

## COMPARISON OF LONG TERM ORAL SUPPLEMENTATION WITH TWO DOSAGES OF CHOLECALCIFEROL ON SERUM 25 - HYDROXYVITAMIN D IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS

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### Abstract

This study reports on the efficacy of two different daily oral doses of cholecalciferol supplementation (high dose group 1000 IU/day and low dose group 500 IU/day) for one year to 100 patients with postmenopausal osteoporosis (PMO) to achieve serum concentration of 25-hydroxyvitamin D (25OHD) of 20 ng/mL and 30 ng/mL. Two thirds of the whole study group (66%) had values below 20 ng/mL and 90% had < 30ng/mL at baseline. After one year of supplementation, in the high dose group none was deficient, 85.1% had values above 20 ng/mL, half had  $\geq$  30 ng/mL and the mean serum 25OHD increased from 17.9 to 29.2 ng/mL ( $p << 0.0001$ ). In the low dose group, 4% of the patients were still deficient, 65.2% had values above 20 ng/mL, 19% above 30 ng/mL and 25OHD increased from 19.3 to 23 ng/mL ( $p = 0.06$ ). Oral cholecalciferol supplementation for one year with 1000 IU/day is adequate to achieve serum 25OHD concentrations higher than 20 ng/mL for most of the patients. If the target concentration was 30 ng/mL, the treatment is successful only for half of the patients. No vitamin D deficiency was recorded for patients under this dosage after one year.

### Rezumat

Acest studiu raportează eficacitatea suplimentării orale cu două doze diferite de colecalciferol (1000 UI și 500 UI) timp de un an la 100 de paciente cu osteoporoză de postmenopauză cu scopul de a atinge concentrații serice ale 25OHD de 20 ng/mL și 30 ng/mL. Două treimi din grupul studiat (66%) au avut valori serice ale 25OHD sub 20 ng/mL și 90% au avut valori sub 30 ng/mL. După un an de suplimentare în grupul cu doză mare niciun pacient nu a fost deficitar, 85,1% au avut valori peste 20 ng/mL, jumătate având valori  $\geq$  30 ng/mL, cu o creștere medie a 25OHD serice de la 17,9 ng/mL la 29,2 ng/mL ( $p << 0,0001$ ). În grupul cu doză mică 4% dintre pacienți aveau încă deficit, 65,2% au avut valori peste 20 ng/mL și 19% valori peste 30 ng/mL, cu o creștere medie a 25OHD serice de la 19,3 ng/mL la 23 ng/mL ( $p = 0,06$ ). Suplimentarea orală zilnică timp de un an cu 1000 UI colecalciferol este adecvată în majoritatea cazurilor pentru atingerea concentrațiilor serice de 25OHD > 20 ng/mL, iar 50% ating concentrații serice > 30 ng/mL.

**Keywords:** vitamin D supplementation, vitamin D deficiency, postmenopausal osteoporosis

### Introduction

Vitamin D, a threshold nutrient, is essential for bone health and the nutritional status of an individual is currently assessed by measuring the serum concentration of 25-hydroxyvitamin D (25OHD). The state-of-the-art method to measure 25OHD is LC-MS/MS (liquid chromatography-tandem mass spectrometry), although automated immunoassays that measure only total serum 25-hydroxyvitamin D are still routinely used in many clinical facilities [1-3].

Vitamin D deficiency and insufficiency are quite prevalent in osteoporotic patients and when prolonged and severe causes secondary hyperparathyroidism, bone loss, maybe inadequate response to antiosteoporotic treatment, muscle weakness and increased risk of falls and fractures [4-10].

The lack of serum 25OHD assays standardization, variability of reference population and the use of different cut-off points have produced quite different prevalence reports from epidemiological studies [11-15]. Most clinicians agree that a serum 25OHD < 10 ng/mL represents severe vitamin D deficiency [16, 17], although osteomalacia was not always found on histology studies. Other experts favour for deficiency thresholds < 12 ng/mL, < 15 ng/mL or less than 20 ng/mL, but most data came from populations where the food is fortified with vitamin D and the supplementation is more frequent [14, 16, 18]. As a consequence, many guidelines recommend target serum 25OHD of  $\geq$  20 ng/mL [16, 17, 19, 20]. The Endocrine Society (US) favours an optimal concentration of at least 30 ng/mL and suggests doses as high as 1500 - 2000 IU

[21]. The oral dose of cholecalciferol required to achieve and maintain an adequate 25OHD status is controversial and many experts consider high doses to achieve extraskeletal benefits also [22].

This study aimed to compare the efficacy of two different daily oral doses of cholecalciferol (1000 IU vs. 500 IU) to achieve serum concentrations of 25OHD above either 20 ng/mL or 30 ng/mL, the most relevant thresholds identified in most of the studies.

## Materials and Methods

*Design and study group.* This was a retrospective observational study conducted in an osteoporosis referral centre. One hundred postmenopausal women with osteoporosis (diagnosed using WHO criteria by dual-energy X-ray absorptiometry, DXA) with no recent history of vitamin D supplementation were selected from our database at the National Institute of Endocrinology, Bucharest, Romania. All patients received antiosteoporotic medication (antiresorptive) and supplementation with cholecalciferol for at least one year with a level of compliance over 80%. The type of oral supplement (pills, solution, with or without calcium) was the physician/patient decision. Secondary causes of osteoporosis were excluded as well as any other disorder or medications influencing bone and mineral metabolism.

The evaluation included demographic data and determination of serum concentrations of 25OHD, PTH, calcium, phosphorus, alkaline phosphatase, creatinine and urinary calcium at baseline and after one year of supplementation with cholecalciferol. Baseline characteristics of the 100 patients with postmenopausal osteoporosis are listed in Table I.

The patients were subgrouped according to the oral dose of cholecalciferol used in: high dose group 1000 IU/day (74 patients) and low dose group 500 IU/day (26 patients). Different cut-offs of serum 25OHD concentrations were analysed at baseline and after repletion: < 10 ng/mL, 10 - 20 ng/mL, 20 - 30 ng/mL,  $\geq$  30 ng/mL and we used the Endocrine Society definitions of deficiency < 10 ng/mL, insufficiency 10 - 30 ng/mL and sufficiency  $\geq$  30 ng/mL [21].

Our primary objective is to report the efficacy of different daily oral doses of cholecalciferol supplementation used over one year in patients with postmenopausal osteoporosis in order to achieve serum concentrations of 25OHD of 20 ng/mL and 30 ng/mL. Secondly, to analyse the vitamin D status at baseline and to observe the change of vitamin D status over supplementation and to make recommendations about monitoring of 25OHD concentrations.

*The serum concentration of 25OHD* was measured using an ELISA method (ImmunDiagnostic 25OHD

EIA-kit) with an intra-assay and inter-assay CV < 10%, minimum detection limit of 1.4 ng/mL; normal range: 19 - 58 ng/mL. The measurements covered all seasons equally. Serum intact PTH was measured by ELISA (DSL-10-8000 ACTIVE I-PTH), intra-assay and inter-assay CV < 6.3%, minimum detection limit of 1 pg/mL; normal range: 16 - 62 pg/mL. The biochemistry parameters (serum calcium, phosphate, alkaline phosphatase and creatinine) were assessed by an automated analyser.

*Areal bone mineral density (BMD)* was measured by DXA at the lumbar spine, L1 - L4 (LS) and femoral neck (FN) (GE-Lunar Prodigy).

The informed consent for collection of data was obtained from all patients.

*Statistical analysis.* The results are expressed as mean  $\pm$  SD. The comparison of continuous variables was performed using Student's t-test or Mann-Whitney U test as appropriate, after checking for assumption for distribution normality (Shapiro-Wilk test) and equality of variances (Levene's test). P values of < 0.05 were considered significant.

## Results and Discussion

Our study group consisted in 100 postmenopausal women with osteoporosis, mean age  $63.1 \pm 9.2$  years, mean BMI  $25.1 \pm 4.1$  kg/m<sup>2</sup>, and baseline biochemical findings are listed in Table I.

**Table I**  
Baseline laboratory characteristics

Characteristic	Mean $\pm$ SD
Serum 25OHD (19.8 - 58 ng/mL)	18.3 $\pm$ 7.8
Serum PTH (16 - 62 pg/mL)	79.2 $\pm$ 43.5
Serum calcium (8.5 - 10.4 mg/dL)	9.5 $\pm$ 0.4
Serum phosphorus (2.5 - 4.5 mg/dL)	3.4 $\pm$ 0.4
Alkaline phosphatase (35 - 110 U/L)	82.9 $\pm$ 44.6
Serum creatinine (0.5 - 1.1 mg/dL)	0.7 $\pm$ 0.1
Urinary Ca (0.07 - 0.3 g/24 h)	0.2 $\pm$ 0.1

Patient distribution in the four 25OHD categories at baseline was: 12% in < 10 ng/mL, 54% in 10 - 20 ng/mL, 24% in 20 - 30 ng/mL and 10% in  $\geq$  30 ng/mL. 66% of the patients had baseline serum 25OHD values < 20 ng/mL and 90% < 30 ng/mL.

After one year of vitamin D supplementation, in the high dose group, the mean serum 25OHD was 29.3 ng/mL and the patient distribution was: zero patients in < 10 ng/mL, 14.9% in 10 - 20 ng/mL, 35.1% in 20 - 30 ng/mL and 50% in  $\geq$  30 ng/mL; therefore, 85.1% of the patients achieved concentrations above 20 ng/mL. In the low dose group, the mean serum 25OHD was 23 ng/mL and patient distribution was: 4% in < 10 ng/mL, 30.8% in 10 - 20 ng/mL, 46.2% in 20 - 30 ng/mL and 19% in  $\geq$  30 ng/mL. 65.2% have reached values over 20 ng/mL. We summarized these data in Table II.

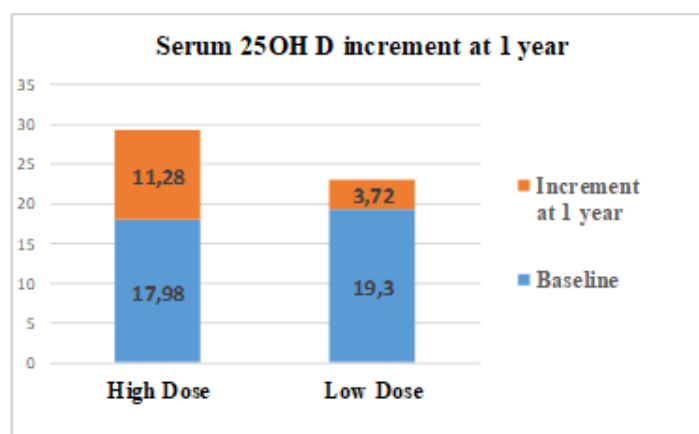
**Table II**

Patient distribution in categories of serum 25OHD at baseline and after supplementation for 1 year with both doses

Vitamin D groups	Baseline		At 1 year			
	% N = 100	Mean (ng/mL)	% high dose N = 74	Mean (ng/mL)	% low dose N = 26	Mean (ng/mL)
< 10 ng/mL	12	7.97	0	-	4	9.8
< 20 ng/mL	66	11.6	14.9	15.5	34.8	12.7
> 20 ng/mL	34	29.0	85.1	30.6	65.2	30.1
> 30 ng/mL	10	35.3	50	36.7	19	35.8

Mean serum baseline 25OHD in the high and low dose groups were 17.9 ng/mL and 19.3 ng/mL respectively (p = NS). After one year of supplementation, serum 25OHD significantly increased to a mean of 29.3 ng/mL in high dose group (p << 0.0001 vs. baseline) and to a mean of 23 ng/mL in low dose group (p = 0.06 vs. baseline); the difference between the

achieved concentrations in the high and low dose groups was highly significant (p = 0.001). The absolute increase in serum 25OHD at one year for both groups was: a mean of 11.28 ng/mL for the high dose group and of 3.72 ng/mL for the low dose group. This suggests a dose dependent increase of 0.5 ng/mL for every 1µg of cholecalciferol (Figure 1).

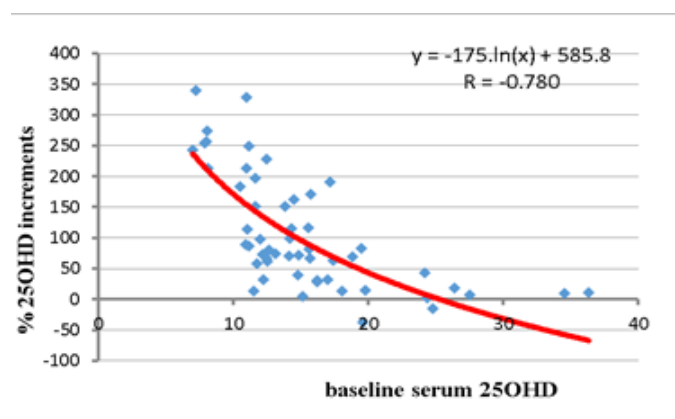


**Figure 1.**

The absolute increase in serum 25OHD (ng/mL) at 1 year for both groups

The correlation between serum 25OHD before supplementation and the absolute and relative increase after one year of supplementation in high dose group,

shows that the increase in 25OHD was inversely related to its initial concentration (r = -0.780) (Figure 2).



**Figure 2.**

Correlation between serum 25OHD (ng/mL) before supplementation and the relative increase after 1 year of supplementation

After one year of supplementation we found a significant decrease in serum PTH (mean PTH 54.2 pg/mL,  $p = 0.001$ ) and serum alkaline phosphatase (mean 60.5 mg/dL,  $p \ll 0.0001$ ) in the high dose group. No differences were observed in serum calcium and phosphorus compared with baseline ( $p = \text{NS}$ ) (data not shown).

Our study identified the oral daily dose of 1000 IU of cholecalciferol adequate for supplementation in patients with postmenopausal osteoporosis (PMO), as 85% of the patients achieved concentrations above 20 ng/mL, believed to prevent bone loss. A lower dose is not effective in all patients to prevent very low concentrations (severe deficiency). Both observations confirm previous data and recommendations [16, 17, 19]. This is an important issue as the clinical phenotype of severe deficiency is osteomalacia in adults. The 1000 IU dose is also recommended by almost all guidelines [16, 17] including the quite recently released European and Endocrine Society (US) guidelines for post-menopausal osteoporosis [23, 24].

In Romania, as elsewhere [25], many practitioners and laboratories use the Endocrine Society vitamin D categories. As a consequence, many physicians recommend oral doses as high as 2000 to 4000 IU daily to achieve the 30 ng/mL concentration.

This is in spite of quite recent data from systematic reviews and meta-analyses which are rather negative for higher doses of supplementation (more than 2000 - 4000 IU) and even observing no effect on bone density, fractures and falls in subjects without severe deficiency or above a threshold of 12 ng/mL [26-31]. These negative results were criticised as they included very few patients with severe vitamin D deficiency, as are the western populations [32]. In addition, the Institute of Medicine (US) report conclude that there is no additional benefit of achieving serum 25OHD of 30 ng/mL (75 nmol/L) when compared to 20 ng/mL (50 nmol/L) [33]. Over and above, Larsson *et al.* stated in a recent mendelian randomization study no causal association between long-term higher serum 25OHD concentrations and BMD in generally healthy population [34].

High doses of vitamin D for supplementation were encouraged not mainly for musculoskeletal endpoints but by the results of observational studies suggesting extraskeletal benefits (cancer chemoprophylaxis, cardiovascular benefits). The consequences of that is the medicalization of vitamin D measurement and supplementation which is seen today. The awaited results of randomized controlled trials with extraskeletal endpoints are just published and are rather negative [35, 36].

The strengths of our study are multiple: it compares high and low dose in the accepted definition, use of multiple 25OHD thresholds, control of patient compliance,

long follow-up (precluded influences of the seasonal variation) and Romanian cohort of PMO.

As expected, the increase in serum 25OHD inversely relates to its initial concentration suggesting that achieved increases in serum 25OHD are higher in patients with lower baseline levels, consistent with reports from several studies [37, 38].

As 85% of the patients achieved concentrations above 20 ng/mL, there is no need for monitoring, except in selected cases. We agree with international organizations like the US Preventive Services Task Force (USPSTF) which found little evidence to support screening/monitoring for vitamin D [39] as patients with osteoporosis receive supplements of vitamin D as part of the therapeutic regimen.

Our present study also observed a high prevalence of low vitamin D levels even when using different cut-offs: 66% < 20 ng/mL and 90% < 30 ng/mL, confirming other reports, including ours [11-15, 40, 41].

Our study has several limitations: the retrospective design and the lack of bone data (BMD, turnover) as patients were on bisphosphonate treatment.

In conclusion, supplementing PMO patients with 1000 IU of vitamin D daily is rational because of the high prevalence of inadequacy and enough to achieve a threshold considered to prevent bone loss. We suggest rechecking only in selected cases.

## Conclusions

Our data support the notion that oral daily supplementation with 1000 IU of cholecalciferol is adequate for almost all the patients with PMO in order to achieve a target concentration of 20 ng/mL. This is consistent with the most recent recommendations from other countries, compelling RCTs, and meta-analyses. For patients who fail to reach or sustain this concentration we suggest rechecking.

## Conflict of interest

No potential conflicts of interest were disclosed.

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