	Fracture of the diaphysis of the ulna and radius.
S52.5	Fracture of the lower radial epiphysis.
S52.6	Fracture of the lower epiphysis of the ulna and radius.
S52.7	Multiple forearm fracture.
S52.8	Fractures in other parts of the forearm.
S52.9	Fracture of forearm, unspecified part.
S62	Fracture of the wrist and the hand.
S62.1	Fracture of other carpal bone(s).
S62.8	Fracture of other parts, and those unspecified, of the wrist and hand.
S72	Fracture of the femur.
S72.0	Fracture of the femoral neck.
S72.1	Pertrochanteric fracture.
S72.2	Subtrochanteric fracture.
S72.3	Fracture of the femoral diaphysis.
S72.4	Fracture of the lower femoral epiphysis.
S72.7	Multiple femoral fractures.
S72.8	Fracture of other parts of the femur.
S72.9	Fracture of the femur, unspecified part.
T08	Fracture of the spinal column, section unspecified.

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Nutrition and osteoporosis. Calcium and vitamin D

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Summary

Calcium and vitamin D are essential nutritional elements in bone health throughout life, in the attainment and maintenance of peak bone mass. In the treatment of osteoporosis, an adequate intake of calcium and the repletion of vitamin D are critical for the maximisation, in terms of antifractural efficacy, of the response to osteo-active treatments: anticatabolics and anabolics.

The daily requirement for calcium is estimated to be between 1,000 and 1,200 mg and may be obtained relatively easily through a normal diet, or by means of food supplements. However, a substantial section of the population does not attain these required levels. In addition, patients with intolerance to milk, with limited gastric secretion due to their age, for autoimmune reasons, or due to the use of agents such as proton pumps which limit it, gastrectomy or other reasons, or malabsorption, make calcium supplements, nutritional or pharmacological, necessary. The requirements for vitamin D are estimated at 800-1,000 UI, but few foods contain this vitamin, and cutaneous synthesis, even in sunny regions, is insufficient to obtain blood levels of 25 (OH)D [marker for the status of vitamin D in the body] above the 30 ng/mL necessary for an optimum biological response in the bone and other target organs and tissues. This means that it is practically always necessary to supplement it through reinforced foods or with pharmacological vitamin D.

Key words: *calcium, vitamin D, proton pump.*

Introduction

Most nutrients and food components which we consume in our daily diet in Spain which act on the metabolism or structure of bone, through endocrine-paracrine actions and by modifying homeostasis of calcium or other bioactive mineral elements of the bone, have a considerable positive or negative effect on bone health[1]. Thus, nutrition should form a part of public health strategies for the prevention, and also the treatment, of osteoporosis. These dietary factors include inorganic minerals, calcium, phosphorus, magnesium, sodium, and potassium principally, and other trace elements, liposoluble vitamins A, D, E, K, and the vitamin B group, folic acid, vitamin C and macronutrients such as proteins and fatty acids.

Three recent reports highlight the importance of calcium and vitamin D in bone health: from the European Commission, on Osteoporosis in the European Community: Action for its Prevention[2]; from the ministry of health of the United States of America, on bone health and osteoporosis[3]; and from the World Health Organisation, on diet nutrition and the prevention of chronic diseases[4].

Below we review the evidence which supports the involvement of calcium and vitamin D in bone health and in the treatment of osteoporosis.

1. Calcium

Calcium is the most abundant mineral in the skeleton, approximately 1,000 g in the form of hydroxyapatite crystals, which contain 99% of the body's calcium and 80% of its phosphorus, and water ($Ca10\ (PO4)_6\ (OH)_2$). These two elements play an important role in the strength of bones and are of primordial importance in osteoporosis[5].

To achieve peak bone mass, and to prevent its loss with age, calcium is the most important nutrient. In addition, calcium has very important cell metabolism functions, and is fundamental to the normal functioning of a wide variety of tissues and physiological processes of the organism, for which reason a minimum concentration of Ca^{2+} should be maintained in the blood and in other extracellular fluids.

The skeleton constitutes the principal organic reservoir of calcium, where the element performs two basic functions: the maintenance of structural integrity and the regulation of metabolic function. Dietary calcium contributes to its homeostasis in the body, to the adequate mineralisation of the osteiod and to the maintenance of the mineral density and quality of bone.

A lack of dietary calcium never significantly affects cellular biological functions. The organism maintains normal levels of extracellular calcium by means of highly efficient mechanisms for the mobilisation of calcium from the bone, at the cost of a deterioration in its quantity, structure and quality.

The bodily requirements for calcium have been established on the basis of the dietary requirements of calcium for the bone, but should also cover the extracellular and intracellular needs of the other tissues[6]. At the present time, we have available a consistent combination of tests which endorse the importance of an adequate supply of calcium throughout a person's lifetime, which are summarised in a number of reports promoted by various health agencies[3,7,8].

The importance of calcium in the treatment of osteoporosis has also been established with precision, and combined with vitamin D, makes up the key component in any preventative or therapeutic regime for osteoporosis. The available evidence is reviewed below.

The clinical practice guide of the Spanish Society for Bone and Mineral Metabolism Research (SEIOMM) from 2008 established that supplements of calcium and vitamin D reduce the incidence of non-vertebral and hip fractures in women over 65 years of age with insufficient intake of calcium and vitamin D, and in institutionalised people. Those patients treated with antiresorptive or anabolic drugs should receive adequate supplements of calcium and vitamin D (recommendation A)[9].

1.1. Effect of calcium on fractures

Two recent meta-analyses were published in Endocrine Review[10] and by the Cochrane Foundation[11]. From the 66 documents published, the 23 RCTs (randomised clinical trials) were selected, out of which 16 were finally chosen in which the duration was longer than a year, which included only women, and in which bone mineral density included lumbar spinal column, hip, distal third of radius or the whole body with or without an evaluation of fractures.

15 RCTs were included, with 1,806 postmenopausal women over 45 years of age (amenorrhea at least 6 months). The women received a placebo or between 500 and 2,000 mg daily of calcium supplements (953 women) which included calcium gluconate, calcium carbonate, calcium citrate, with or without vitamin D. If they took vitamin D (placebo and/or control group) the initial dose should not be higher than 300,000 UI and the continuing dose should not be higher than 400 UI per day. For the analysis of the effect on fractures five studies were chosen which included 576 women. A non-significant trend towards a reduction in vertebral fractures was observed in the calcium group. The relative risk of vertebral fractures was 0.79 (CI 95%: 0.54-1.09, p=0.2), and the risk of non-vertebral fractures was 0.86 (CI 95%: 0.43-1.72).

Between these publications and the appearance of new meta-analyses a number of significant articles were published. In the RECORD study, in which were included some 4,700 older patients (over 70 years of age) with history of fragility fracture, no reduction in the risk of fracture after the administration of a gram of calcium, with or without vitamin D, was observed. The four arms of the study found no protector effect on new fractures[12]. It is important to highlight the fact that the average blood levels of vitamin D (25(OH)D) of the participants were low at the start of the

study (15 ng/ml) and with an average increase of 9 ng/ml in those who received 800 UI of vitamin D and of 1.6 ng/ml in those receiving only calcium. It is also notable that in this study a reason for exclusion was the taking of more than 200 UI of vitamin D or more than 500 mg of supplementary calcium, as well as the use of medication active on the bone.

Despite this, two years from the start of the study 5% of patients were taking medicines active on the bone and 2.8% openly taking calcium-vitamin D. From this study it may be concluded that calcium supplements (alone, or associated with vitamin D), in older patients, with a low level of vitamin D repletion and previous fragility fractures, are not effective in the prevention of new fractures.

In the WHI study[13], 36,282 postmenopausal women between the ages of 50 and 79 years were included, with an average daily intake of calcium of 1,100 mg, who received 1,000 mg of calcium element and 400 UI of vitamin D daily, divided into two arms (18,176 with active treatment and 18,106 with placebo). The incidence of fractures in the hip and other specified locations, was studied, comparing between groups. The use of calcitonin or biphosphonates was allowed and more than half the patients were on HRT (in accordance with the randomisation among women in the hormonal therapy trial). A reduction of 12% in the risk of hip fracture was observed in the group which took calcium + vitamin D, although it was not significant. There were no significant reductions in clinical vertebral fractures of the arm or wrist, or of total fractures. However, in the subgroup of women who adhered to the protocol, the risk of fracture was reduced (RR=0.71; CI 95%: 0.52-0.97), although taking into account the high number of patients who were taking other osteoactive medication, what may have happened is that the higher adherence to the calcium-vitamin D would also corresponded to a higher adherence to the rest of the medication.

More recently, Prince et al.[14] obtained some similar results and in an intention to treat analysis found that daily supplements of 1,200 mg of calcium do not significantly reduce the incidence of fractures, but does so when only the women adhering to treatment (56.8%) are analysed. This is a study with a follow up of 5 years, carried out in 1,460 Australian women over 70 years of age (average age 75), randomised, double blind and placebo-controlled. The treatment group received a tablet of calcium carbonate (600 mg) at each meal. 17.5% of the placebo group had suffered after 5 years at least one clinical fracture, as against 15.1% of those who received calcium supplements (HR=0.87; CI 95%: 0.67-1.12). Nor were there significant differences in the appearance of new vertebral fractures evaluated by densitometric morphometry (11.1% with placebo vs 10.2% with calcium. HR=0.95; CI 95% 0.78-1.17).

In the 830 women with good adherence to treatment (who took ≥ 80% of the tablets), the number of new fractures at 5 years was significantly lower in those who took calcium with respect to those of the placebo group (10.2% vs 15.4%. HR=0.66; CI 95%: 0.45-0.97). The difference was for a combination of any fracture, not specifically for hip fracture (0.7% with placebo vs 1.2% calcium), or for clinical vertebral fracture (2 vs 2.1% for placebo and calcium respectively). There was a tendency to a reduction in new vertebral deformities in the group with calcium (7.2% vs 10.5% with placebo. HR=0.83; CI 95%: 0.65-1.05). The analysis restricted to those women who complied with treatment was pre-planned in the protocol of the study. It should also to be noted that the average intake of calcium was approximately 900 mg/day in all the groups, and that in an analysis of a randomised subgroup of 81 women blood levels of 25(OH)D were 27 ng/ml (to convert to nmol/l, multiply by 2.5) on average in winter and 35 ng/ml in summer. None of these women had raised levels of blood PTH.

In the year 2007 three meta-analyses on the effects of calcium were published with apparently contradictory results.

Boonen et al.[15], with the objective of extending the results of the meta-analysis of Bischoff-Ferrari which showed that a dose of 700-800 UI daily of vitamin D would reduce the risk of hip fracture by 25%, examined the additional need of calcium in these results. After a systematic search and using a random effects model, 4 randomised trials (9,083 patients) were analysed which had a relative risk of hip fracture of 1.10 (CI 95%: 0.89-1.36) for vitamin D alone, without heterogeneity being found. The 6 trials of calcium and vitamin D (45,509 patients) showed a RR of 0.82 (CI 95%: 0.71-0.94), also without heterogeneity. An indirect comparison, adjusted, of the relative risks of the preceding meta-analyses for the RR of hip fracture with vitamin D and calcium as opposed to vitamin D alone was 0.75 (CI 95%: 0.58-0.96), which leads the authors to conclude that vitamin D appears to reduce the risk of hip fracture, but only when the supplementation is carried out with calcium.

Tang et al.[17], published a meta-analysis which includes randomised trials in which calcium or calcium plus vitamin D were administered in the prevention of fractures or of bone loss. In this study they drew data from 29 trials (n=63,897) and used a random effects model. In those studies whose outcome variable was the fracture (17 trials, n=52,625), the treatment was associated with a reduction in risk of 12% (RR 0.88, CI 95%: 0.83-0.95; p=0.0004). The reduction in risk of fracture (was) 24% higher in those trials in which adherence was higher (p=0.0001). The effect of treatment was also better when a dose of 1,200 mg or higher was used (0.80 vs 0.94; p=0.006), and when a dose of vitamin D higher than 800 UI/day (0.84 vs 0.87; p=0.03) was used. For the authors the evidence supports the use of calcium (1,200/day or more), alone, or accompanied by vitamin D (≥ 800 UI/day) in the preventative treatment of osteoporosis in people over 50 years of age.

The third meta-analysis contains data truly contradictory with the previous analyses. Bischoff-Ferrari et al.[17], after publishing an earlier meta-analysis[18] which evidenced the beneficial effects of vitamin D at doses higher than 600-800 UI/day on non-vertebral fractures and those of the hip, and participating in the meta-analysis of Boonen[15], presented in a new meta-analysis the evaluation of the effect of the intake of calcium on the risk of hip fracture, including cohort studies and clinical trials. In women (7 prospective cohort studies, 170,991 women with 2,954 hip fractures), no association was found between the total intake of calcium and hip fracture (RR for each 300 mg of calcium/day 1.10; CI 95%: 0.97-1.05). In men (5 prospective cohort studies, 68,606 men, 214 hip fractures), the RR per 300 mg of daily intake of calcium was 0.92 (CI 95%: 0.82-1.03). Based on 5 clinical trials (n=5,666 women and 1,074 men) with 814 non-vertebral fractures which compared calcium supplements (800-1,600 mg/day) with a placebo, it was 0.92 (CI 95%: 0.81-1.05). In the 4 trials which had separate trials for hip fracture (6,504 subjects with 139 hip fractures) the RR between calcium and placebo was 1.64 (CI 95%: 1.02-2.64). The sensitivity analysis including 2 small additional trials or protocol results, did not modify the results, for which reason Bischoff et al. suggested that calcium intake is not significantly associated with hip fracture. The combination of the results of the controlled trials did not show a reduction in the appearance of hip fractures, it even being possible that their incidence increased with the supplements. On non-vertebral fractures, the effect was neutral in the controlled trials.

Therefore, although the supplements of calcium and vitamin D appear to clearly reduce the incidence of non-vertebral and hip fractures in women over 65 years of age with an insufficient intake of calcium and vitamin D, and in institutionalised people, the effects of calcium alone on osteoporotic fractures are not well demonstrated, which means that further studies with better quality methodologies are necessary.

1.2. Effect of calcium on bone mass

The review by the Cochrane[11] revealed that the administration of calcium is more effective than the placebo in reducing the rate of bone loss after two or three years of treatment.

Calcium supplements by themselves had a reduced positive effect on bone density. Small but significant effects were found of the calcium supplements on bone loss during a period of two years, and a greater effect was observed for calcium citrate on total bone mass and of that in the hip, but with an opposite tendency in the spinal column.

In the WHI study higher bone mass values were observed in the group with received calcium and vitamin D with respect to the placebo, throughout the period of the study (9 years); at the end of this period bone mass remained stable in the total hip in the group with calcium and vitamin D vs a loss of 1.3% in the placebo group[13].

The supplementation of milk products (800 mg of calcium and 240 UI of vitamin D) is associated with a reduction of 50% in the loss of bone mass after two years, accompanied in the treated group by a decrease in the values of PTH and an increase in values of vitamin D[19].

In women who took calcium, at 5 years the following was observed: 1) in ultrasound of the calcaneum and in analysis adjusted for age, the body mass index (BMI) and compliance in taking the tablets, there was a significant increase in BUA (Ultrasound Attenuation Index) and elasticity, but not in transmission velocity. 2) in the DXA densitometry, a lower loss of bone mineral content (BMC) and area, but not in BMD in the femoral neck and total body, either in the analysis without adjustment or in that adjusted for age, BMI and compliance with taking the tablets. There was no difference in other areas measured. 3) in the peripheral QCT at the radius there was a higher cortical volume, with a favourable effect on the resistance indices[14].

In the meta-analysis of Tang et al.[16] cited above, and in those trials in which the variable evaluated was the change in BMD (23 trials, n=41,419), the treatment was associated with a reduction of bone loss in the hip of 0.54% (0.35-0.73; $p<0.0001$) and in the spine, a reduction of 1.19% (0.76-1.61%: $p<0.0001$).

Those studies which investigated the effect of calcium supplements of atypical origin, such as oyster shells, sea weed, powdered egg, vitamin supplements, etc, described minimum changes in bone mass or of markers for bone remodelling when compared with a placebo, and no difference when compared with calcium carbonate[20-22].

Calcium supplements, especially if associated with vitamin D, are efficacious in the reduction of loss of bone mass.

1.3. Effect of calcium on markers for bone remodelling

In a randomised study which included 99 postmenopausal women (66 years of age and with 15 years of menopause), no significant changes were observed in bone mass in the long term, nor in the values of PTH, in women who received 1,450 mg calcium plus 400 UI of vitamin D, with respect to a group of patients who received dietary instruction to achieve a calcium intake higher than 800 mg/day, with the ideal aim of achieving 1,450 mg. A greater decrease in PTH observed in the two groups with supplements was only observed in the first year of treatment[23]. This study would support the similarity of effects of dietary and medicinal calcium.

In the study of Prince et al.[14] a significant reduction was observed at 5 years in blood levels of PTH in the group treated with calcium compared to those treated with a placebo. In a study of 30 young women without metabolic bone disease, the administration of calcium, divided into two or four doses over the course of the day, influenced neither the PTH response nor that of the markers for bone remodelling[24].

The effects of calcium were independent of whether it was taken in the morning or at night. The calcium supplements had no significant effects on the markers for bone resorption, nor for formation. These results cannot be extrapolated to postmenopausal women with osteoporosis.

A small heterogeneous study showed that changes in calcium intake modify, in the short-term, the circadian rhythm of bone resorption[25]. In another prospective, randomised, double blind, factorial study[26] the differential effect of 300 mg calcium daily – in two formulations of skimmed milk - on markers for bone remodelling in healthy postmenopausal women (n=117; aged between 49 and 71 years, with 10 or more years postmenopause) was determined; their previous dietary intake of calcium was less than 750 mg/day. Group A (34 people) were administered skimmed milk fortified with calcium, phosphorus, lactose and vitamin D_3 (1,200 mg of calcium and 5.7 micrograms of vitamin D_3 each day). Group B (39 people) were administered skimmed milk fortified with vitamin D (900 mg of calcium and 5.7 grams of vitamin D_3 daily). The bone alkaline phosphatase was not modified. In both groups the PICP showed a significant reduction during the study, but without any difference between the groups. No differences were observed in NTx and only small differences were observed in Pyridinoline and D-Pyridinoline. The average value of 25(OH)D, which was observed after 6 months, increased by 5.56 ng/ml in group A and decreased 1.005 ng/ml in group B.

Taking all the available data together, the calcium supplements appear to have little effect on the markers for bone remodelling.

1.4. Adverse effects of calcium

In the RECORD study the gastrointestinal symptoms were more acute in the calcium group (16.4%) compared with the vitamin D group (11.9%)[12]. In the WHI study a significant increase in the appearance of renal lithiasis was observed (RR 1.17; 1.02-1.34) in the group which received supplements of calcium carbonate and vitamin D, with a baseline intake of calcium of 1,100 mg/day and receiving 1,000 mg of calcium and 400 UI of vitamin D.

A recent meta-analysis using data from 5,500 women who participated in trials with calcium monotherapy would suggest that the risk of hip fractures increases (RR of 1.5, CI 95%: 1.06-2.12)[14].

Bolland et al.[27], in a secondary analysis using data from an earlier trial published two years before by the same group in the American Journal of Medicine, evaluated in 1,471 postmenopausal women with an average age of 74 years, the risk of acute myocardial infarction and stroke, or both, with 732 taking calcium supplements and 739 a placebo. They had a greater risk of suffering an acute myocardial infarction (RR 2.12, CI 95%: 1.01-4.47) and a greater tendency to suffer a cardiovascular event of the three types evaluated (acute myocardial infarction, sudden death, or stroke).

This conclusion has generated great controversy, with supporters[28] and critics[29], and suggests a review of the appropriateness of administering calcium as a monotherapy or associated with vitamin D, as well as determining what the optimum dose should be so as not to cause harmful cardiovascular effects, and, in any case necessitates new studies which include these variables as primary objectives.

1.5. Physiology of calcium absorption

In addition to the quantity of calcium provided by the diet, the absorption of dietary calcium is a critical factor in determining its biological availability, which means that it is essential to review how this happens.

Calcium from food is found in the form of salts and/or is associated with other constituents in the form of complexes or ions of calcium (Ca^{2+}). In physiological conditions it is absorbed mainly in the small intestine, which is responsible for 90% of the absorption, progressively decreasing in the duodenum>jejunum>ileum.

The capacity of the small intestine to absorb calcium contained in the diet depends also on the amount of calcium present, the solubility and ionisation of the calcium salts, both pH-dependent, and the availability of vitamin D. But not all the salts and complexes of calcium dissolve or ionize in the same proportion. For example, it is a paradigm that calcium carbonate is barely soluble at a high pH, and that gastric acid is critical for its absorption[30].

Various factors affect the efficiency of intestinal absorption of calcium, which depends on the physiological needs of the organism. When these increase, the efficiency of the absorption does the same; thus growth, pregnancy and lactation stimulate the intestinal absorption of calcium, while aging reduces it. For these physiological adaptation mechanisms to meet the necessities of the organism, an adequate level of vitamin D is required. Thus, a low supply of calcium in the diet in relation to the needs of the organism, increases the proportion of intestinal calcium absorbed by means of a mechanism which modifies the metabolism of vitamin D, lipid composition and the fluidity of the intestinal membranes.

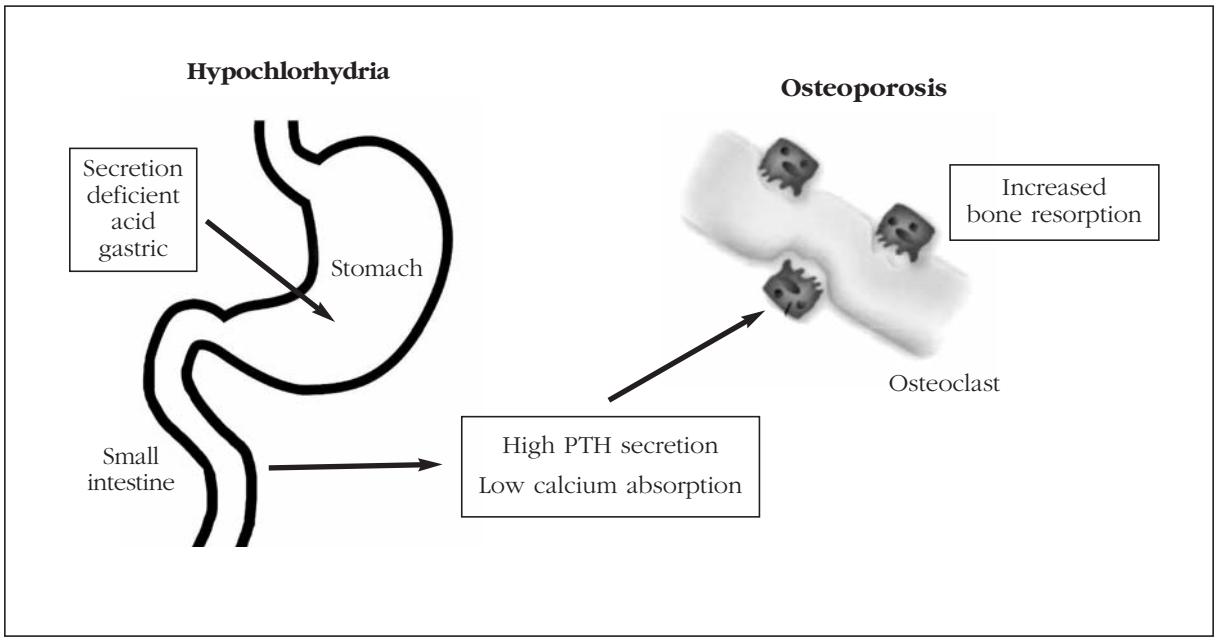
The absorption of dietary calcium, generically, diminishes with a higher content of fat, fibre, phytates, oxalates or caffeine, and increases with lactose and the protein content of the diet[31].

1.5.1. Gastric secretion and absorption of calcium

The absorption of calcium ingested in food or in dietary or pharmacological supplements depends to a great extent on the gastric secretion of hydrochloric acid.

The highly acid medium of the stomach, and less so of the proximal duodenum, is a fundamental endogenous factor in releasing the ingested calcium from the matrix of the food and in facilitating its intestinal absorption. Most of the salts or compounds of calcium require hydrochloric acid to be converted into soluble ionic calcium (Ca^{2+}) in

Figure 1. Calcium from foods is mostly absorbed in the small intestine under the influence of vitamin D. For it to be absorbed it is necessary for it to be dissolved and ionised in the stomach and proximal duodenum by the action of the hydrochloric acid in the stomach. Hypochlorhydria for whatever reason reduces the ionisation of calcium and therefore its availability to be absorbed. The resulting hypocalcemia increases the secretion of the parathyroid hormone (PTH), which increases bone resorption and contributes to the development of osteoporosis



such a way that if the secretion of gastric acid is inhibited or suppressed the calcium salt is not sufficiently dissociated in the stomach or proximal duodenum, resulting in a malabsorption of calcium, with a negative organic balance of calcium and loss of bone quality and quantity[32]. (Figure 1).

An increase in the secretion of gastric acid corresponds with a higher solubility and better absorption of calcium, which diminishes during fasting, as well as in patients with reduced gastric secretion for whatever reason, and which is proportional to the capacity of dissociation of the calcium salts[33-35].

For example, the absorption of calcium in patients with achlorhydria, is significantly higher with calcium citrate than with calcium carbonate (Recker, 1985). In achlohydric patients, the average absorption of calcium citrate was some ten times higher than with calcium carbonate (calcium: 0.453 ± 0.88 vs 0.401 ± 0.038 in blood and 0.047 ± 0.009 vs 0.052 ± 0.018 in urine)[33]. The absorption of calcium citrate during fasting has been shown to be higher than that of lactogluconate and of calcium carbonate in different studies and using various techniques, which implies a lower participation of the gastric acids, due to their better dissociation and ionisation[36-38].

The importance of gastric excretion in the absorption of dietary calcium is critical, and has great clinical importance in patients with hypochlorhydria or achlorhydria, for whatever cause: destruction or loss of physiological functioning of the gastric parietal cells, autoimmunity, associated with aging; iatrogenic, due to total gastrectomy or bariatric surgery using bypass techniques[39], or due to medical treatment with protein pump inhibitors (PPI), or histamine H2 receptor antagonists, used for the treatment of gastro-oesophageal reflux or gastric ulcers. (Figure 1).

Treatment with omeprazol reduces significantly the absorption of calcium carbonate taken when fasting in postmenopausal women aged between 65 and 89 years[40]. Although there are some discrepancies between authors[41], this action is consistent with data published recently in animals deficient in TCIRG1, which codes for a basic component of the proton pump for the maintenance of the stomach's acidity[32] and explains the association described between the use of PPI drugs and/or histamine H2 receptor antagonists and osteoporotic fractures.

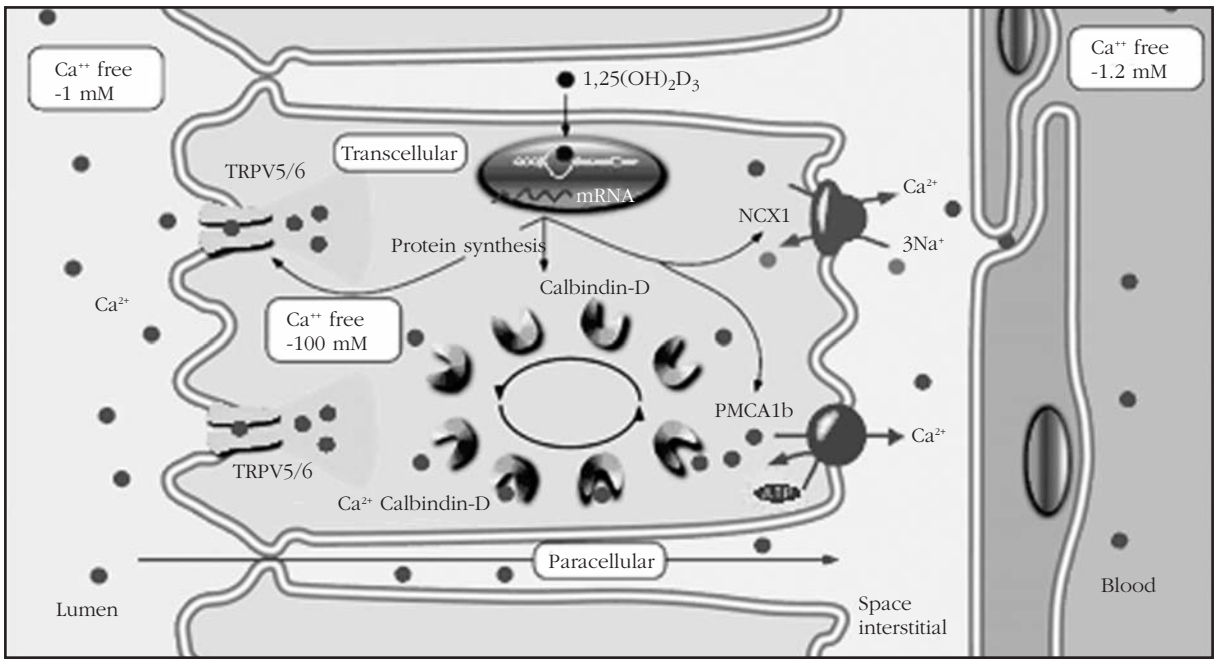
A case controlled study with a small number of cases (n=356) evaluated the association between the taking of histamine H2 receptor antagonists (cimetidine) and hip fractures with an adjusted odds ratio (OR) of 2.5 (1.4-2.6)[42].

Of greater importance is the association described between the use of PPI and osteoporotic fractures[43], evaluated in three case controlled studies[44-46].

Evaluating all fractures in patients in the United Kingdom older than 50 years of age who had used PPI for more than a year Yang et al.[44] found an adjusted odds ratio=1.44 (1.30-1.59).

The duration of treatment and average daily dose was associated significantly with the risk of fracture, >1.75 times the average, and during more than a year of treatment the adjusted odds ratio was 2.65 (1.80-3.90).

Figure 2. Once in a soluble and ionised form the Ca^{2+} is absorbed through the intestinal epithelium by two transport mechanisms: 1) transcellular, active, metabolically controlled by vitamin D and 2) paracellular, passive, non-saturable, through the hermetic bonds between the cells and driven only by the electrochemical gradient of the Ca^{2+} (Modified from Hoenderop, 2005)



In Danish patients, and considering only hip fractures, Vestergaard et al.[45] found an adjusted OR=1.18 (1.12-1.43) for the use of PPI for the year before the study.

However, a study carried out in Manitoba, Canada, which included vertebral, wrist and hip fractures in patients over 50 years of age, the relationship between the taking of PPIs and osteoporotic fracture was not significant after up to 7 years of continuous treatment (adjusted OR=1.92, 1. 16- 3.18)[46].

With the evidence available at the present time, in patients who comply with appropriate indications for treatment with drugs which inhibit gastric secretion (e.g. gastro-oesophageal reflux, gastroduodenal ulcers, treatment of *Helicobacter pylori*, dyspepsia and gastritis) and at the correct dose, and with the experience of intervention studies which confirm the association of drugs which inhibit gastric secretion with the reduced absorption of calcium and its impact on osteoporotic fractures, it is not possible to indicate the withdrawal of these treatments. However, due to the great impact which they have on the absorption of calcium, we need to be highly rigorous in the indications for use, dosage and duration of their use.

In any case, in these patients, it is necessary to enhance the obtaining of calcium through the diet, essentially through milk or its derivatives, given that the calcium contained in these products is dissociated enzymatically with greater ease[35], and that lactose and milk proteins favour its absorption; if unavailable, we need to use easily ionisable salts of calcium such as calcium citrate, gluconate or pidolate, and which can be taken between meals. Calcium carbonate should always be administered with a meal.

In patients who have been gastrectomised for whatever reason, or with demonstrable evidence of functional alterations in the parietal cells, autoimmune or associated with aging, similar should be indicated action.

1.5.2. Intestinal absorption of calcium. Epithelial transport

Once in a soluble and ionised form, the calcium is absorbed through the intestinal epithelium by two transport mechanisms: transcellular, controlled metabolically, and the other, non-saturable passive, by means of the hermetic seals between the cells, driven only by the electrochemical gradient of Ca^{2+}, called paracellular[47,48]. (Figure 2).

1.5.2.1. Paracellular transport of calcium

The intestinal epithelium is configured by a continuous layer of individual cells with narrow intracellular spaces between them, which allows the diffusion of ions and small molecules[47,48]. The paracellular route needs to be regulated by the epithelium to maintain a selective permeability. The hermetic seals are a barrier to movement by this route, and are a specialised part of the membrane located in the apical region of the enterocyte.

The movement of the Ca^{2+} through the hermetic cellular seals is a passive process which happens when the diffusible calcium which reaches the lumen of the small intestine is normal or high.

Therefore, it is when the calcium salts are more susceptible to dissociate themselves into diffusible

Ca^{2+} that the flow of calcium through this path is highest. The physiological regulation of the paracellular route is not controlled by the endocrine system of vitamin D, as the transcellular route is, but its absorption depends on the dietary supply of diffusible Ca^{2+}.

1.5.2.2. Transcellular transport of calcium

The active transport of calcium through the cell (transcellular) in the small intestine takes place in three stages: 1) entry of Ca^{2+} through the (hetero) tetrameric epithelial Ca^{2+}, channels TRPV5 and TRPV6, located on brush border; 2) bonding of Ca^{2+} to calbindin D9K with which it spreads up to the basolateral membrane, where 3) by means of a ATP-dependent Ca^{2+}-ATPase pathway (PMCA1b) and an Na^{+}/Ca^{2+} (NCX1) interchange it is expelled into the intracellular space. Hence a net absorption of Ca^{2+} is produced from the intestinal lumen into the extracellular compartment. (Figure 2).

The entry of Ca^{2+} through the apical membrane of the enterocyte is facilitated significantly by the electrochemical gradient, because the concentration of Ca^{2+} within the cell (10^{-7} to 10^{-6} mol/L) is considerably lower than in the intestinal lumen (10^{-3} mol/L), and the cell has an electronegative potential in relation to the intestinal lumen. Therefore, the movement of Ca^{2+} through the apical membrane has no energy cost.

However, each step in the transcellular movement of Ca^{2+} has a component dependent on 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$ or calcitriol], which is a function of the status of vitamin D in the body (blood levels of $25(OH)D_3$). Although $1,25(OH)_2D_3$ induces the expression of the calcium channels, calbindin and extrusion systems, it is thought that calbindin D_{9K} is the most limiting molecule to the transcellular transport of calcium[47,48].

When the supply of Ca^{2+} is sufficient, the synthesis of $1,25(OH)_2D_3$ is inhibited and the transcellular transport is saturated, which means that the paracellular absorption mechanism becomes predominant, while on the other hand, it is when the supply of Ca^{2+} is limited that the saturable transcellular mechanism plays the predominant role.

If there is a high quantity of the type of salt which is less soluble and ionisable, it may be sufficient to saturate the transcellular mechanism , but not sufficient to significantly enhance the paracellular transport. However, if a food or a soluble and ionisable calcium salt is administered, once saturated, the transcellular process may continue to absorb it through the paracellular mechanism. This circumstance is illustrated by the work of Sheik et al.[49], in young people, in whom an increase in the intake of calcium in foods from 502 to 1,071 mg/day results in a doubling of its absorption.

With aging, the adaptive physiological mechanisms for the enhancement of the absorption of calcium are highly deteriorated. The availability of vitamin D is greatly reduced, and the process for the gastric conversion of calcium into Ca^{2+} is generally not efficient, which means that it is advisable that the supply of calcium be provided through foods which contain easily diffusible and ionisable salts.

In addition to the main absorption of calcium (90%) in the small intestine, a residual but important part happens in the colon, which may be enhanced by acid fermentation. Various constituents of foods have classically been considered enhancers to the absorption of calcium, notably some of the components of milk such as lactose, lactulose and casein phosphopeptides[50], and some oligosaccharides[31].

1.5.3. Provision of calcium through dietary ingestion

In most Western countries, including Spain, the highest proportion of dietary calcium (60-70%) comes from milk and its derivatives, yoghurts or cheeses[51]. With the exception of almonds and other nuts, some blue fish and small fish such as whitebait or anchovies eaten with their bones, octopus, some vegetables such as chard, cardoon, lettuce, curly endive, endive, spinach or beet tops, normal food products have little calcium (Table 1), except for flour which may have been enriched with calcium[52].

The evaluation of the dietary intake of calcium may be carried out by a self-filled survey, using a questionnaire which contains all the contents shown in Table 1. A record of all the foods eaten each day is taken over seven days, and using a simple calculation the average daily amount of calcium taken every day is made. Although this procedure has the possibility of bias on the part of the patients, whose answers generally tend to be on the high side, it is a procedure which is easy to manage and acceptable for normal clinical practice[53].

When an evaluation of this kind is made and dietary advice given, it is important to consider the new types of milks and supplemented milk derivatives with a range of types and quantities of calcium, which increase in varying amounts the dietary supply of calcium.

The amount of calcium contained in mineral waters should also be evaluated[54,55]. It is also worth considering that as well as supplying calcium, waters rich in calcium bicarbonate, due to their effect on the acid-base equilibrium and the calcium-phosphorus homeostasis, are more healthy than others which may contain other calcium salts[54-57].

1.5.4. Influence of dietary calcium on the treatment of osteoporosis

The ingestion of foods rich in calcium and/or calcium supplements is fundamental for the maintenance of a positive calcium balance and consequently, for skeletal integrity, and is recommended for the prevention of osteoporosis and its fractures by all the agencies and scientific societies[58].

However, the influence and importance of dietary calcium in the prevention of osteoporotic fractures is open to discussion[59]. A paramount pro-

Table 1. Calcium content in rations used in the habitual diet

Portion size	Foods	mg calcium
Milk products		
1 cup (200 ml)	Whole, semi-skimmed, skimmed milk (with or without vitamin D)	250
1 cup (200 ml)	Milk supplemented with calcium	320
1 container (125 g)	Normal, bio, fruit, skimmed, junket	225
1 container (125 g)	Yogurt or junket with calcium	250
2 slices (50g)	Semi-cured Manchego cheese	400
1 piece (100 g)	Burgos cheese	300
1 piece (100 g)	Curd cheese	100
2 slices (50 g)	Creamy cheese like Brie or Camembert	200
2 slices (50 g)	Emmental, Edam, Parmesan, Gruyere, cured Manchego	550
2 slices (50 g)	Sandwich cheese	125
1 portion (20 g)	Creamy cheese in triangles like “El casiero”	55
1 small jar	“Petit Suisse” type	60
1 portion/container	Creme caramel, custard desserts, rice pudding, creamy ice cream...Cereals	120
Cereals		
100 g	White or wholemeal bread	30
1 ración	Pastries (2 medium madeleines, 1 croissant, 1 “ensaimada”, 4 “Maria”- type biscuits, etc...	120
Fruits and vegetables		
200 g	1 half orange or two medium mandarins	50
1 plate	Chickpeas, beans, in stews (“potage”, “cocido”, “fabada”)	75
1 plate	Chard, cardoon (approximately 200-250 g)	250
1 plate	Spinach, beet tops (“grelos” and “nabizas”)	150
1 plate	Lettuce, curly endive, endive	40
1 plate	Green beans	140
1 plate	Cabbage (“col” and “repollo”)	75
Fish		
1 plate (200 g)	Fresh sardines, anchovies, herring	100
1 can	Tinned sardines	200
1 plate	Small fish with their bones (anchovies, etc...)	80
1 plate	Squid, prawns, shrimps (150g)	100
1 plate	Octopus (150g)	170
1 plate	Other fish - hake, monkfish, etc...	50
1 plate	Clams, mussels, snails, barnacles	40
Meat		
1 plate	Meat (steak, quarter chicken, 100 g of other meats)	30
Various		
1 portion	5 Figs, filled with almonds or hazelnuts	50
1 small plate	Olives	50
1 egg		30

blem is the necessity for major studies to provide consistent evidence of this relationship given that the effect is probably modest. A recent meta-analysis reported that a low intake of milk products was associated with a higher risk of fracture, although this only became statistically significant in the case of those aged over 80 years[60].

Another significant problem is that there are few studies in which only calcium is administered, without vitamin D, whether as a supplement in milk[26], or as a pharmacological supplement. In a study of 1,471 postmenopausal women treated with a gram of calcium citrate daily over five years, although the BMD increased the study showed no significant reduction in the risk of fracture[6]. In other prospective clinical trials calcium increased BMD in postmenopausal osteoporotic women[62].

Bischoff-Ferrari et al.[63] in a meta-analysis which included five clinical trials (5,666 women and 1,074 men, with 814 non-vertebral fractures), reported that the aggregated RR of non-vertebral fractures of those supplemented with calcium (800-1,600 mg/day) vs the placebo was 0.92 (0.81-1.05). When 4 clinical trials with separate results for hip fracture were considered (6,504 subjects with 139 hip fractures) the aggregate RR between calcium and placebo was 1.64 (1.02-2.64). This led the authors to conclude that dietary calcium or calcium taken as a supplement does not prevent the risk of hip fractures in men or in women, and in evaluating intervention studies it may even increase them by up to 64%.

However, other results are given by the meta-analysis of Tang et al.[16] which included 29 studies with 63,897 patients, 92% women with an average age of 67.8 years. The effects of calcium alone or in combination with vitamin D were analysed in 16 and 13 trials respectively. These studies, which included 5 describing the effects of treatment on fracture, 12 on BMD and 12 on both, although no studies with dietary calcium were used, indicated that calcium alone or in combination with vitamin D is associated with a reduction of 12% in the risk of fractures (RR=0.88, 0.83-0.95; p=0.0004), with a slight reduction in the diminishing of bone loss in the hip, 0.54% and in the spinal column, 1.2%. The vitamin D supplements ≤ 800 UI daily (20 μg) did not modify the actions induced by the calcium. The effect of the treatment was increased in institutionalised people, in people over 70 years of age, in thin people who had previously had a low dietary intake of calcium, and when the intake of calcium was ≥ 1,200 mg/day and a dose of vitamin D 800 ≥ UI/day was used.

The efficacy of the treatment observed in the meta-analysis also increased when compliance was high (24% reduction in risk of fracture when compliance was higher than 80%). Poor compliance with treatments which provides calcium and vitamin D through supplements is a normal occurrence in the majority of clinical trials, which may explain, in part, the negative results of certain clinical trials[12,13] and justify the provision of dietary calcium.

The meta-analysis of Tang et al. is in agreement with Avenell et al.[64] and Boonen et al.[15].

The apparent inconsistencies between studies are essentially the result of various determining factors: 1) an appropriate compliance with recommendations, 2) variability in the absorption of calcium determined by factors such as the secretion of gastric acid or the influence on the absorption of other food components, 3) the possible modulation of the risk of fracture by other dietary factors, such as the taking of a sufficient quantity of proteins, the dietary composition of the food in general or the status of vitamin D in the body, of great importance, not only in the transcellular intestinal absorption of calcium, but also on musculo-skeletal function and its direct action on bone health, modifying the risk of fracture.

Together, the evidence supports the recommendation for the use of calcium (≥ 1,200 mg/day), and preferably accompanied by vitamin D (≥ 800 UI/day), in the preventative treatment of osteoporosis in people over 50 years of age and endorses the recent NIH consensus indicating the importance of calcium supplements in reducing the risk of osteoporosis[65].

On this basis, the guide to clinical practice of the Spanish Society for Bone and Mineral Metabolism Research (SEIOMM) of 2008 established that supplements of calcium and vitamin D reduce the incidence of non-vertebral and hip fractures in women over 65 years of age with an insufficient intake of calcium and vitamin D, and in institutionalised people. It was established, with a grade of recommendation of A, that those patients treated with anti-catabolic or anabolic drugs should receive adequate supplements of calcium and vitamin D[9].

In 2006 the North American Menopause Society (NAMS) published a position document supporting the role of calcium in association with sufficient vitamin D, in the reduction of bone loss in peri-postmenopausal women, and in the reduction of fractures in women over 60 years of age with a low intake of dietary calcium[5].

NAMS recommends for the treatment of osteoporosis in postmenopausal women that they take 1,200 mg of calcium and 700-800 UI of vitamin D each day, which they estimate is enough to maintain sufficient blood levels of 25(OH)D from vitamin D (≥ 30 ng/mL) (see below). Foods are recommended as the preferred main source of calcium, with foods enriched with vitamin D as alternative sources[5].

Earlier, the clinical guide to osteoporosis in Canada, published in 2002, recommended taking, preferably in the diet, at least 1,500 mg of calcium and 800 UI daily of vitamin D[66], and the endocrinologists of the United States confirmed the requirements for vitamin D and established a daily intake of calcium of 1,200 mg[67].

More recently, the European guide for the diagnosis and treatment of osteoporosis recommends the use of at least 1,000 mg of calcium and 800 UI of vitamin D daily[60]. The National

Osteoporosis Foundation (NOF) in its guide for the prevention and treatment of osteoporosis supports the recommendation of the National Academy of Sciences (NAS)[68] and recommends that everyone should have an adequate intake of calcium, at least 1,200 mg each day, adding supplements to the diet when necessary, and 800-1,000 UI of vitamin D.

Intakes of calcium higher than 1,200-1,500 mg per day have limited potential benefits, and may increase cardiovascular risks or result in associated renal lithiasis[69]. Although the American and European agencies give 2,500 mg as a safe maximum daily intake of calcium, the possible appearance of cardiovascular effects or other adverse effects such as renal lithiasis, means that the quantity of calcium recommended as safe could probably be lower[27].

In any case, given the intimate relationship between the status of vitamin D in the body and the absorption of calcium, recommendations as to the levels of calcium intake should not be made generically, rather in relation to blood levels of vitamin D[70].

1.5.5. Intake of calcium in Spain. The necessity of improving the intake of calcium in Spain

The dietary intake of calcium is below the recommendations of the agencies and societies in most of the surveys carried out. When the surveys consider all food eaten, the intake of dietary calcium is 991 ± 359 mg daily for Orozco et al.[51], 1,074 ± 374 mg/day for Bruyere[71], 1,019 ± 470 mg/day for Quesada et al.[72] and 1,326 ± 588 mg/day for Úbeda[73].

The estimated intake of lactic calcium is 70%, and 30% in other foods, which means some 200-400 mg/day[51,71,72]. On this basis surveys have been carried out to calculate the intake of calcium derived from milk products, with an average consumption of milk products being report of 684 mg/day[51], 699 mg/day[74], 788 mg/day[75], 769 mg/day[76] 783 mg/day[77], 569 mg/day[78] and 909 mg/day[79].

A case controlled study of 410 patients (342 women and 68 men 83 ± 7 years with hip fracture vs 544 controls (339 women and 205 men of 77 ± 9 years) evaluated calcium intake derived from milk products which was 574 ± 326 in the controls vs 645 ± 359 mg/day in those without fractures (p=0.002)[80].

Calcium administered in the diet has various advantages over its pharmacological administration in the form of supplements, the most important of which is that it itself optimises gastric pH, which facilitates its absorption. The patient does not have the feeling that they are in treatment, which means a great improvement in quality of life and improving adherence, essential in chronic treatments.

We should note though that a patient who does not take milk products for whatever reason will not in most cases achieve the 400 mg of daily calcium obtained from other foods from the daily diet.

2. Vitamin D

More than 90% of vitamin D is provided to the organism by exposure to sun and something less than 10% from the normal or supplemented diet. Normal foods contain very little vitamin D, unless they are supplemented, and in Spain few are, and in minimum quantities. In the epidermis, the ultraviolet B (UVB) radiation with a wavelength of between 290 and 315 nm converts 7-dydrocholesterol by means of a photochemical reaction into previtamin D_3, which is rapidly converted into vitamin D_3. Excessive UVB irradiation does not produce vitamin D intoxication because the re-vitamin D_3 and vitamin D synthesised to excess are broken down in the skin into biologically inactive metabolites[81].

Although there is a family of products with vitamin D activity, generally speaking, when we talk of vitamin D we refer both to vitamin D_3 (colecalciferol) and to vitamin D_2 (ergocalciferol), the first produced in human beings, and the second obtained by the irradiation of ergosterol contained in yeasts.

The vitamin D from the diet, absorbed by chylomicron fraction or synthesised in the skin, and later also its metabolites, circulate bonded to a transporter protein (DBP). In the liver it undergoes hydroxylation by the action of 25 hydroxylase (25-Oase; CYP27A1) to form calcifediol ($25OHD_3$). Calcifediol has a high concentration and a long half-life, of two or three weeks, which is why it is used to evaluate the status of vitamin D in the body (see below), and it constitutes a suitable substrate for the formation of 1,25 dihydroxyvitamin D (1,25 $(OH)_2D$; calcitriol), a hormonally active metabolite of the vitamin D endocrine system[81-83].

In the plasmatic membrane of the tubular renal cells, the ($25OHD_3$)-DBP complex bonds with megalin, the protein which introduces the complex into the cell, where the $25OHD_3$ is released, and in the mitochondria, by the action of 25-hydroxyvitamin D-1αhydroxylase (1-αOHase; CYP27B1) 1,25 $(OH)_2D$ is synthesised, whose principal endocrine function is the maintenance of calcium homeostasis, essential in many metabolic functions, neuromuscular transmission and bone mineralisation, acting in the intestine, parathyroid, bone and kidneys[81-83].

In the intestine the $1,25(OH)_2D$ acts on the receptors of the membrane and binds with its nuclear receptor, the vitamin D receptor (VDR), forming the structure $1,25(OH)_2D$-VDR, which in the nucleus forms a heterodime with the receptor for retinoic acid (RXR) forming the complex $1,25(OH)_2D$-VDR-RXR in the nucleus, which bonds with response elements of vitamin D (VDRE) of various genes, among which are those of the epithelial canal of calcium, which facilitates the entry of calcium into the cell, and also the calcium binding protein (CaBP, calbindin 9K), which facilitates the translocation of the capillaries. $1,25(OH)_2D$ also facilitates the absorption of phosphorus in the small intestine[81-83].

The contribution of vitamin D is essential for the intestinal absorption of calcium through the saturable cellular pathway, above all when the calcium is provided through foods or not easily ionisable compounds. Calcium and phosphorus are essential for the correct production of mineralisation.

When a deficiency of vitamin D occurs there is a 15% drop in the absorption of calcium and up to 60% in that of phosphorus, reducing the level of ionised calcium in the blood, which is detected by the calcium sensors (CaSR) of the parathyroid glands, resulting in an increase in the expression, synthesis and secretion of the parathyroid hormone (PTH)[81,84].

The mission of PTH is to conserve calcium, increasing its proximal and distal tubular reabsorption and mobilising calcium from the bone. PTH increases the expression of a membrane protein, activator of the receptor of membrane ligand NFκβ (RANKL) in the osteoblasts. The osteoblast RANKL bonds with RANK from the plasmatic membrane of the monocyte pre-cursors in the osteoclasts inducing their transformation to mature osteoclasts, which bond to the bone, releasing hydrochloric acid and collagenase, reabsorbing bone and releasing calcium and phosphorus into the bloodstream[81-83]. The PTH in the kidney reabsorbs the filtered calcium and reduces the reabsorption of phosphorus, causing phosphaturia. In the kidney, the PTH and low level of phosphorus, which is also induced by PTH, are powerful stimulants to the formation of $1,25(OH)_2D$.

When there is an insufficient supply of calcium to the organism the $1,25(OH)_2D$ helps to maintain calcium homeostasis, acting on the VDR in the osteoblasts in which it induces in a similar way to PTH, the formation of the membrane protein (RANKL).

In addition to these target organs and endocrine actions which we may call "traditional" or "classic" which regulate bone and calcium-phosphorus homeostasis, the endocrine system of vitamin D has other auto-paracrine functions in the organism as a whole[81].

The majority of tissues and cells, normal or neoplastic, such as muscle, heart, brain, blood vessels, breast, colon, prostate, pancreas, skin and immune system, among others, possess VDR and calcifediol activator enzymes (25OHD) such as 1-hydroxylase (1- αOHase; CYP27B1), in these locations not regulated by PTH, to synthesise $1,25(OH)_2D$, and as happens in the kidney, inactivator enzymes such as 24 hydroxylase (24-OHase; CYP44A1) which catabolises both 25OHD and $1,25(OH)_2D$ to form, respectively, $24,25(OH)_2D$ and $1,24,25(OH)_3D$, and ends up forming calcitroic acid, soluble in water and biologically inactive.

The $1,25(OH)_2D$ bonds with its VDR with close affinity and regulates the transcription of approximately 3% of the human genome. It is involved in the regulation of cellular growth and maturation, inhibits the production of rennin and increases the secretion of, and sensitivity to, insulin, modulating the function of active B & T lymphocytes and macrophages, among other actions, which confer on it important implications for health[86].

2.1. Measurement of calcifediol (25OHD) as an indicator for the status of vitamin D in the body

The vitamin D endocrine system is critical, not only for the maintenance of bone health, but also of the entire organism taken as a whole, in which it ensures an adequate level of 25(OH)D, the metabolite with the longest half-life, and the essential substrate for the synthesis of calcitriol, both in the kidneys and in other cells or tissues, which means that the measurement of 25(OH)D is commonly accepted as the indicator for vitamin D status[84,85].

A fundamental problem in the determination of 25OHD is the precision and reproducibility of the methods available for its measurement[86]. In spite of the variability between the methods available to measure vitamin D, and although there is no widely accepted universal consensus on adequate levels of calcifediol, there is ever increasing agreement that a concentration of 25OHD >30 ng/mL (to change to nmol/L multiply by 2.5) constitutes the optimum level of vitamin D to ensure bone health[87]. Although, higher levels of calcifediol are probably required to ensure other health objectives[63]. The minimum desirable blood concentration of calcifediol should be higher than 20 ng/mL in everyone, which would imply an average of around 30 ng/mL in the population as a whole[88].

Patients are considered to have severe vitamin D deficiency when they have blood levels of calcifediol lower than 10 ng/mL, moderate deficiency or insufficiency when they are between 10 and 20 ng/mL, a suboptimum state for vitamin D we locate between 20 and 30 ng/mL of calcifediol in the blood, with the optimum being above 30 ng/mL. Suitable blood levels of calcifediol have not been clearly defined but it can be deduced that in populations exposed to the sun it is very difficult to exceed a blood concentration of calcifediol of 65-70 ng/mL[89].

Therefore, blood levels of calcifediol of between 30 and 70 ng/mL 25OHD appear to be the most physiologically appropriate, and therefore recommendable. In a review of thirty works there was no evidence of toxicity in patients with levels of calcifediol below 100 ng/mL. A minimum toxicity threshold has been suggested to be 200 ng/mL[90].

Inadequate levels of calcifediol in Spain

At present, the insufficiency and, frankly, the deficiency of calcifediol constitutes a pandemic which affects more than half the population, children, young people, postmenopausal women and old people. In this last group, if they have osteoporotic fractures the prevalence of hypovitaminosis D reaches 100%[84].

In Spain, this situation of inadequate levels of calcifediol is present (Table 2). The variations in the different methods used in different laborato-

ries makes a rigorous comparison difficult, but the table illustrates clearly that despite Spain having a benign climate for the synthesis of vitamin D, the levels are similar or lower than those described for central Europe or Scandinavia, as has been described in previous studies[72,91].

Attempts have been made to explain this apparent "paradox", that Spain shares with other countries of the Mediterranean basin[72], by the low dietary intake of vitamin D which cannot be compensated for by cutaneous synthesis. Most of Spain is above the latitude of 35ºN, where there is little possibility of synthesising vitamin D in winter and spring.

The insufficiency of vitamin D in Spain is not dependent on geographical zone (Table 2), given that low levels of vitamin D may be found independent of exposure to sun[92], with seasonal variations, but barely managing to become normalised after summer-autumn[93]. It is found in children and young people[94], persisting in adults[95-97], postmenopausal women[98,99], postmenopausal osteoporotic women[72,91], and old people who live at home, and even more if they live in a residence[93,100,102-104].

Factors contributing to low blood levels of calcifediol

The intake of vitamin D in Spain is far below the traditional recommendations of the FAO (United Nations Food and Agriculture Foundation) of 200 UI/day in infancy and adults up to 50 years of age, 400 UI in people from 51 to 65 years of age and 600 UI/day for those older than 65 years of age[106]. Lower even than the recent recommendations of the United States Department of Health which recommends as a minimum requirement for vitamin D 500 UI/day, which should be increased to 1,000 UI/day in people over 70 years of age, in people with dark skin and low exposure to sun, and those who are institutionalised[3].

In general, the intake of vitamin D is much lower in the countries of southern Europe, less than 200 UI on average, than in Scandinavian countries and in the United States, where it is nearly 400 UI daily due to the high consumption of blue fish, and where the supplementation of foods with vitamin D, essentially milk, milk products and flour, is mandatory[107].

In Spain, it is impossible to achieve the requirements of 800 UI daily recommended for the treatment of osteoporosis, only through the diet and without supplements. However, there is a wide belief among patients, but also among health staff, doctors and nurses that the ease of taking sun in most of the regions of Spain makes it unnecessary to take supplements.

However, as is shown in Table 2, for the great majority of the population the daily diet and normal, non-programmed, taking of sun is not sufficient to obtain optimum blood levels of vitamin D. To achieve them, it is necessary to take sun for at least 20 or 30 minutes, depending on the time of day and season, directly, without glass in between, and without the use of sun-blocking creams[84]. But it is not easy to find the available time to do this, and many people are not exempt from risks.

The cutaneous synthesis of vitamin D_3 depends on the season of the year. During the months of November to March north of the latitude 35ºN/S, that is to say in most of Spain, due to the increased angle of the solar zenith, most of the UVB photons are absorbed by the stratospheric ozone, making necessary a longer path in order to arrive at the earth's surface, which makes them inactive, and the synthesis of vitamin D very limited or zero[84].

The climate is a critical factor: whether the weather is suitable for taking sun. Climates which are too cold do not enable this since people will be fully clothed, and those which are too hot will make people avoid the sun. In older Spanish people lower levels of vitamin D have been reported for the summer months due to the high temperatures which occur in the southern cities of Spain during the summer, where they frequently exceed 35°C. The older people avoid being in the sun and prefer to be inside their houses where the temperature is more comfortable. In addition, older people are also very careful about the risk of skin cancer due to the direct exposure of the skin to the sun, but in autumn, or during the winter months, these regions benefit from more favourable temperatures (15-25°C), which allow them to take sun with light clothes and thus synthesise vitamin D[96,97,104].

Hyperpigmentation may reduce cutaneous production by up to nearly 100%, and this has been proposed as a cause of vitamin D deficiency in the countries of southern Europe (Lips, 2001). The use of sun-protection creams, which in the summer is a usual practice for the vast majority of the population, also reduces the formation of vitamin D. Neither is vitamin D_3 synthesised if the skin is covered for cultural, social, religious or any other reason[82-84].

Other common causes of a deficiency of vitamin D is obesity (body mass index >30) which, as is happening in other Western countries, is increasingly prevalent in our country, since the body fat captures vitamin D (Passeri, 2005). Another proposed cause recently reported is the use of xenobiotics and drugs which activate the pregnane receptors (PXR), and others which may increase the catabolisation of vitamin D and reduce its concentration in the blood[84].

Repercussions of vitamin D insufficiency in Spain

These data alert us to the fact that in Spain: 1) eating of foods is not sufficient to obtain adequate levels of vitamin D; 2) despite the general belief that is it easy to obtain vitamin D by a programme of sunbathing, the great majority of patients do not achieve adequate levels of vitamin D; 3) in the general population there is a high prevalence of insufficiency, and even of deficiency, in vitamin D, and what is even more "paradoxical" , in patients in treatment for osteoporosis[72].

The magnitude of the prevalence of vitamin D insufficiency, combined with its repercussions on bone health constitutes a significant public health problem. Its impact on markers for remodelling, bone mineral density, fractures and their potential impact on health in general are reviewed in-depth[85].

The anticatabolic agents most used in normal clinical practice are the biphosphonates (principally alendronate, risedronate, ibandronate and zoledronate) and the selective modulators for oestrogen receptors (raloxifene). And the anabolic agents are teriparatide and PTH 1-84, and in between them both strontium ranelate.

The efficacy of these drugs and their record has been demonstrated through major randomised clinical trials designed to verify their efficacy in reducing fractures. In all the pivotal clinical trials, calcium and vitamin D were administered to both the control and intervention groups and, in some trials, the repletion of vitamin D was a criterion used as a prerequisite for the inclusion of patients. So it is not possible to conclude the degree of efficacy of the drugs cited in patients depleted in vitamin D and/or with an insufficient supply of calcium.

For this reason, all the guides and therapeutic consensuses for the treatment of osteoporosis indicate treatment with calcium and vitamin D[79], which means that the majority of the pharmacological supplements of calcium come associated with vitamin D.

However, the taking of calcium and vitamin D are the elements with the lowest compliance in the medical treatment of osteoporosis[108], and in Spain in women treated for osteoporosis insufficient levels of calcifediol are found in 63%[85], similar to that observed in Europe[109] or the United States of America[110].

The ingestion of calcium is relatively easy achieved through the diet with the commitment and adherence of the patient to the dietary indications of their doctor, or by using supplemented milk products. The attainment of adequate levels of vitamin D through the diet is almost impossible, and repletion of vitamin D is therefore critical in order to maximise the response to anticatabolic treatment in terms of an increase in BMD or antifractural efficacy[111,112],

Adami et al.[112] studied 1,515 women with postmenopausal osteoporosis in treatment with antiresorptive agents (alendronate, risedronate, raloxifene) for a period of a little over a year (13.1 months) and a good adherence to treatment (>75%). The patients were classified as deficient in vitamin D (n=514) or replete in vitamin D (n=1001). The increase in BMD in the spinal column, femoral neck and total hip was significantly higher in women replete in vitamin D.

The adjusted incidence (for age, type of treatment, previous clinical fractures, intake of calcium and body weight) was 77% higher in women depleted in vitamin D (25(OH)D < 20 ng/mL) (odds ratio 1.77; CI 95%: 1.20-2.50 p=0.004) . These were similar to the results obtained in an earlier study (Adami, 2006), which found evidence that during anti-resorptive treatment the supplementation of vitamin D was a significant predictor of new fractures.

In conclusion, an optimum status of vitamin D during treatment of osteoporosis is necessary to maximise the response to anti-resorptive agents in terms of changes in BMD and anti-fractural efficacy.

Although there is an intercellular pathway for the absorption of calcium, this depends to a great extent on vitamin D. In young people, with blood levels of 25(OH)D < 10 ng/mL, a daily intake of calcium less than 800 mg is insufficient and leads to secondary hyperparathyroidism. For the higher levels of 22 ng/mL, an intake of calcium of 800 mg a day, while much less than the quantity of calcium recommended, is sufficient, given that to maintain the organic requirements of calcium it is not necessary to raise the levels of PTH[70].

It is important to be very clear that those patients who for whatever reason cannot take calcium supplements need to attain blood levels of 25(OH)D higher than 40 ng/mL to optimise the therapeutic response[113].

The cost of anti-resorptive treatments is so high in comparison with vitamin D that the attainment of optimum levels of 25(OH)D is efficient from a therapeutic point of view. Unfortunately, it is practically impossible to achieve these optimum levels of 25(OH) through diet which means that it is necessary to include supplementation as part of the treatment, which facilitates its achievement.

In any case, this makes it essential to promote active public health policies of education in healthy living, but above all to enhance the development of functional foods supplemented with calcium, as well as regular supplementation with vitamin D.

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Table 2. Status of vitamin D evaluated as blood levels of 25 hydroxyvitamin D (25OHD) in the Spanish population. SD: standard deviation. PCA: competitive protein assay. RIA: radioimmune analysis. HPLC: high performance liquid chromatography

Reference	Population studied	City	Season	Age (years)	Number	$25OHD_3$ average ± SD ng/mL	Prevalence levels serum low 25OHD	Definition low serum 25OHD ng/mL	Method
Quesada 1989	Both sexes Home	Córdoba 37º 6'	Spring	27 - 49 67 - 82 70 - 85	32 32 21	22 ± 11 14 ± 6 15 ± 10	32% 68% 100%	15	PCA
Quesada 1992	Both sexes Home	Córdoba 37º 6'	Spring	20 - 59 60 - 79 >8	81 31 17	38.0 ± 13 18 ± 14 9 ± 4.6			PCA
Mata-Granados 2008	Donors blood Men Women	Córdoba 37º 6'	Spring	18 - 65 18 - 64	116 9	18 ± 10.5 15 ± 9.2	14% 51% 65%	10 20 30	HPLC
Mezquita-Raya 2001	Women postmenopausal	Granada 37º 10'	Winter-Spring	61 ± 7	161	19 ± 8	39%	15	RIA
Aguado 2000	Women postmenopausal	Madrid 40º 26'	Winter-Spring	47 - 66	171	13 ± 7	87% 64% 35%	20 15 10	RIA
Lips 2001	Women postmenopausal osteoporotic	Spain 43º 37º	Winter-Spring	64 ± 7	132	24 ± 14	41,7% 10,6	20 10	RIA
Larrosa 2001	Both sexes Elderly Residence	Sabadell 41º 35'		61 - 96	100	10.2 ± 5.3	87%	25	RIA
Vaqueiro 2006	Both sexes Elderly Living at home	Sabadell 41º 35'	Winter-Spring	72 ± 5	239	17 ± 7.5	80% 17%	25 10	RIA
González-Clemente 1999	Both sexes Elderly Outpatients	Barcelona 41º 23'	Winter-Spring	75 ± 6	127		34,6%	10	RIA
Gómez-Alonso 2003	Both sexes Elderly Home Men Women	Oviedo 43º 22'	All year Winter-Summer	68 ± 9 68 ± 9 < 65 65 - 74 >65	134 134	17 ± 8 17 ± 9	72% 80% 72%	18	RIA
Pérez-Llamas 2008	Both sexes Elderly Residence	Murcia 37º 59'	All year Fall Winter Spring-Summer	77 ± 8	86	20 ± 1 25 ± 15 16 ± 9	58,2%	20	RIA
Docio 1998	Children Home	Cantabria 43º 27'	Winter Summer	8 ± 2	43	15 ± 5 29 ± 10	31% 80%	12 20	RIA
Pérez-Castrillón 2008	Both elderly sexes Living at home Residence	Valladolid 41º 38'	All year	75 ± 85 83 ± 7	197 146	15 ± 8 17 ± 7	31 79 32 91	10 20 10 20	RIA
Quesada 2007	Women osteoporotic postmenopausal Not Treated Treated	All Spain 43º 28'	Final spring	71 ± 5 71 ± 5	190 146	22 ± 10 27 ± 11	11% 44% 76% 5% 29% 63%	10 20 30 10 20 30	HPLC

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Profile of action of denosumab in treatment of osteoporosis

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Summary
The recent discovery of the RANK/RANKL/OPG system (RANK: Receptor Activator for Nuclear Factor B; RANKL: Receptor Activator for Nuclear Factor B Ligand; OPG: osteoprotegerin) as final effector in osteoporosis pathogenesis have lead to the development of new therapeutic strategies. Denosumab is a human monoclonal antibody that, like OPG, binds to RANKL preventing RANK activation, thus decreasing bone turnover and increasing bone mineral density. Denosumab is administered subcutaneously every 6 months. Clinical trials have demonstrated efficacy on bone mineral density and reduction of fractures in postmenopausal women and a favourable safety profile.

Key words: *postmenopausal osteoporosis, osteoprotegerin, RANKL, denosumab.*

Clinical case

A 60 year old woman who attended a clinic for a general preventative check, with an evaluation of possible osteoporosis. Personal history: multiple birth (3 children) with menopause at 51 years of age. Did not have climeratic syndrome, which meant that she did not require hormonal treatment. Moderate smoker of 15 cigarettes/day and occasional drinker. With sedentary job in office administration. Generally healthy, at 52 years of age after a fall in her doorway she had a right Colles fracture without complications. Family history: hip fracture in her mother at 78 years of age. Physical examination: weight, 60.6 kg; height, 159 cm (BMI 24). Complementary examinations: bone densitometry showed a T-score of -2.4 in the lumbar spine and -1.9 in the hip. According to the FRAX tool, the probability of major osteoporotic fracture after 10 years is 10%, and 2% for a hip fracture. Given these characteristics of the patient and her personal and family histories, and taking into account the risk of fracture at 10 years according o the FRAX index, it is considered recommendable that she adopt some hygiene measures, such as stopping smoking, taking exercise and control of weight, with the aim of not going below the ideal weight. In terms of pharmacological measures the administration of an antiresorptive drug such as denosumab is also contemplated, along with calcium and vitamin D supplements.

Introduction

The clinical case shows the importance of investigating the situation of bone mass in an apparently healthy, recently menopausal patient, during a normal health check. Her history of bone fracture and her risk factors suggest the presence of low bone mass, as the bone densitometry demonstrated.

Taking into account the impact of osteoporosis (OP) on the quality of life of patients and its general repercussions on society, we have considered it to be of interest to carry out this review concerning the monoclonal antibody denosumab, an antiresorptive drug recently developed following the discovery of the final effector in osteoporosis, the RANK/RANKL/OPG system[1].

Pharmacological action on the RANK/RANKL/OPG system. Denosumab

The pharmacological treatment of OP includes antiresorptive drugs such as the biphosphonates, raloxifene (and other selective estrogen receptor modulators - SERMs – such as bazedoxifene) and calcitonin; anabolic drugs such as teriparatide or whole molecule recombinant PTH; and dual action drugs such as strontium ranelate[2]. All these have been trialled during their clinical development with a range of doses of calcium and vitamin D as pharmacological supplements. From the knowledge of the RANK/RANKL/OPG system different ways of regulating the condition have been proposed[3,4]:

In relation to RANKL

- Inhibition of its expression: by means of 17ß-estradiol.

- Blocking RANKL: by means of OPG or OPG-like proteins, the application of neutralising proteins or anti-RANKL antibodies or generation of antibodies by autovaccination.

In relation to RANK

- By the interruption of its bond with RANKL or by the suppression of the postreceptor signal (by 17ß-estradiol).

In relation to OPG

- By increasing its endogenous production (by 17ß-estradiol, raloxifene, biphosphonates...), by transgenic overexpression of OPG or by the administration of OPG or OPG-like proteins.

Within this line of development is found denosumab (AMG 162) (DMAB), an anti-RANKL monoclonal antibody which prevents RANKL's action by impeding its coupling with its receptor RANK. In the development of the monoclonal antibodies, the first were 100% murine in origin, but given the problems this caused, antibodies have increasingly been developed with higher human content until they are completely human (100%), with more appropriate pharmacokinetic characteristics and better immunogenicity[5]. DMAB belongs to this last group and its clinical development began in 2000. It consists of an isotope of entirely human immunoglobulin IgG_2, with a high affinity with, and specificity for, RANKL[6] and which acts in a similar way to OPG, in that it prevents the interaction between RANKL and RANK and reduces the differentiation, activity and survival of the osteoclasts, thereby inhibiting bone resorption. One of the potential risks of the use of OPG molecules is the generation of antibodies which may react with the endogenous OPG. However, no anti-DMAB[7,8] neutralising antibodies have been observed, probably due to the structure of DMAB not being similar to that of OPG.

• Pharmacokinetics and pharmacodynamics of DMAB (Preclinical and phase I studies)

Although the pharmacokinetic properties of DMAB are not totally defined, considering the pharmacokinetic properties of the monoclonal antibodies, its saturable bond with the corresponding antigen would take a non-linear distribution and elimination[9]. In addition, based on studies with similar IgG antibodies, DMAB is probably absorbed by the lymph system and later drained into the vascular system[6]. Its bioavailability would be between 50 and 100% with a similar distribution to the plasmatic volume and clearly dependent on the reticulo-endothelial system[6]. A study of a single dose compared with a placebo showed the non-linear pharmacokinetics of DMAB[8], with a) a prolonged absorption which provided some maximum blood concentrations which increased disproportionately (2.6 times) in relation to the increase in dose, and which were observed 5-21 days after their administration; b) a prolonged half-life of up to 32 days; and c) and an average residence time in the blood of 12 to 46 days depending on the dose. At the recommended dose of 60 mg subcutaneously (see below), the time to maximum concentration is 26 days[10].

The action of DMAB is rapid, prolonged and reversible. The long duration of its effect may be attributed to its half-life and its osteoclast inhibitor effect. Preclinical models suggest that the delay in the recuperation of the osteoclasts after the suspension of the inhibition of RANKL appears to be due to the time needed for their regeneration from the precursor cells[11]. However, once the drug has been eliminated and the osteoclasts have been regenerated, its antiresorptive effect rapidly disappears. This reversibility distinguishes DMAB from the biphosphonates since, differently from them, it is not incorporated into the bone matrix[12]. As has been shown by the changes in markers for bone remodelling (amino-terminal telopeptide of type I collagen (NTx) in urine, and carboxy-terminal telopeptide of type I collagen in blood, among others), the subcutaneous administration of DMAB reduces the function of the osteoclasts, quickly (in between 12 and 72 hours) and sustainably (up to 6 months), as well as being reversible, as is shown by the increase in the aforementioned markers when the drug disappears from circulation, and recoverable when the therapy is reinstated

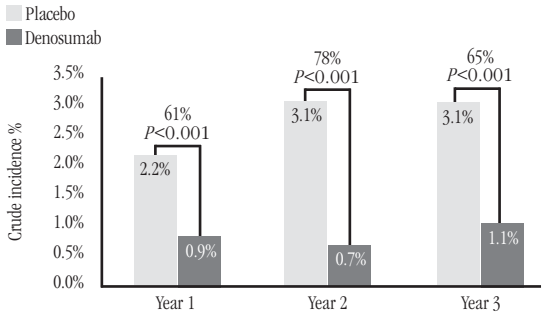
.

• Clinical efficacy of DMAB (Phase II and III clinical trials)

The efficacy and safety of DMAB was initially evaluated in the phase II study, a randomised, double blind, dose-ranging study, in which 412 postmenopausal women with osteoporosis received DMAB subcutaneously over 12 months, every three months (at doses of 6, 12, or 30 mg), or every 6 months (at doses of 14, 60, 100 or 210 mg), the treatment masked with weekly alendronate (at a dose of 70 mg) or placebo[7]. Treatment with DMAB was associated with a rapid increase in bone mineral density in the spine, hip and distal third of the radius which was higher than that observed with the placebo, and similar, even higher (in the hip and distal extreme of the radius) than that found with 70 mg of alendronate weekly[7]. From this study, the doses considered to be optimum were 30 mg/3 months and 60 mg/6 months, the latter being chosen for subsequent development[7]. The efficacy of DMAB has subsequently been confirmed in 4 phase III studies in women with osteopenia or OP. In a clinical trial with randomisation stratified as a function of the duration of the menopause (>5 years or ≤5 years), double blind, of two years' duration, carried out in 332 postmenopausal women with low BMD (T-score in lumbar spine – LS – of between -1.0 and -2.5), the efficacy of 60 mg subcutaneous DMAB every 6 months was compared with that of a placebo[14]. The DMAB increased significantly the BMD in the LS at 2 years in comparison with the placebo (6.5% vs. -0.6%, p< 0.0001), which was independent of the time passed since the menopause[14].

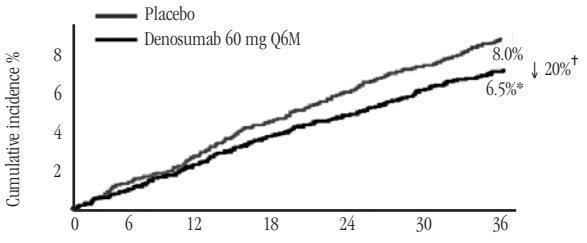
Figure 1. Time until the first fracture with DMAB as opposed to the placebo (Adapted from Cummings and cols.)

Figure 1A. The effect of denosumab on new vertebral fractures year on year
Phase III: FREEDOM study



Intention to treat, last observation carried out in the following analysis.
The percentage of new fractures is calculated using the number of patients with a radiological evaluation of the spine at the baseline and at least one other in the post-baseline period.
Adapted from: Cummings SR et al. N Engl J Med 2009;361:756-65

Figure 1B. Effect of denosumab on the time until the first non-vertebral fracture during 36 months.
Phase III: FREEDOM study

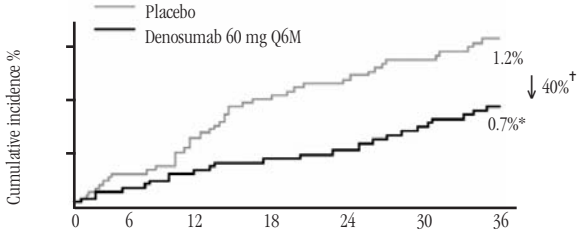


Number of patients at risk

Placebo, n	3.906	3.750	3.578	3.410	3.264	3.121	3.009
Denosumab, n	3.902	3.579	3.594	3.453	3.337	3.228	3.130

† Non-vertebral fractures were reduced by 20% (95% CI: 0.67, 0.95)
* *P*= 0.01
Adapted from: Cummings SR, *et al.* N Engl J Med 2009;361:756-65

Figure 1C. Effect of denosumab on the time until the first hip fracture during 36 months
Phase III: FREEDOM study



Number of patients at risk

Placebo, n	3.906	3.799	3.672	3.538	3.430	3.311	3.221
Denosumab, n	3.902	3.796	3.676	3.566	3.477	3.397	3.311

† Hip fractures were reduced by 40% (95% CI: 0.37, 0.97)
* *P*= 0.04
Adapted from: Cummings SR, *et al.* N Engl J Med 2009;361:756-65

Another randomised, double blind with double simulation trial carried out in 1,189 postmenopausal women with a T-score of -2.0 or less in the spinal column or total hip, compared the efficacy of DMAB at the same dose with that of alendronate of 70 mg weekly[15]. In this study, after a year of treatment, DMAB was higher than alendronate in the increase of BMD in total hip (3.5% vs. 2.6%, p< 0.0001) and in the other locations in the bone, with both drugs showing a similar safety profile. This apparent superiority of DMAB over alendronate in the increase in BMD has also been demonstrated in another clinical trial, double blind with double simulation, in which 504 postmenopausal women with a T-score in LS or total hip between -2.0 and -4.0, who had received 70 mg of alendronate weekly, orally, over one month, were randomly chosen to continue with weekly alendronate or to change to DMAB at a standard dose for one year[16]. One of the inclusion criteria in this study was whether the patient had been in treatment with weekly alendronate for at least 9 months continuously. The women who changed to DMAB experienced an increase in BMD in the total hip a year after treatment of 1.9% compared with 1.05% observed in the group which received alendronate (p<0.0001); the increases in BMD were also significantly higher with DMAB in the LS, femoral neck (FN) and distal third of the radius, with both drugs having a similar safety profile[16].

In a survey carried out in all the women patients from these last two trials it was found that those treated with DMAB administered subcutaneously preferred this treatment, were more satisfied with it, and that it bothered them less than the weekly oral treatment with alendronate[17].

Finally, within what has been the clinical development of DMAB in the treatment of OP it is worth noting a recently published randomised placebo controlled clinical trial, the FREEDOM study[18]. In this trial were included 7,868 women from 60 to 90 years of age with a T-score below -2.5 but not less than -4.0 to whom was administered DMAB at a dose of 60 mg every 6 months over 3 years. In comparison with the placebo, DMAB significantly reduced the risk of vertebral fractures (reduction of relative risk (RRR) of 68%) in any of the 3 years considered, even when separating (Figure 1A) non-vertebral (RRR, 20%) (Figure 1B), and hip fractures (RRR,40%) (Figure 1C).

In addition, treatment with DMAB was associated with a relative increase in mineral density in the LS and hip, as well as a reduction in markers for bone formation (PINP, amino-terminal propeptide of type 1 procollagen) as well as those for resorption (CTX), significantly in comparison with the placebo[18], already from the first month and during the whole time the trial lasted. The authors concluded that DMAB, due to its action as an inhibitor of RANKL, reduces bone resorption and increases bone mineral density, which means that it offers a valid alternative in the treatment of OP[18].

• Clinical safety

Due to the interference of DMAB with the RANK/RANKL/OPG system, and taking into account the fact that RANKL is expressed both in bone cells and in immune cells[19], the possible incidence of infections or neoplasms with this drug merits special attention. However, in the clinical studies published to date no significant differences have been observed between DMAB, the placebo and alendronate in relation to the notification of serious adverse effects, either in terms of infections or neoplasms. Only in one study were 6 infections which required hospitalisation reported in the DMAB group during its extension phase to 24 months[20]. However, all these corresponded to infections acquired in the community, did not follow a specific common pattern of infection and responded adequately to standard antibiotic treatment[20].

The aforementioned FREEDOM study[18] did not observe any increase in the risk of cancer, infections, cardiovascular disease, delay in the consolidation of factures or hypocalcemia, nor were any cases of osteonecrosis of the jaw reported after 36 months following the use of DMAB. The adverse events most frequent or relevant to the FREEDOM study are presented in Table 1. Similar, coherent, results regarding safety have been reported in another randomised double blind clinical trial in 1,468 patients with prostate cancer subject to androgenic deprivation and treated with DMAB or a placebo for 36 months[21]. However, in the FREEDOM study the female patients treated with DMAB, in comparison with those treated with the placebo had a significantly higher frequency of eczema, flatulence and cellulitis as serious adverse effect (Table 1)[18]. While on the contrary, in comparison with the placebo, those treated with DMAB had a significantly lower incidence of falls[18], a circumstance which, without doubt, would merit a much deeper analysis due to its possible implications in the near future.

In the other three phase III trials[14,15,16], the global incidents of adverse events, and of serious adverse events, was similar between the two treatment groups studied. In the phase II clinical trial, in which were included an arm given treatment masked with alendronate, with the exception of one significantly higher incidence of dyspepsia with alendronate no differences were observed in the profile of adverse events between the patients who received denosumab and those who received the placebo or alendronate[7]. However, in another phase III comparison with alendronate no differences were found in the frequency of adverse events between DMAB and alendronate, including gastrointestinal disorders[15]. Similarly, in the clinical trial substituting alendronate by DMAB, there were no differences in the profile or frequency of adverse events between patients who changed to DMAB and those who continued with alendronate, the most frequent adverse events being nasopharyngitis (13.4% vs 10.8%), back pain (10.7% vs 11.6%), bronchitis (6.3% vs 5.6%), arthralgia (5.9% vs 10.4%) and constipation (5.1% vs 4.8%)[16].

Table 1. Adverse events with denosumab in the FREEDOM study (adapted from Cummings and cols.[18])

	Placebo (n=3.876)	Denosumab 60 mg Q6M (n=3.886)	*p*-value
Adverse events			
Infection	2,108 (54.4)	2,055 (52.9)	NS
Tumoral process	166 (4.3)	187 (4.8)	NS
Reaction at site of injection	26 (0.7)	33 (0.8)	NS
Symptomatic hypocalcemia	3 (0.1)	0 (0)	NS
Delayed recuperation of fracture	4 (0.1)	2 (0.05)	NS
Fracture of femoral diaphysis	3 (0.1)	0 (0)	NS
Fracture or humerus (not at the site of the joint)	1 (0.03)	0 (0)	NS
Osteonecrosis of the jaw	0 (0)	0 (0)	NS
Adverse events with an incidence of ≥ 2% and $p \leq 0.05$ in comparison with the placebo			
Eczema	65 (1.7)	118 (3.0)	<0.001
Falls*	219 (5.7)	175 (4.5)	0.02
Flatulence	53 (1.4)	84 (2.2)	0.008
Serious adverse events			
Tumoral process	125 (3.2)	144 (3.7)	NS
Infection	133 (3.4)	159 (4.1)	NS
Cardiovascular events	178 (4.6)	186 (4.8)	NS
Heart attack	54 (1.4)	56 (1.4)	NS
Coronary disease	39 (1.0)	47 (1.2)	NS
Peripheral vascular disease	30 (0.8)	31 (0.8)	NS
Auricular fibrillation	29 (0.7)	29 (0.7)	NS
Serious adverse events with an incidence ≥ 0.1% and $p \leq 0.01$ in the comparison with the placebo			
Cellulitis (including erysipelas)	1 (<0.1)	12 (0.3)	0.002
Commotion	11 (0.3)	1 (<0.1)	0.004

* Excludes falls happen on the day of the fracture

Finally, in one of the comparative clinical trials with placebo the patients who received DMAB had a significantly higher incidence of constipation (11% vs 4.8%), sore throat (9.1% vs 3%) and exanthema (8.5% vs 3%)[14]. These circumstances, apparently, to not have major clinical implications, although it is necessary to indicate them.

Final comments

Pharmacological research in the field of biological therapies has recently designed the first entirely human monoclonal antibody against RANK-L which has a unique physiological action mechanism which acts at the physiopathological roots of the disease. It may be said to be an "alternative pharmacological variant of osteoprotegerin", which, by bonding specifically to RANK-L impedes OP's accelerated bone destruction.

DMAB has shown in many well designed clinical trials (randomised, placebo-controlled, prospective, multicentred) notable increases in BMD in all locations measured and in the main types of

bone, cortical and trabecular, always higher than that observed in the placebo arm. The same applies, when DMAB is compared with an arm with active treatment with alendronate.

In addition, in a major phase III trial, the confirmation of the notable protection against fractures in all locations (with no distinction between vertebral, hip and non-vertebral), positions it as the first choice drug in the well-stocked therapeutic arsenal against OP.

The demonstrated reversibility of its effects on the bone once the administration of the drug is withdrawn, as well as its good general pharmacological safety profile is comparable to the placebo and to alendronate, the latter being a paradigm in the classic pharmacopeia against osteoporosis , especially as a function, nowadays, of its accumulated experience of over 13 years. DMAB's easily delivered dosage, administered subcutaneously twice a year makes it, in theory, a good candidate to address the chronic pathology that OP presents.

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Scientific reviews: considerations regarding their evolution

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Introduction

Since the first, the Journal of Savanç, was published in 1665, scientific reviews have been the vehicles par excellence for the spreading of information and scientific knowledge, and instruments of communication between researchers from the same field. In the general framework of scientific communication "authors resort to the scientific journals with intention of ensuring ownership of their ideas (a public register) and with the certainty that the knowledge published is valid, is scientifically verified and respects the academic norms of scientific method, and this because it has been evaluated fairly and impartially by the members of the scientific community themselves, thus becoming an instrument of certification, validation and knowledge"[1].

The importance of scientific reviews reaches its greatest expression in the case of medicine, where they are the medium most used for the interchange of ideas, to review and to update knowledge, making them a true reflection of the evolution of this scientific community.

Since their appearance in the 17th century, the evolution of scientific reviews has been linked with the changes in the way science is conducted and especially, to the events in the purely technological realm. In recent years, especially since the appearance and spreading use of the internet in the 90s, and after its consolidation as the main medium for searching for information and spreading content, we are experiencing great transformations in the process of scientific communications which directly affect the way in which reviews are written and published: the step from the printed form to the electronic, the appearance and consolidation of the Open Access movement which has modified the way scientific knowledge is published, spread and visualised, and the incorporation in the scientific reviews of the technologies of the so-called Web 2.0, "a system of applications on the internet which can be integrated to facilitate the publication of content by the users"[2], turning it into more attractive products for the final user. All this has produced significant changes in the editorial guidelines of scientific publications, as well as in the way of consulting with, and participating in, these journals. I think that these conditions make this the time for reflexion and to try to give a response to questions such as: Will the print media survive or will the digital format definitely take over? Will the reviews be accessible by subscription or free access? Will the reviews be 2.0?

Taking into account how, and by how much, the scientific communication scene has transformed in recent years, the difficulty associated with predicting how scientific reviews will be in just a few years will have escaped no one, but we attempt here to give some broad brushstrokes to give us a glimpse, at least, of the general picture.

The step to digital format

We are saying nothing new when we state that newly created scientific journals are already being created in digital form and, on only a very occasions, coexisting with ever more reduced numbers of paper examples. In addition, the most traditional scientific reviews have already migrated their formats to a digital version. The new generations have grown up in direct contact with computers and the internet: the so-called "digital natives" are much better familiarised with consulting digital versions of the reviews than the traditional printed versions. I recently heard how a young director of a university hospital said that until very recently he had neither seen or handled an example of the British Medical Journal on paper. This anecdote may give an idea of how much has changed in the panorama of scientific communication in recent years.

The advantages of digital reviews have been widely described in professional bibliography and it is not the purpose of this work to analyse them in detail, although it is worth recalling a few of them in order to be able to answer some of the questions raised. Some

of the most obvious are the savings in the costs of printing and distribution, the richness of the content (hypertext links, new formats of audio and video, etc.), greater accessibility and speed in getting content to the reader, greater potential audience, the possibility of searching within the reviews' collections, interconnection with search engines and open science portals, the possibility of including social participation tools, connection with bibliographical reference managers avoiding problems of space in libraries, not to mention the notable contribution to a more ecological and sustainable planet which will result from the elimination of paper. Although the advantages are many, there are also some disadvantages: dependence on computers, unstable web sites, confusing navigation systems, discomfort in reading from screens, etc.

There was a period when a good part of the scientific community showed their reticence with respect to electronic reviews, questioning the guarantee of quality and the scientific validity of the work published in them. Today, an enormous number of electronic scientific reviews is available on the web, with similarly formal characteristics to their printed homologues, which use the peer review mechanism for the control and selection of their contents, which comply with international norms and standards of publication, which can count on the collaboration of highly prestigious editorial and scientific teams and in which so-called "opinion formers" in different subjects are published, have overcome this initial reticence from the scientific community themselves.

Each day there are more reviews produced solely in electronic format, which on occasion co-exist with their paper version, although this is very much the minority. In the case of the **Review of Osteoporosis and Mineral Metabolism**, organ of the Spanish Society for Bone and Mineral Metabolism Research, which was launched in 2009 and with, as was stated in its launch editorial, " the aim of the review [is] to achieve the greatest diffusion and to reach the highest number of specialists, the publication will have a printed edition in Spanish, which will be distributed only to SEIOMM members and subscribers, and a bilingual version on-line"[3].

Taking into account the fact that in carrying out a bibliographical search these days it is necessary to consult the internet, a presence on the web is absolutely essential for any scientific review which has the intention of reaching its potential readers and which wants to survive in the long term. But it will not be sufficient solely to have a presence on the web, but the review will have to have visibility among the hundreds of thousands of medical reviews which are published today, and will therefore need to have different entry points to its content on the internet though its presence in databases, catalogues, indexes and directories, review portals or specialist search engines such as Google Scholar.

Although it is clear that the two systems of publication and diffusion of scientific information are perfectly compatible, the context for scientific publication is today the internet, and it is perhaps not unreasonable to assert that the co-existence of paper and electronic formats have a "sell-by" date. The electronic reviews are already not the future, but a fact, a reality which has naturally established itself within the process of scientific communication.

Open access to scientific reviews

The movement for open access to scientific literature advocates free access to the products of scientific research without either economic or copyright restrictions. As Malero asserts, it is important here to distinguish between "free" and "open": the first is synonymous with "gratis" or "without payment" , while "open" includes access without economic barriers and claims of authors' rights over their articles[4]. For the implementation of this new model of scientific communication two strategies have been put into motion: the publication of open access reviews (known as the gold route) and their deposition or self-archiving in institutional or thematic repositories (known as the green route). The main adherents to the initiatives for open access to science were reflected in three well-known declarations: the Declaration of Budapest, signed in February 2002, and the later declarations of Bethesda and Berlin signed in 2003[5-7], to which, little by little, have been added a large number of universities, research institutions and those funding research. One of the most significant boosts to open access happened in the year 2008 when the National Institutes of Health (NIH) of the US, one of the biggest funders of research in the world adopted a mandate which obliged all researchers funded by them to put the resulting articles of this research in an open repository after 6 months. Thus was created the PubMed Central repository (http://www.pubmedcentral.com). The British Welcome Trust has followed the same example, with the establishment of UK PubMed Central (http://ukpmc.ac.uk/), another major repository of biomedical research funded by that organisation. In our country, last May, Law 14/2011 of Science, Technology and Innovation was passed which established in its article 37 a mandate to self-archive in repositories the results of investigations financed by public money, within one year, as well as calling on the public servants in the Spanish system of science, technology and innovation to create repositories in which to house them[8].

The movement for open access to scientific research is being implemented very rapidly in the scientific communication system and, far from being a minority trend, each day there are more examples of open access reviews on the market, from the pure open access reviews, in which the author or their institution pays a fee to be published in them and reserves their copyright; hybrid reviews, paid for by subscription but with the option of publishing in open access (pre-payment of fees); or free access reviews which are not pure open access but which are offered free on the web, with or without an embargo period, while the exclusive copyright is held by the publisher and not the author. This is the case with the **Review of Osteoporosis and Mineral Metabolism**, available free on the web at http://www.revistadeosteoporosisymetabolismomineral. Most of this last type are included on publicly-funded open access platforms or portals, such as SciELO (http://www.scielo.org) o Redalyc (http://www.redalyc.unam.mx).

There are ever-increasing numbers of scientific reviews which are published according to the open access approach. The Directory of Open Access Reviews maintained by the University of Lung, the DOAJ (http://www.doaj.org), currently has registered (data for October 2011) 7,295 open access scien-

ce reviews, of which almost 3,000 have been added in the last year (between November 2010 and November 2011). Of all the reviews registered, 472 belong to the general medicine category and 182 are in the area of public health, as compared with 3,303 reviews in the areas of social sciences and humanities, which is to say 45%, as opposed the 8% which represent biomedicine. It seems clear that, at the moment, the scientific reviews which have most adopted this model of open publication have been those in the area of the humanities, with the biomedical reviews being much more recent.

However, there are increasing numbers of reviews in the area of biomedicine which are banking on a strategy of increasing their visibility and spread using open publication. Although studies carried out to date on the relationship between the number of citations obtained by a review and its open publication are contradictory, it seems obvious that those articles which are at the disposal of the general public, at no cost and from the moment of their publication, will have more possibilities of being cited than those which require a subscription to be taken out in order to access them.

It is probable that the decision of the editorial team of the **Review of Osteoporosis and Mineral Metabolism** to publish its content at no charge and without embargo (while not being able to be considered as open access in the strict sense, since the intellectual property rights are not ceded by the authors) will result, in time, in the arrival of a greater number of manuscripts to the publication, in an increase in the number of citations these receive, and that its accessibility will make the review more attractive to its potential public. There are already many reviews which, published under the open access model, have high standards of quality and even "Impact Factor" in the prestigious ISI database, which shows that the model of open access to content is a success and has the support of authors and institutions, although we cannot say that that this is well consolidated or its sustainability guaranteed. Taking into account the distance travelled in recent years and the willingness of those responsible for science policy to promote open access to public research, it seems that the future panorama will be the coexistence of the traditional model of access by subscription with the open access model.

Reviews 2.0

The move to digital format has also been a decisive and catalytic factor in the process of adopting on the part of the scientific reviews some of the tools belonging to the so-called Web 2.0, or social web, thus framed within the movement which has come to be called Science 2.0[9]. The Web 2.0 concept covers a series of applications which provide interactive services which allow users to produce and share information. Among these should be mentioned blogs and social networks, the greatest exponents of the Web 2.0, with Facebook (http://www.facebook.com), Tuenti (http://www.tuenti.com) or their more scientific versions, such as Mendeley (http://www.mendeley.com), being some examples. Also to be included in this group of 2.0 tools are microblogging services such as Twitter (http://www.twitter.com), the wikis or the syndication channels such as RSS. The social tagging sites for storing, classifying and sharing favourites (bookmarks) such as Delicious (http://www.delicious.com/) or Sympy (http:// www.simpy.com), and the social bibliographical reference managers such as Zotero (www. zotero.org/), Connotea (www.connotea.org) or CiteUlike (http://wwwciteulike.org), which offer the functionality of a reference manager combined with the possibility of sharing this information with other colleagues over the web[10], are other examples of successful tools of the Web 2.0 increasingly used in the world of science. The virtual spaces in which various types of content such as photographs (Flick:www.flickr.com), presentations (slideshare: http://www.slideshare.net), videos (Youtube: http://www.youtube.com) are also 2.0 tools. All are applications which exist on the web to allow the sharing of information and resources, and facilitate the participation of the user.

Although many reviews have migrated from print to digital format, the majority of them are merely a copy of their print version. However, some have started to explore the new possibilities offered by these tools which use the web as a platform. It is already some years since scientific reviews started investing in improving and modifying their traditional service with its one-way approach, in which the user is a mere reader of the published content who consults the review passively, by offering them possibility of participating, giving opinions and creating content. This is possible thanks to these social information management tools which allow the shared generation of knowledge, the publication of all types of content and their universal diffusion. The use by these scientific reviews of these technological tools to improve communication between scientists, authors and readers has given rise to the suggestion of the scientific review 2.0, which may be defined as "that which incorporates in its electronic version original technological elements of the Web 2.0, and at the same time, maintains the policy of generating the participation and interaction between readers, authors and editorial team in an open way"[11].

It is a fact that some of the most prestigious scientific publishers such as Nature, Science, JAMA, Lancet, Plos-One or the British Medical Journal, to list some of the most significant examples, are in the vanguard of this new approach and are incorporating 2.0 tools into their electronic editions.

For example the ability to make comments or notes on published works (as is the case of the Rapid Responses in the BMJ which has been a great success with researchers), the establishment and maintenance of blogs by the reviews as an extension of their publications and a more agile form of communication with their readers, the publication of content in different formats from PDF/HTML such as videos and/or podcasts (sound files), the interconnection with social software such as bibliography managers or social tagging services, the installation of RSS content syndication channels or a presence on social networks such as Facebook, are some of the tools of this new web most used by the scientific reviews. In addition, the great scientific review platforms, both private (see Elsevier or Springer) and public (see SciELO portal or Redalyc) are already following the 2.0 trend and attempting to multiply their diffusion channels and the visualisation of their contents, as well as recruiting and keeping new readers, especially the younger ones.

While the adoption of these technologies by Spanish biomedical reviews is still at quite an embryonic stage, and there are very few reviews which have incorporated 2.0 functionalities[12], we think that these tools are destined to play a significant role as channels for visualisation and diffusion of their content in the scientific and professional world, as well as providing new ways of communication with authors and readers.

Torres Salinas offers clear examples which sketch out the usefulness of these tools, such as the introduction of comments on published articles, which will make scientific debate more agile, and facilitate the control of fraud, systems of scoring or voting as a way of highlighting works, connection with the so-called social bibliography managers which allow the sharing and relating of articles with other users with similar interests, the generalist social networks such as Facebook or Twitter which facilitate the diffusion of content, or RSS which allows the reader the more effective use of the review[13].

Last year a study was published on the use of these types of tools on the part of British researchers which found that only 13% of researchers regularly use Web 2.0 tools, 45% of them occasionally use these tools, and 39% never use them[14]. In spite of this, we believe that the gradual incorporation into scientific publications of these participative technologies will improve the process of communication and diffusion of the research published in them, making them more attractive for younger users, reaching a wider potential audience and a wider public, offering value-added services to readers and, lastly, making the scientific review more dynamic and complete. Reviews 2.0 are the reviews of the future.

Final reflection

At the heart of the considerations expressed, and not forgetting the great degree of difficulty in predicting what is going to happen in the world of scientific reviews, we believe that the future panorama will consist of:

1. Digital reviews with a web presence, as well as being incorporated into major portals and directories.

2. Increasing amounts of content, if not the whole review, will be offered openly and at no charge, either immediately or after an embargo period.

3. Web 2.0 tools will be incorporated into the reviews which will broaden their services and make them more attractive to their readers.

It seems clear that the central nucleus of the system of scientific communication will continue to be the peer-reviewed scientific review, but offered over the internet, openly and with a great degree of participation on the part of the user. These systems, based on the web and Web 2.0 will enrich the reviews, and the formal process of communication will become more universal and democratic. It will be necessary for the reviews to be attentive to this situation and to maintain an open attitude; only in doing so will they be successful in adapting to, and surviving, these changes.

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Elevated prevalence of hypovitaminosis D in a population attending a health centre in Tenerife, Canary Islands

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To the Editor:

The existence of hypovitaminosis D in the population is well known, both in the general population and in certain groups of patients[1,2], being most significant in older people who are institutionalised in residential homes[3].

The Canary Islands, being an archipelago with a large number of hours of sun and, therefore, with a high availability of what constitutes the natural source of vitamin D, might be expected to have a low prevalence of hypovitaminosis D expected in its population. However, various studies in our environment have confirmed that levels of vitamin D are as low as those in other areas of Spain situated further north[4-8]. Hence, highly notable is a study carried out in Canarian medical students who, being young, healthy and with sufficient knowledge of the physiology of vitamin D, and even though spending a large number of hours in the sun, still had a high prevalence of hypovitaminosis D[9].

Therefore, we carried out a study in a group of 163 people from the area of "Valle de Guerra" who use the Tejina Health Centre in La Laguna, Tenerife. The group consisted of patients on whom an analysis was going to be carried out for some other medical reason, without any other criteria – either of exclusion or inclusion. Authorisation was requested from all the patients to add the measurement of 25-hydroxycalciferol (25-HCC), which was the metabolite which was analysed by immunochemiluminescence. The blood was taken during the months from March to June of 2011.

Table 1 shows the data obtained. 62.3% of the population who participated in the study were women. The overall average age of the participants was almost 52 years. It was observed that more than half the patients who participated in the study (50.6%) had values of 25-HCC lower than 30 ng/mL, this prevalence being higher in women (54.5%) than in men (44.3%).

Our study showed the elevated prevalence of hypovitaminosis D in an unselected group of individuals who simply attended the health centre for the performance of a control analysis for another medical condition (hypercholesterolemia, diabetes mellitus, health prevention activities, etc.), and who could well be seen as representative of the "real world" of patients attending a health centre. This coincides with that seen in other studies be they randomised or observational[1,3-9]. With this study we have attempted to confirm the "epidemic" of which hypovitaminosis D consists, and which is very often undiagnosed.

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Tabla 1. Results obtained

	All	Men	Women
Number (%)	163 (100)	61 (37.5)	102 (62.5)
Age (years)	51.8 ± 16.4	51.3 ± 15.5	52.1 ± 17
25-HCC (ng/mL)	31.3 ± 11.4	31.7 ± 8.8	31 ± 12.7
25-HCC less than 30 ng/mL (%)	50.6	44.3	54.5

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