

# Biological effects of various regimes of 25-hydroxyvitamin D3 (calcidiol) administration on bone mineral metabolism in postmenopausal women

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## Summary

**Introduction.** It is evident from several studies that vitamin D inadequacy is widespread among women with osteoporosis across all continents regardless of season or latitude, with similar prevalence in patients treated for osteoporosis and in untreated women. These results underscore a need to improve physician and patient awareness of the importance of adequate vitamin D supplementation in postmenopausal women with osteoporosis.

**Materials and Methods.** As the daily administration of vitamin D combined with 1 gr calcium is hampered by an insufficient patient adherence, we performed a longitudinal study in 90 randomly recruited postmenopausal women aged 65-75 years with inadequate calcium intake and circulating levels of 25-hydroxyvitamin D3 (lower than 30 ng/mL). The prevalence of secondary hyperparathyroidism (parathyroid hormone > 65 pg/mL) was 36% in the all population. The possible repercussion of oral single weekly or monthly calcidiol administration on phospho-calcium metabolism was observed after three months treatment (from April through July) with 500 mg calcium daily and with three different therapeutic regimens of calcidiol (Group I: 25 drops weekly; Group II: 50 drops monthly; and Group III: 100 drops monthly). The general baseline characteristics of the three groups were superimposable. We measured fasting morning serum 25-hydroxyvitamin D3, parathyroid hormone, calcium, phosphate, bone alkaline phosphatase, urinary deoxypyridinoline, and 24hr-calcium, - phosphate, and - creatinine.

**Results.** The adherence to the weekly calcidiol treatment was over 80% in 90% of the patients. All three therapeutic regimens of calcidiol led to normalization of 25-hydroxyvitamin D3 after 3 months, yet with a significantly higher potency ( $P > 0.01$ ) of regimens I and III, when compared to Group II. Also the decrease of circulating levels of parathyroid hormone was significantly higher ( $P < 0.001$ ) in Groups I and III versus Group II. No biochemically and clinically relevant adverse effects were observed at the end of the 90-day follow-up.

**Conclusions.** In postmenopausal women with inadequate circulating levels of 25-hydroxyvitamin D3, calcium and pulsed calcidiol supplementation normalized 25-hydroxyvitamin D3 levels and reduced circulating parathyroid hormone levels.

**KEY WORDS:** postmenopausal osteoporosis, vitamin D3, calcidiol, bone biomarkers .

## Introduction

Vitamin D is one nutrient thought to contribute to bone health, through the interaction with the vitamin D receptor and the consequent increase in intestinal calcium absorption and decrease of parathyroid hormone (PTH) secretion (1). Vitamin D deficiency is known to lead to rickets and osteomalacia and vitamin D insufficiency is involved in the development of osteoporosis, with low 25-hydroxyvitamin D3 (25-OHD3) levels being associated with reduced bone mineral density (BMD) and consequent fragility fractures (2).

Hypovitaminosis D in the elderly population is recognized as an epidemic in many parts of the world, independently on latitude, sex, and race (3-5). This because the lifestyle habits of the elderly population (i.e. reduced physical activity, scarce sun exposure, low dietary intake of calcium) contribute to increase the risk of bone mass loss in individuals with low or inadequate circulating levels of vitamin D. As vitamin insufficiency is also associated to an increased risk of developing other chronic disorders, such as cancer, diabetes, and cardiovascular disease, the need to offer a solution to this problem is urgently felt.

Increased exposure to UVB, either natural or artificial, is recognized to improve vitamin status, however this practice is not advised due to the increased risk of skin cancer. Vitamin D supplementation via dietary sources is very difficult to be obtained, even in countries with high fish consumption. Therefore, vitamin D supplementation is the only safe, effective, feasible, and cost effective primary prevention in older people, in whom most osteoporotic fractures occur. The results obtained up to now indicate that isolated vitamin D supplementation prevents fractures (6) and this is particularly true in high risk groups. Moreover, as many interventions effective in high risk groups are not feasible in the general population owing to problems related to poor compliance, to adverse reactions and to lack of cost effectiveness (6), the cost of vitamin D supplementation is certainly affordable.

Finally, results available out of controlled clinical studies have used either native vitamin D3 (7) or 1,25-dihydroxyvitamin D3 (calcitriol), the physiologically most active vitamin D metabolite (8). In postmenopausal women there are limited observations using calcidiol (25OHD3), the predominant circulating form of vitamin D and, therefore, considered to be the most reliable index in a person's vitamin D nutritional status (9).

We report results from a prospective study of weekly or monthly supplementation with calcidiol on mineral and bone metabolism in 90 postmenopausal women aged 65-75 years with documented inadequate status in the intake of calcium and in the circulating levels of 25OHD3 itself.

## Patients and methods

### Study population

For this prospective study, 90 Caucasian women aged 65-75 from the Outpatient Bone Metabolic Unit of the University Hospital of Florence, were randomly selected. All the women included in the study were given a complete physical examination, including height and weight with light clothing. Bone mineral density (BMD) was measured at the lumbar spine (L2-L4) using the 4500 Hologic densitometer. The body mass index (BMI) was used, defined as body weight (in Kg) divided by height (in sq.Mt). None of the subjects had ever taken or was taking at the time of the study vitamin D supplementation or any drugs known to interfere with bone metabolism. Among the exclusion criteria we included also chronic conditions that affect mineral metabolism or cause long-term immobilization. The study was carried out between April and July; all subjects were administered a dietary questionnaire to evaluate the daily intake of calcium (10). Patients were encouraged not to change their usual dietary habits during the study.

### Intervention

Participants were randomized into three groups of thirty to receive different dosages of calcidiol per os: Group I treated with weekly 25 drops/125 µg (5 000 IU vitamin D equivalent); Group II treated with monthly 50 drops/250 µg (10 000 IU vitamin D equivalent); and Group III treated with monthly 100 drops/500 µg (20 000 IU vitamin D equivalent). To all the patients a daily dosage of 500 mg calcium was administered. The treatment lasted for 90 days. If they discontinued the trial intervention they continued to be followed for endpoints.

### Endpoint ascertainment

We ascertained the changes of biochemical markers of bone and mineral metabolism after three months from the beginning of treatment. After three months the adherence to treatment was also evaluated through a diary aimed to count the proportion of doses taken over the observation period.

### Biological samples and measurements

Fasting blood samples were obtained at baseline and after 90 days of calcium and calcidiol administration. Serum 25OHD3 concentrations were determined by RIA (Diasorin Inc., Stillwater, MN). Measurements of serum PTH levels were performed using an IRMA assay (N-tact PTHSP; Diasorin; Normal Values: 5-65 pg/ml). Serum bone alkaline phosphatase (BAP) levels

were measured by an immunoenzymatic assay (Alisei-Radim, Pomezia, Italy; normal values: 6-22.5 U/l). Urinary deoxypyridinoline (DPD) was measured in 2hr sample by EIA (Immulite 2000, Siemens Medical, USA); normal values 3-9 nmol/mmol Cr).

### Statistical analysis

Only participants who concluded the study were included in the analyses. Results are presented as mean values and SD. Statistical analysis was carried out using the SPSS Version 8.0 package for Windows (Microsoft Corp., Redmont, WA, USA). The results were compared using the Student t test, one way analysis of variance (ANOVA). The correlations were established through the Pearson coefficient. In every case differences were considered significant at level of  $P < 0.05$ .

## Results

### Characteristics of participants

Table I shows the baseline characteristics of the patients who completed the study: age, height, weight, BMI, current calcium intake, creatinine clearance, and BMD. Among the women included in the study 25% were osteopenic and 75% were osteoporotic, with not differences in the distribution among the three groups. Daily calcium intake was lower than 800 mg in the study population without significant differences when the three groups were analyzed. Comparing the results for the three groups, we found no significant statistical differences in any of the variables, so we can affirm that this is a homogeneous population. As shown, vitamin D inadequacy was detected in all groups, without differences one from another (Table II).

### Compliance and biochemical variables

Table II presents data on biochemical markers of bone remodelling. We used BAP as a bone formation marker and the calcium/creatinine and DPD/creatinine ratios determined from 2hr-urinary sample tests as bone resorption markers. No statistically significant differences were detected among the three groups in any of the studied variables. We also found no significant statistical differences among the three groups for serum calcium and phosphate and for urinary calcium. All these results were in the normal range for our laboratory. However, the mean PTH values obtained, although homogeneous in the patients of our study, were above the range considered as normal in our laboratory (up to 65 pg/ml). Indeed, 36% of the patients in the overall population showed secondary hyperparathyroidism, without differences among the three Groups.

Table I - Baseline characteristics of 90 postmenopausal women aged 65-75 years at baseline according to allocation to treatment with calcium and calcidiol.

	Group I	Group II	Group III	P value
Number of completers	25	28	27	
Age (years)	65.1 ± 8.4	60.7 ± 10.2	58.7 ± 8.1	NS*
BMI (kg/sq. Mt)	24.3 ± 4.4	23.2 ± 3.6	26.1 ± 6.6	NS*
Calcium intake (mg/day)	777 ± 312	718 ± 451	761 ± 100	NS*
Creatinina clearance (mL/min)	79.1 ± 5.1	75.9 ± 6.3	78.4 ± 6.4	NS*
LX-DEXA (t-score)	-276 ± 1.13	-2.78 ± 1.21	-2.75 ± 1.08	NS*

\* Not significant.

Table II - Baseline biochemical markers of bone remodelling of 90 postmenopausal women aged 65-75 years according to allocation to treatment with calcium and calcidiol.

	Group I	Group II	Group III	P value
Number of completers	25	28	27	
Serum calcium (mg/dL)	9.7 ± 2.4	9.5 ± 2.8	9.7 ± 1.5	NS*
Serum phosphate (mg/dL)	3.8 ± 1.7	4.0 ± 2.77	3.7 ± 2.1	NS*
25OHD3 (ng/mL)	20.1 ± 4.8	20.4 ± 7.1	21.0 ± 3.0	NS*
PTH (pg/mL)	71.2 ± 3.0	71.5 ± 6.4	72.7 ± 8.4	NS*
Bone Alkaline Phosphatase (U/L)	20.1 ± 4.8	20.4 ± 7.1	21.4 ± 3.0	NS*
DPD/Cr (nmol/mmol)	8.1 ± 3.9	7.8 ± 0.8	8.5 ± 4.0	NS*
Urinary Ca/Cr (mmol/mmol)	0.1 ± 0.08	0.1 ± 0.06	0.2 ± 0.04	NS*

\* Not significant.

Table III - Percentage changes in biochemical markers of bone remodelling compared with basal values.

	Group I	Group II	Group III	P value
Number of completers	25	28	27	
Serum calcium (mg/dL)	3.3 ± 1.7	2.8 ± 1.3	2.8 ± 2.0	NS*
Serum phosphate (mg/dL)	1.8 ± 0.6	2.0 ± 1.1	2.1 ± 1.6	NS*
Bone Alkaline Phosphatase (U/L)	-0.1 ± 1.0	1.2 ± 0.6	0.5 ± 1.6	NS*
PTH (mg/ml)	-10.2 ± 3.0	-3.6 ± 1.7**	-15.6 ± 8.1	< 0.001
25OHD3 (mg/ml)	50.3 ± 15.6	36.5 ± 9.8***	47.7 ± 8.9	< 0.01
DPD/Cr (nmol/mmol)	-0.08 ± 0.6	0.6 ± 0.4	0.7 ± 0.5	NS*
Urinary Ca/Cr (mmol/mmol)	8.2 ± 3.6	3.7 ± 1.1***	10.1 ± 4.6	< 0.01

\* Not significant.

\*\* Significantly (P < 0.001) different versus both Groups I and III.

\*\*\* Significantly (P < 0.01) different versus both Groups I and III.

In Table III the differences obtained between the initial and final values of the biochemical markers of bone remodelling are shown, expressed as percentages. There were no statistically significant differences for bone formation and resorption markers among the three Groups. This Table also shows a significant increase in the serum 25OHD3 levels in all the patients, with significant lower levels in Group II when compared to Groups I and III (P < 0.01).

As expected, serum PTH levels were significantly reduced in the patients receiving oral administration of 25OHD3 for three months (a decrease of over 10% compared with the basal values), but in Group II where PTH levels were not significantly reduced versus baseline (a decrease of 3,6%) (Table III).

The global compliance was very good (> 80% in 90% of the patients) less than 10% of the patients that assumed monthly treatment interrupted the treatment within 3 months. Overall only 10% of the patients interrupted the treatment without differences in the distribution among the three Groups.

No clinically or biochemically relevant adverse events were observed at the end of the three months of observation.

## Discussion

Osteoporotic fractures are projected to increase exponentially worldwide (11). There is a rationale for supplementing the diets of elderly subjects with a combination of calcium and vitamin D. Absorption of calcium and possibly of vitamin D and production of vitamin D by the skin decline with aging independently on latitude (12-16). Vitamin D deficiency is a risk factor for osteoporosis (17 and when prolonged and severe can lead to osteo-

malacia and proximal myopathy (18). Several studies report that daily supplementation with vitamin D and calcium reduces fractures (19, 20). Also bolus cholecalciferol supplementation appeared to prevent fractures in elderly people living in the general community (6). There is also a trend towards a reduction in the risk of falls among patients treated with vitamin D3 alone compared with placebo, suggesting that vitamin D3 should be an integral part of effective osteoporosis management (21).

While for most vitamins dietary intakes offer a reasonable reference point for how much people might be need, for vitamin D we cannot use dietary intake as a guide, because, except for fish, our diets do not provide enough to prevent rickets or osteomalacia. Therefore, in order to determine a vitamin D requirement, an objective basis of recommending intakes of vitamin D for adults was searched and finally recognized in the minimal 25OHD3 serum levels considered physiologic for humans and which prevents osteoporosis (22).

It is now understood that the circulating levels of 25OHD3 are important indicators of mineral metabolic homeostasis, favoring its measurement as the parameter of choice for estimating a person's vitamin D status. A low serum concentration of vitamin D leads to lower serum calcium concentrations, and this in turn will increase PTH secretion, leading to secondary hyperparathyroidism. It follows that the bone turnover accelerates (2, 23). However, osteoporosis is one area where there is strong evidence that official recommendations for vitamin D intake are too low (24).

Interestingly, even though the majority of the intervention studies were based on the use of cholecalciferol, the native vitamin D3, vitamin D metabolites and analogues have been less often

implicated in the therapy of osteoporosis. The history of the use of vitamin D metabolites in clinical medicine began with their use in renal bone disease following the appreciation that the kidney is the major site of the 1- $\alpha$ -hydroxylase of vitamin D. Since then there has been much interest in the rationale for the use of 1- $\alpha$ -hydroxylated compounds in osteoporosis. Clinical trials suggested an important role of calcitriol as adjunctive therapy to antifracture agents in the treatment of involutional osteoporosis (25, 26). Moreover, continuous treatment of postmenopausal osteoporosis with calcitriol for three years was shown to reduce the rate of new vertebral fractures in women with this disorder (8). A randomized multicenter, double-blind study showed the superiority of the 1- $\alpha$ -hydroxylated metabolite alfacalcidol compared to vitamin D in lumbar BMD in postmenopausal osteoporosis (27). A published meta-analysis clearly showed the advantageous efficacy of hydroxylated vitamin D metabolites (alfacalcidol, calcitriol) versus parent vitamin D (28). The high prevalence of secondary hyperparathyroidism and the high incidence of moderate impairment of renal function suggest that a more wide spread use of these agents could make a substantial impact in the treatment of osteoporosis in the elderly.

As disturbances in vitamin D metabolism can have a relevance to osteoporosis, impairment of 25-hydroxylation in the liver poses the question of the use of 25-hydroxylated metabolites. Indeed, the disappearance of clinical symptoms of osteomalacia and the normalization of the investigated laboratory parameters were recorded in patients with chronic liver diseases treated with calcidiol (29). Moreover, calcidiol is effective in preventing hypocalcemia in the low birth weight newborn (30). More recently, the 25OH-derivative, calcidiol, has been used to evaluate the effects of vitamin D3 supplementation on bone phenotypes. In the study by M. Sosa and coll., after 1 year of treatment calcidiol administered at a dosage of 10 640 IU per week, in combination with 1 gr of calcium daily, corrected secondary hyperparathyroidism and increased bone mass in the femoral neck in elderly women with hip fracture (9). Interestingly, 25OHD3 has an important role in modulating PTH serum levels, possibly via a "residential" parathyroid 1- $\alpha$ -hydroxylase, as it has been suggested (31, 32). Moreover, 25OHD3 at high dosage was proposed to act directly on bone mineralization (33). Interestingly, the most abundant circulating metabolite of 25OHD3-24, 25(OH)2D3- exerts direct effects on bone cell metabolism (34, 35), probably also through autocrine mechanisms (36).

Our study was carried out on postmenopausal patients evaluated in an outpatient osteoporosis clinic. The primary aim was to evaluate the influence of three different regimens of 25OHD3 administration on bone mineral metabolism after 90-day of treatment. Once the three groups were randomly formed, we confirmed that they were homogeneous both clinically and biochemically. The population was characterized by a low calcium intake, by inadequate circulating levels of calcidiol and by secondary hyperparathyroidism.

The principal finding of this prospective study was that the 25OH-derivative of vitamin D3, administered with 500 mg calcium daily, is active in directly regulating the circulating levels of PTH in postmenopausal women with inadequate circulating levels of 25OHD3, both when administered weekly or monthly. This action parallels the correction of the circulating levels of 25OHD3. However, the total dose administered during the month has to be not lower than 20 000 IU. These results, not yet published, open the possibility to develop future strategies of monthly administration of 25OHD3 in osteoporotic patients, without any undesirable effect on blood and urinary calcium levels. Hypercalcemia and hypercalciuria, which occur more rapidly with calcitriol and alfacalcidol, is monitored by serum and urine calcium levels every 3 months (37). In the present

study no adverse biochemical effects were observed with 25OHD3 administration for 3 months. This is clearly evidenced by the circulating 25OHD3 levels in the studied population, that never reached the levels needed to observe hypercalcemia and hypercalciuria (38).

## Conclusion

In conclusion, adequate dosages of calcidiol administered either weekly or monthly with 500 mg daily of calcium reduce previously high serum levels of PTH without modifying serum and urinary calcium and bone remodelling markers. Based on 90-day 25OHD3 levels, a monthly bolus administration of calcidiol is as effective as a weekly administration, a finding with significant therapeutic implications for overcoming the low adherence to daily calcium and vitamin D administration in postmenopausal women.

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