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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 health¹. 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 Prevention²; from the ministry of health of the United States of America, on bone health and osteoporosis³; and from the World Health Organisation, on diet nutrition and the prevention of chronic diseases⁴.

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 ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). These two elements play an important role in the strength of bones and are of primordial importance in osteoporosis⁵.

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 osteoid 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 tissues⁶. 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 Review¹⁰ and by the Cochrane Foundation¹¹. 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 fractures¹². 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 study¹³, 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 \geq 80% of the tablets), the num-

ber 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 (\geq 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-analysis¹⁸ 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 Boonen¹⁵, 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 Cochrane¹¹ 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 group¹³.

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 D¹⁹.

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 indices¹⁴.

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 carbonate²⁰⁻²².

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 treatment²³. 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 remodelling²⁴.

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 resorption²⁵. In another prospective, randomised, double blind, factorial study²⁶ 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 D₃ (1,200 mg of calcium and 5.7 micrograms of vitamin D₃ each day). Group B (39 people) were administered skimmed milk fortified with vitamin D (900 mg of calcium and 5.7 grams of vitamin D₃ 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 supporters²⁸ and critics²⁹, 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 (Ca²⁺). 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 absorption³⁰.

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.

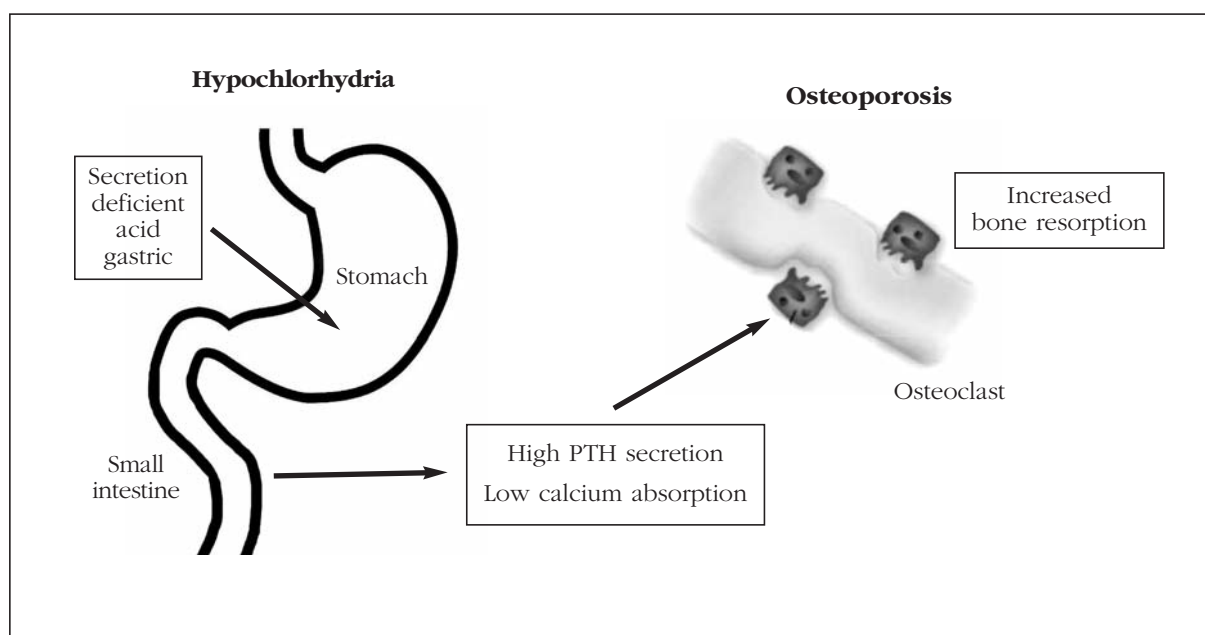
The absorption of dietary calcium, generically, diminishes with a higher content of fat, fibre, phytates, oxalates or caffeine, and increases with lactose and the protein content of the diet³¹.

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 (Ca²⁺) 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 quantity³². (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 salts³³⁻³⁵.

For example, the absorption of calcium in patients with achlorhydria, is significantly higher with calcium citrate than with calcium carbonate (Recker, 1985). In achlorhydric 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 ionisation³⁶⁻³⁸.

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 techniques³⁹, or due to medical treatment with proton 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 years⁴⁰. Although there are some discrepancies between authors⁴¹, 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 acidity³² and explains the association described between the use of PPI drugs and/or histamine H2 receptor antagonists and osteoporotic fractures.

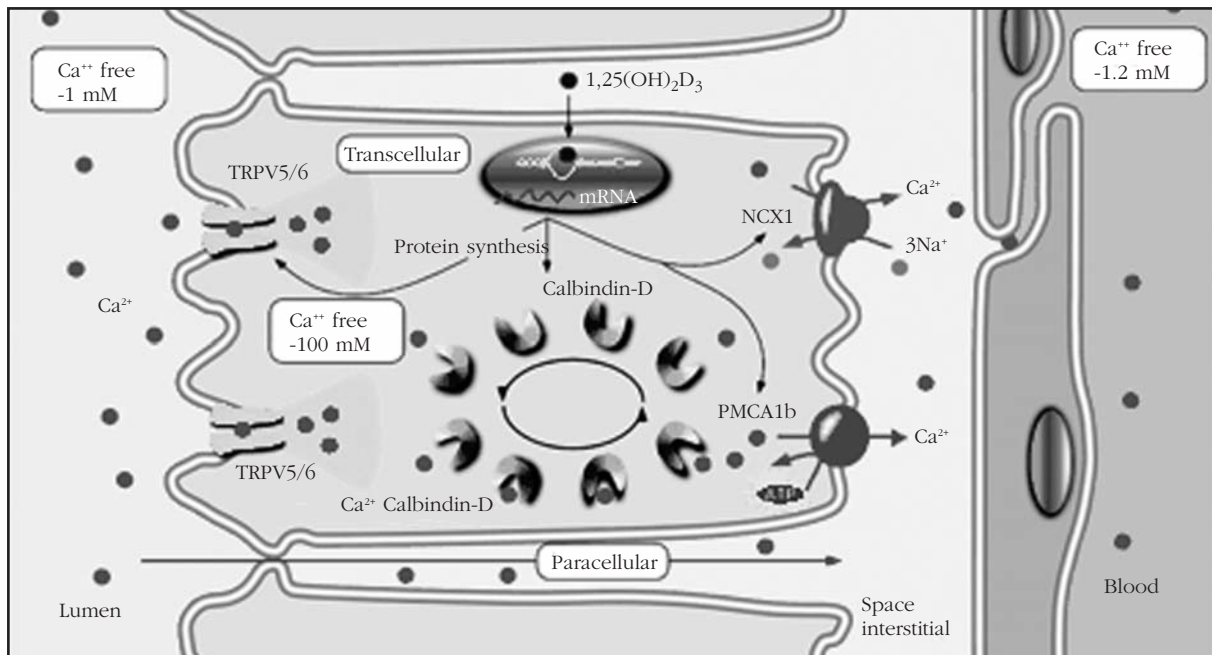
A case controlled study with a small number of cases (n=356) evaluated the association between the taking of histamine H2 receptor antagonists (cimetidine) and hip fractures with an adjusted odds ratio (OR) of 2.5 (1.4-2.6)⁴².

Of greater importance is the association described between the use of PPI and osteoporotic fractures⁴³, evaluated in three case controlled studies⁴⁴⁻⁴⁶.

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 ease³⁵, 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

Ca²⁺ 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 Ca²⁺.

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 Ca²⁺ through the (hetero) tetrameric epithelial Ca²⁺ channels TRPV5 and TRPV6, located on brush border; 2) bonding of Ca²⁺ to calbindin D9K with which it spreads up to the basolateral membrane, where 3) by means of a ATP-dependent Ca²⁺-ATPase pathway (PMCA1b) and an Na⁺/Ca²⁺ (NCX1) interchange it is expelled into the intracellular space. Hence a net absorption of Ca²⁺ is produced from the intestinal lumen into the extracellular compartment. (Figure 2).

The entry of Ca²⁺ through the apical membrane of the enterocyte is facilitated significantly by the electrochemical gradient, because the concentration of Ca²⁺ 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 Ca²⁺ through the apical membrane has no energy cost.

However, each step in the transcellular movement of Ca²⁺ has a component dependent on 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃ or calcitriol], which is a function of the status of vitamin D in the body (blood levels of 25(OH)D₃). Although 1,25(OH)₂D₃ 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 Ca²⁺ is sufficient, the synthesis of 1,25(OH)₂D₃ 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 Ca²⁺ 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 Ca²⁺ 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 phosphopeptides⁵⁰, and some oligosaccharides⁵¹.

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 cheeses⁵¹. 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 calcium⁵².

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 practice⁵³.

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 salts⁵⁴⁻⁵⁷.

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 societies⁵⁸.

However, the influence and importance of dietary calcium in the prevention of osteoporotic fractures is open to discussion⁵⁹. 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	Crema 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 years⁶⁰.

Another significant problem is that there are few studies in which only calcium is administered, without vitamin D, whether as a supplement in milk²⁶, 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 fracture⁶. In other prospective clinical trials calcium increased BMD in postmenopausal osteoporotic women⁶².

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 $\geq 1,200$ mg/day and a dose of vitamin D $800 \geq$ 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 ($\geq 1,200$ 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 osteoporosis⁶⁵.

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 D⁹.

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 calcium⁵.

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 sources⁵.

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 D⁶⁶, and the endocrinologists of the United States confirmed the requirements for vitamin D and established a daily intake of calcium of 1,200 mg⁶⁷.

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 daily⁶⁰. 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 lithiasis⁶⁹. 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 lower²⁷.

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 D⁷⁰.

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, $1,074 \pm 374$ mg/day for Bruyere⁷¹, $1,019 \pm 470$ mg/day for Quesada et al.⁷² and $1,326 \pm 588$ mg/day for Úbeda⁷³.

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/day⁵¹, 699 mg/day⁷⁴, 788 mg/day⁷⁵, 769 mg/day⁷⁶ 783 mg/day⁷⁷, 569 mg/day⁷⁸ and 909 mg/day⁷⁹.

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-dihydrocholesterol by means of a photochemical reaction into pre-vitamin D₃, which is rapidly converted into vitamin D₃. Excessive UVB irradiation does not produce vitamin D intoxication because the re-vitamin D₃ and vitamin D synthesised to excess are broken down in the skin into biologically inactive metabolites⁸¹.

Although there is a family of products with vitamin D activity, generally speaking, when we talk of vitamin D we refer both to vitamin D₃ (coleciferol) and to vitamin D₂ (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 (25OHD₃). 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 system⁸¹⁻⁸³.

In the plasmatic membrane of the tubular renal cells, the (25OHD₃)-DBP complex bonds with megalin, the protein which introduces the complex into the cell, where the 25OHD₃ is released, and in the mitochondria, by the action of 25-hydroxyvitamin D-1 α hydroxylase (1- α OHase; 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 kidneys⁸¹⁻⁸³.

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 intestine⁸¹⁻⁸³.

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 bloodstream⁸¹⁻⁸³. 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 whole⁸¹.

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 health⁸⁶.

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 measurement⁸⁶. 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 health⁸⁷. Although, higher levels of calcifediol are probably required to ensure other health objectives⁶⁵. 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 whole⁸⁸.

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/mL⁸⁹.

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/mL⁹⁰.

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%⁸¹.

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 basin⁷², by the low dietary intake of vitamin D which cannot be compensated for by cutaneous synthesis. Most of Spain is above the latitude of 35°N, 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 sun⁹², with seasonal variations, but barely managing to become normalised after summer-autumn⁹³. It is found in children and young people⁹⁴, persisting in adults⁹⁵⁻⁹⁷, 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 age¹⁰⁶. 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 institutionalised³.

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 mandatory¹⁰⁷.

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 creams⁸⁴.

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 D₃ depends on the season of the year. During the months of November to March north of the latitude 35°N/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 zero⁸⁴.

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 D₃ synthesised if the skin is covered for cultural, social, religious or any other reason⁸²⁻⁸⁴.

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 blood⁸⁴.

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 it is 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 osteoporosis⁷².

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-depth⁸⁵.

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 D⁷⁹, 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 osteoporosis¹⁰⁸, and in Spain in women treated for osteoporosis insufficient levels of calcifediol are found in 63%⁸⁵, similar to that observed in Europe¹⁰⁹ or the United States of America¹¹⁰.

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 anti-fractural efficacy^{111,112}.

Adami et al.¹¹² studied 1,515 women with postmenopausal osteoporosis in treatment with anti-resorptive 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 PTH⁷⁰.

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 response¹¹³.

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

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