CLINICAL TRIAL

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# Long-Term Treatment and Effect of Discontinuation of Calcifediol in Postmenopausal Women with Vitamin D Deficiency: A Randomized Trial

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## ABSTRACT

Vitamin D plays a major role in bone health and probably also in multiple extraskeletal acute and chronic diseases. Although supplementation with calcifediol, a vitamin D metabolite, has demonstrated efficacy and safety in short-term clinical trials, its effects after long-term monthly administration have been studied less extensively. This report describes the results of a 1-year, phase III-IV, double-blind, randomized, controlled, parallel, multicenter superiority clinical trial to assess the efficacy and safety of monthly calcifediol 0.266 mg versus cholecalciferol 25,000 IU (0.625 mg) in postmenopausal women with vitamin D deficiency (25(OH)D < 20 ng/mL). A total of 303 women were randomized and 298 evaluated. Patients were randomized 1:1:1 to calcifediol 0.266 mg/month for 12 months (Group A1), calcifediol 0.266 mg/month for 4 months followed by placebo for 8 months (Group A2), and cholecalciferol 25,000 IU/month (0.625 mg/month) for 12 months (Group B). By month 4, stable 25(OH)D levels were documented with both


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calcifediol and cholecalciferol (intention-to-treat population): 26.8 ± 8.5 ng/mL (Group A1) and 23.1 ± 5.4 ng/mL (Group B). By month 12, 25(OH)D levels were 23.9 ± 8.0 ng/mL (Group A1) and 22.4 ± 5.5 ng/mL (Group B). When calcifediol treatment was withdrawn in Group A2, 25(OH)D levels decreased to baseline levels (28.5 ± 8.7 ng/mL at month 4 versus 14.4 ± 6.0 ng/mL at month 12). No relevant treatment-related safety issues were reported in any of the groups. The results confirm that long-term treatment with monthly calcifediol in vitamin D-deficient patients is effective and safe. The withdrawal of treatment leads to a pronounced decrease of 25(OH)D levels. Calcifediol presented a faster onset of action compared to monthly cholecalciferol. Long-term treatment produces stable and sustained 25(OH)D concentrations with no associated safety concerns. © 2023 Faes Farma SA. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

**KEY WORDS:** CALCIFEDIOL; CHOLECALCIFEROL; VITAMIN D DEFICIENCY; MENOPAUSE

## Introduction

Vitamin D deficiency is a very common condition worldwide. In addition to the well-established relationship between vitamin D and bone health, several studies have associated low 25(OH)D levels with a variety of acute and chronic diseases.[1-3] Hypovitaminosis D is a special concern in the elderly population since decreased 25(OH)D levels are associated with an increased risk of hip fracture in men and women and, in postmenopausal women, is also a risk factor for nonvertebral fractures.[4-7]

Vitamin D status is usually measured as total serum 25(OH)D levels. The optimal serum 25(OH)D levels have not been established, but some authors suggest that 25(OH)D concentrations should be >20 ng/mL and ideally >30 ng/mL.[8-12] Although the main source of vitamin D is its endogenous synthesis in the skin, it can also be obtained from different food sources, such as fatty fish (e.g., cod, salmon), eggs, milk, or fungi. In postmenopausal women, guidelines and therapeutic recommendations for the prevention and treatment of osteoporosis recommend co-administration of antiresorptive or anabolic agents with calcium and vitamin D, the latter at a dose of 800 IU daily or equivalent.[13,14] Vitamin D significantly reduces bone loss, as well as fall risk, in patients aged 65 or older.[15] The effect on fracture risk of vitamin D supplementation, with or without calcium, has been addressed in several meta-analyses.[16-19]

Hypovitaminosis D can be treated with ergocalciferol ($D_2$), cholecalciferol ($D_3$), or calcifediol [25(OH)$D_3$], with cholecalciferol being the most widely used.[20] Cholecalciferol is recommended for the treatment and prevention of vitamin D deficiency and as co-adjuvant therapy for osteoporosis.[8,10,13,21] At the time of the execution of the clinical trial, monthly calcifediol 0.266 mg was authorized for the treatment of vitamin D deficiency and insufficiency and as a co-adjuvant therapy for osteoporosis for up to 4 months, but this time limit was eliminated in a recent revision of the Summary of Product Characteristics (SmPC), and the indication for the prevention of vitamin D deficiency was also included. Some studies have shown that calcifediol is more potent and faster than cholecalciferol in correcting 25(OH)D levels.[22,23] However, most of the published studies with calcifediol have used it in a daily, weekly, or biweekly basis, but not monthly and/or in the long term.

The objectives of the present study were to assess the long-term (12-month) efficacy and safety of monthly calcifediol 0.266 mg administration in the correction and maintenance of 25(OH)D levels in postmenopausal women, compared to monthly cholecalciferol 25,000 IU. This study also investigated the effect of calcifediol withdrawal on 25(OH)D levels after 4 months of treatment. An interim analysis of this study after 4 months of treatment was published previously,[23] whereas the present analysis describes the results up to 12 months.

## Methods

This was a double-blind, randomized, controlled, parallel, multicenter, international, superiority phase III-IV clinical trial conducted between March 2018 and October 2020 at 10 centers in Spain and Italy. The study methodology was described in detail elsewhere.[23] A flow chart of study procedures is presented in Table S1. In brief, patients were randomized at a 1:1:1 ratio at the baseline visit into three study groups: Group A1 (monthly calcifediol treatment for 12 months); Group A2 (monthly calcifediol treatment for 4 months, then monthly placebo for the next 8 months); and Group B (monthly cholecalciferol treatment for 12 months). Calcifediol (Hidroferol®; FAES Farma, Leioa, Spain) was administered as soft gelatin capsules (0.266 mg). Cholecalciferol (Dibase®; Abiogen Pharma, Pisa, Italy) was administered as one jar (single-dose container) of 2.5 mL (25,000 IU).

All the investigators, staff, and participants were blinded to the allocation. The independent ethics committees of each participating center and Spanish and Italian regulatory agencies reviewed and approved the protocol. All participants signed the informed consent form prior to entering the trial. The study was performed in strict compliance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guidelines, the Declaration of Helsinki, as well as local laws and regulations. The study was registered with EudraCT No. 2017-004028-31.

### Study participants

Postmenopausal women with 25(OH)D levels <20 ng/mL were randomly assigned to one of the three different treatment groups. Inclusion and exclusion criteria of this study have been reported elsewhere.[23]

### Endpoints and measurements

The effect of the treatments was primarily assessed on serum 25(OH)D levels, both in the overall population and different subgroups, but defined bone mineral metabolism (total serum calcium [Ca], phosphate [P], total alkaline phosphatase, and intact parathormone [iPTH]) and formation and resorption markers (procollagen type 1 N-terminal propeptide [P1NP] and β-isomerized C-terminal telopeptides [β-CTx]) were also evaluated. The primary efficacy endpoint was percentage of patients achieving 25(OH)D levels above 30 ng/mL at month 4.[23]

Superiority was demonstrated by a difference between groups greater than 20%. The key secondary endpoint was the percentage of patients achieving 25(OH)D levels >30 ng/mL at months 8 and 12. Other secondary objectives included time to achieve the treatment goal of 25(OH)D concentrations >30 ng/mL; percentage of patients achieving 25(OH)D levels >20 ng/mL at 1, 4, 8, and 12 months; mean change from baseline in 25(OH)D levels at months 1, 4, 8, and 12; mean change from baseline in serum concentrations of Ca, iPTH, albumin, P, and total alkaline phosphatase at 1, 4, 8, and 12 months; mean change from baseline in serum concentrations of 25(OH)D free fraction at 4 and 12 months; and mean change from baseline in serum concentrations of vitamin D metabolite 24,25-hydroxyvitamin $D_3$ (24,25$(OH)_2D_3$), among other assessments.

Qualified health professionals obtained blood samples during the required visits. Serum 25(OH)D concentrations were determined in a central laboratory using an automated chemiluminescence system (LIAISON® XL, Saluggia, Italy). The central laboratory was accredited by the 25 Hydroxyvitamin D External Quality Assessment Scheme (DEQAS) Advisory Panel during the clinical trial. Free 25(OH)D was determined using a competitive enzyme-linked immunosorbent assay by DIAsource ImmunoAssays® S.A. kits (DIAsource, Louvain-la-Neuve, Belgium). 24,25$(OH)_2D_3$ was determined by liquid chromatography with tandem mass spectrometry (Agilent Technologies, Santa Clara, CA, USA). Concentrations of total Ca, P, iPTH, total alkaline phosphatase, P1NP, and β-CTX were measured with the techniques and timepoints previously described.[23] Safety endpoints included analysis of adverse events (AEs), treatment-related AEs (TEAEs), serious AEs (SAEs), and percentage of patients withdrawn from the study due to safety concerns.

### Statistical analysis

The primary superiority analysis was based on the intention-to-treat (ITT) population, whereas all secondary efficacy analyses were based on both ITT and per protocol (PP) populations. For the ITT analysis, last observation carried forward (LOCF) imputation for missing data was used. For the primary efficacy analysis, the chi-square test without continuity correction was used, and results were presented with the 95% asymptotic confidence interval (CI) for the proportion difference. Quantitative variables such as serum 25(OH)D levels and biochemical analyses were summarized by mean, SD, and 95% CI of mean. The Student's *t* test or Mann–Whitney test was used for pairwise comparisons. Binary correlation between continuous variables was performed using Pearson's correlation test.

For statistical significance a $p$ value < 0.05 was considered appropriate. SAS® (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for analyses within a validated and secure environment. Statistical tests were two-sided, and a significance level of 5% was reported for CIs.

## Results

A total of 401 postmenopausal women were screened and 303 were randomized. Of these, 298 patients were included in the ITT population and 170 in the PP population (Fig. 1). Table 1 shows baseline characteristics of the patients (ITT population, see Table S2 for baseline characteristics of the PP population), which were homogeneous for all treatment arms. When analyzing the baseline characteristics for both the PP population and the patients who were excluded from it due to protocol deviations ($n = 128$), we found no statistically and/or clinically significant differences.

In what follows, we report results for the ITT population (unless otherwise specified) and, for endpoints related to 25(OH)D levels, for the PP population.

The mean age of the patients was 63.4 ± 8.2 years, and 32 patients (10.7%) were osteoporotic. Mean 25(OH)D baseline values were 13.0 ± 3.9 ng/mL. The percentages of patients achieving a serum concentration of 25(OH)D > 30 ng/mL at month 4 were described in the interim analysis (Groups A1 and A2 pooled), which showed that monthly calcifediol 0.266 mg met the prespecified superiority criterion over monthly cholecalciferol 25,000 IU.[23] At month 8, the percentage of patients achieving a serum concentration of 25(OH)D > 30 ng/mL was 24.5% in Group A1 versus 2.0% in Group A2 and 8.2% in Group B ($p < 0.0001$ and $p = 0.0021$, respectively). At the end of the study (month 12), these percentages were 21.6% in Group A1 versus 3.1% in Group A2 and 9.2% in Group B ($p < 0.0001$ and $p = 0.0188$, respectively). The median time to achieve 25(OH)D concentrations >30 ng/mL was estimated via survival analysis and was 8.1 months (95% CI, 4.6 nonestimable) in Group A1. This median could not be estimated for Group A2 or Group B. There were no timepoints at which the survival curve reached 50% of responders (patients with levels >30 ng/mL), which resulted in nonestimable parameters in both scenarios.

The percentage of patients achieving a serum concentration of 25(OH)D > 20 ng/mL at month 12 followed a similar pattern, with 69.6% in Group A1 versus 13.3% in Group A2 and 61.2% in Group B ($p < 0.0001$ and $p = 0.2358$, respectively). The median time to achieve 25(OH)D concentrations >20 ng/mL was 1.6 months (95% CI, 1.4–4.1) in Group A1, 1.4 months (95% CI, 1.4–1.8) in Group A2, and 4.2 months (95% CI, 4.1–4.3) in Group B.

After 4 months of treatment, patients treated with calcifediol had mean (± SD) 25(OH)D levels of 26.8 ± 8.5 ng/mL (Group A1) and 28.5 ± 8.7 ng/mL (Group A2), whereas those treated with cholecalciferol had mean 25(OH)D levels of 23.1 ± 5.4 ng/mL. At month 8 the groups with active treatments (Groups A1 and B) had mean 25(OH)D levels of 25.7 ± 6.9 and 23.4 ± 5.9; and at month 12, 25(OH)D levels remained stable for both groups (23.9 ± 8.0 for Group A1 and 22.4 ± 5.5 ng/mL for Group B), and the mean increase from baseline was 11.4 ± 7.4 ng/mL in Group A1 compared to 9.2 ± 6.1 ng/mL in Group B ($p = 0.0118$).

After calcifediol withdrawal in Group A2, a sharp decrease in serum 25(OH)D levels was observed. The mean decrease in 25(OH)D concentrations from month 4 to month 12 was 14.2 ± 8.3 ng/mL (14.1 ± 5.1 ng/mL in the PP population), and by month 12, 85 patients (86.7%) in this group (38 patients [92.7%] in the PP population) had returned to 25(OH)D levels <20 ng/mL. The mean 25(OH)D levels for these patients at month 12 were 12.4 ± 4.29 ng/mL.

The evolution of the serum 25(OH)D levels for the three groups at baseline and months 1, 4, 8, and 12 are shown in Fig. 2 for the PP population. The results are comparable to those of the ITT population: at 4 months, mean levels were 26.8 ± 6.7 ng/mL in Group A1, 27.2 ± 7.4 ng/mL in Group A2, and 22.4 ± 5.1 ng/mL in Group B. At 8 months, mean levels were 25.6 ± 6.2 ng/mL in Group A1, 14.6 ± 5.4 ng/mL in Group A2, and 23.5 ± 5.7 ng/mL in Group B. After 12 months, levels remained stable for the active treatments (25.7 ± 5.5 and 22.5 ± 5.0 ng/mL for Groups A1 and B, respectively), with a mean

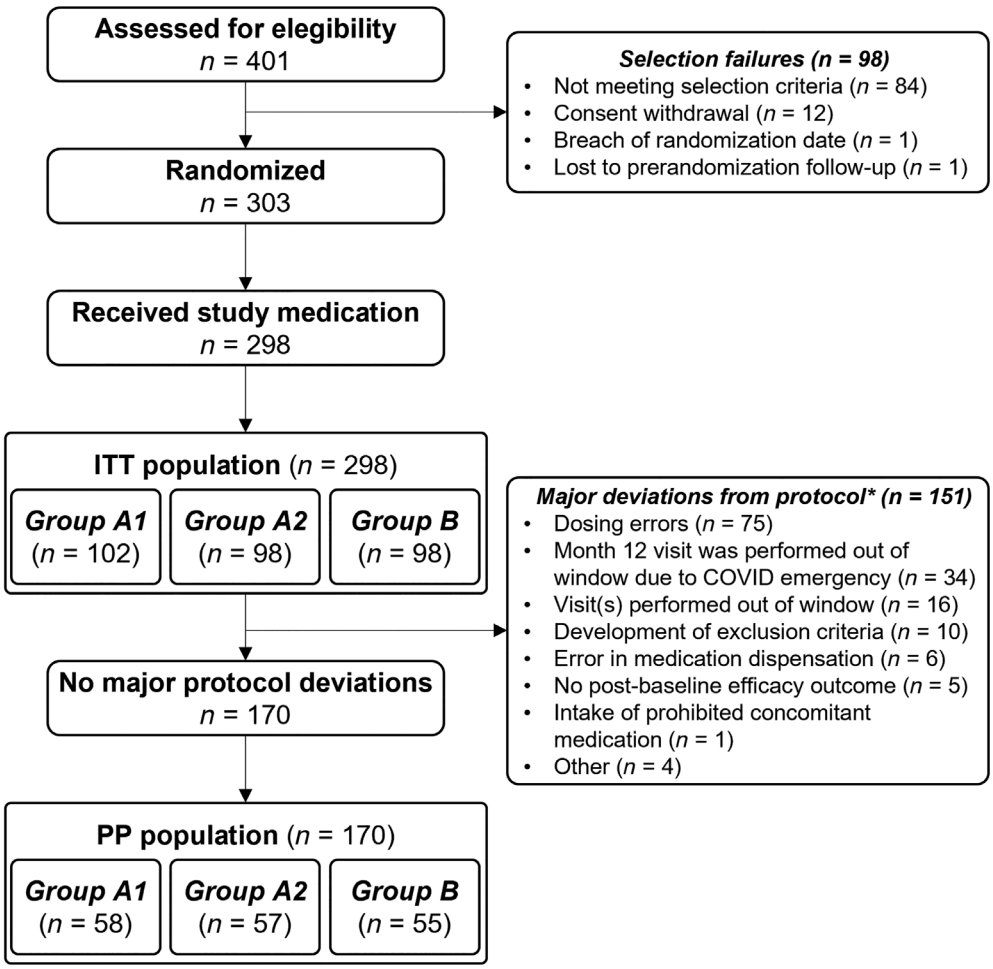


**Fig. 1.** Patient disposition.

[Correction added on 9 March 2023, after first online publication: figure 1 has been replaced due to incorrect data in Group A2 and Group B at the bottom part of the figure]

increase from baseline of 12.2 ± 4.9 ng/mL for Group A1 compared to 9.0 ± 5.4 ng/mL for Group B ($p = 0.0013$).

Regarding analysis of response profiles, the mean increment in 25(OH)D concentrations (in ng/mL) per microgram of administered drug was significantly higher in Group A1 (0.0150 [95% CI, 0.0114–0.0186]) and in Group A2 (0.0141 [95% CI, 0.0128–0.0155]) than in Group B (0.0041 [95% CI, 0.0036–0.0046]; $p < 0.0001$ for both comparisons) at month 4. At month 12, mean increment from baseline was also significantly higher in Group A1 (0.0041 [95% CI, 0.0033–0.0048]) compared to Group B (0.0013 [95% CI, 0.0011–0.0015]; $p < 0.0001$).

Analysis of the 25(OH)D free fraction concentrations showed results consistent with those from total 25(OH)D and favorable to the calcifediol treatment. The mean (± SD) changes after 12 months of treatment were 3.4 ± 2.1 pg/mL in Group A1 versus 2.7 ± 1.9 pg/mL in Group B ($p = 0.0226$). Regarding serum 24,25 $(OH)_2D_3$ levels, Group A1 had a mean concentration at month 12 of 1.8 ± 0.9 ng/mL versus 1.6 ± 1.0 of Group B ($p = 0.0454$). No differences were found in mean change from baseline.

No relevant differences between treatments were observed for bone and mineral metabolism markers (Ca, P, and iPTH) as well as bone turnover markers (β-CTX, P1NP), despite some statistically significant differences for serum calcium values at baseline and at 12 months. However, these values are within normal levels, and the difference between groups is not clinically meaningful (Table 2; see Table S4 for data of the PP population).

Patients were divided into subgroups for secondary efficacy analyses. Regarding baseline 25(OH)D levels, they were separated in those who had <10 ng/mL and >10 to 20 ng/mL. At month 12, Group A1 showed an increase from baseline of 11.4 ± 8.2 and 11.4 ± 7.1 ng/mL ($p = 0.9925$) in these subgroups, respectively, whereas Group B had an increase of 12.4 ± 4.7 and 8.4 ± 6.1 ng/mL ($p = 0.0073$), respectively (Fig. 3*A*).

When analyzing by body mass index (BMI) subgroups at month 12, Group A1 showed a mean increase of 25(OH)D of 12.5 ± 8.3 in patients with normal BMI ($n = 32$) and of 10.3 ± 6.9 ng/mL in obese ($n = 40$) patients ($p = 0.2265$). For Group B, the mean increase was 12.4 ± 7.4 ($n = 17$) compared to 7.6 ± 5.7 ng/mL ($n = 43$), $p = 0.0051$, respectively (Fig. 3*B*). Patients with normal BMI values showed no statistically significant differences with respect to the mean change from baseline of 25(OH)D levels in both treatment arms, whereas those with obesity as per BMI showed a greater increase in 25(OH)D levels when receiving

**Table 1.** Baseline Characteristics of Patients ($n = 298$)

| Variable | All ($n = 298$) | Calcifediol *group A1* ($n = 102$) | Calcifediol *group A2* ($n = 98$) | Cholecalciferol *group B* ($n = 98$) |
|---|---|---|---|---|
| Age (years), mean ± SD | 63.4 ± 8.2 | 64.3 ± 8.2 | 62.2 ± 7.6 | 63.6 ± 8.9 |
| Caucasian ethnicity, *n* (%) | 292 (98.0) | 99 (97.1) | 97 (99.0) | 96 (98.0) |
| Osteoporosis diagnosis, *n* (%) | 32 (10.7) | 12 (11.8) | 9 (9.2) | 11 (11.2) |
| BMI (kg/m$^2$), mean ± SD | 29.3 ± 6.1 | 28.9 ± 6.6 | 29.0 ± 6.0 | 29.9 ± 5.5 |
| Waist circumference (cm), mean ± SD | 96.4 ± 13.6 | 96.3 ± 14.6 | 95.8 ± 13.2 | 97.1 ± 13.1 |
| 25(OH)D (ng/mL), mean ± SD[a] | 13.0 ± 3.9 | 12.4 ± 3.9 | 13.2 ± 3.9 | 13.2 ± 3.7 |
| 25(OH)D < 10 ng/mL, *n* (%) | 74 (24.8) | 31 (30.4) | 23 (23.5) | 20 (20.4) |
| Free 25(OH)D concentration (pg/mL), mean ± SD | 3.9 ± 1.1 | 3.7 ± 1.0 | 4.0 ± 1.1 | 4.0 ± 1.1 |
| Total serum calcium (mg/dL), mean ± SD | 9.6 ± 0.4 | 9.5 ± 0.4 | 9.6 ± 0.4 | 9.6 ± 0.4 |
| Phosphate (mg/dL), mean ± SD | 3.5 ± 0.5 | 3.5 ± 0.5 | 3.5 ± 0.5 | 3.5 ± 0.5 |
| iPTH pg/mL, mean ± SD[b] | 60.1 ± 25.5 | 63.3 ± 26.9 | 54.6 ± 27.3 | 62.3 ± 21.2 |
| Total alkaline phosphatase (IU/L), mean ± SD | 87.4 ± 23.5 | 87.8 ± 24.5 | 85.8 ± 23.3 | 88.6 ± 23.0 |
| β-CTX (μg/L), mean ± SD ($n = 261$)[c] | 0.46 ± 0.32 | 0.45 ± 0.19 | 0.49 ± 0.48 | 0.45 ± 0.21 |
| P1NP ng/mL, mean ± SD ($n = 261$)[c] | 51.3 ± 20.6 | 54.8 ± 19.4 | 49.2 ± 19.9 | 49.7 ± 22.2 |

*Note*: The table includes baseline characteristics for the ITT population.

Abbreviations: SD, standard deviation; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; iPTH, intact parathormone; ODS, Office of Dietary Supplements; P1NP, procollagen type 1N-terminal propeptide; β-CTX, β-isomerized C-terminal telopeptides.

[a]25(OH)D: 1 ng/mL = 2.5 nmol/L (ODS, National Institutes of Health, updated on August 17, 2021).

[b]In the cholecalciferol arm, one iPTH value was missing (n = 54), overall sample size for this parameter is n = 169.

[c]Assessed only in non-osteoporotic patients: Group A1, n = 89; Group A2, n = 88; Group B, n = 84.

calcifediol 10.3 ± 6.9 compared to 7.6 ± 5.7 ng/mL with cholecalciferol at 12 months ($p = 0.0314$).

A total of 129 (42.6%) of the 303 randomized patients enrolled reported at least one TEAE in this study, 45 (44.1%) in Group A1, 43 (42.6%) in Group A2, and 41 (41.0%) in Group B (overall, 215 TEAEs were reported). Only two treatment-related AEs were reported by two (0.7%) patients (abdominal discomfort and dyspepsia), and only one (0.3%) subject had an AE that led to her withdrawal from the study. A total of 17 (5.6%) patients reported at least one SAE (overall, 23 SAEs were reported), but none of them was attributable to any of the study drugs. One death not related to study treatment was reported. The maximum 25(OH)D level reached by a patient was 64.4 ng/mL at the first month of treatment due to a medication error (weekly instead of monthly intake). The withdrawal criterion for 25(OH)D levels in this clinical trial was ≥80 ng/mL, and no patient reached that threshold. No clinically relevant hypercalcemia cases were reported in any of the treatment groups, and the maximum calcium level reached was 11.1 mg/dL in one patient, whereas the remaining patients had levels ≤10.8 mg/dL.

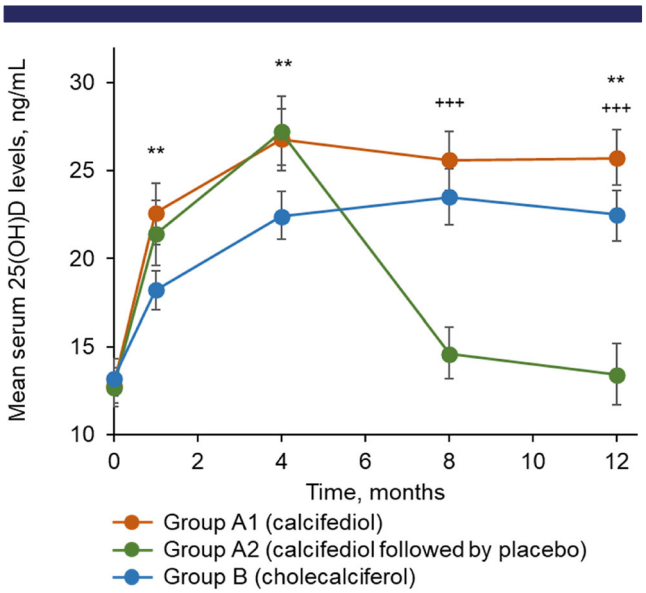


**Fig. 2.** Mean serum 25(OH)D concentrations during study (PP population, $n = 170$). A strong decrease in 25(OH)D levels to baseline can be observed 4 months after calcifediol withdrawal (Group A2) compared with sustained administration (Group A1). Statistical comparisons are for Group A1 versus Group A2 (+, $p < 0.05$; ++, $p < 0.001$; +++, $p < 0.0001$) and for Group A1 versus Group B (*, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$). Error bars: 95% CI.

## Discussion

Previous studies compared the efficacy of calcifediol and cholecalciferol in increasing serum 25(OH)D levels,[22,24-26] although these were relatively small studies using different dosages and populations. These studies, along with this research, demonstrated that calcifediol is faster and more potent than cholecalciferol. Only two trials analyzed the effects of calcifediol and cholecalciferol in the long term (52 weeks).[25,27] In the study published by Navarro-Valverde et al. (2016) in postmenopausal women with osteopenia and vitamin D deficiency (38.7 ± 4.2 nmol/L; that is ≈ 15.5 ± 1.7 ng/mL), patients using calcifediol 0.266 mg oral solution once every 2 weeks reached 25(OH)D levels above 60 ng/mL at month 6 and above 80 ng/mL at month 12.[27] In addition, patients who took a weekly dose reached 25(OH)D levels above 80 ng/mL at month 6 and above 90 ng/mL at month 12, suggesting that weekly or biweekly doses of calcifediol 0.266 mg could be useful for greater increments in 25(OH)D levels when needed. However, in that study

**Table 2.** Effect of Treatment on Bone Mineral Metabolism Parameters

| Variable | Baseline | | | Month 12 | | |
|---|---|---|---|---|---|---|
| | Calcifediol *group A1* ($n = 102$) | Cholecalciferol *group B* ($n = 98$) | *p* value | Calcifediol *group A1* ($n = 102$) | Cholecalciferol *group B* ($n = 98$) | *p* value |
| Total serum calcium (mg/dL) | 9.5 ± 0.4 | 9.6 ± 0.4 | 0.0222 | 9.5 ± 0.4 | 9.6 ± 0.4 | .0372 |
| Phosphate (mg/dL) | 3.5 ± 0.5 | 3.5 ± 0.5 | 0.9558 | 3.6 ± 0.5 | 3.6 ± 0.5 | .5195 |
| Total alkaline phosphate (U/L) | 87.8 ± 24.5 | 88.6 ± 23 | 0.8063 | 81.1 ± 21.1 | 86.4 ± 31.9 | .1659 |
| iPTH (pg/mL) | 63.3 ± 26.9 | 62.3 ± 21.2 | 0.7842 | 57.4 ± 27.5 | 56.3 ± 16.9 | .7372 |
| | Calcifediol *Group A1* ($n = 89$) | Cholecalciferol *Group B* ($n = 84$) | *p* value | Calcifediol *Group A1* ($n = 89$) | Cholecalciferol *Group B* ($n = 84$) | *p* value |
| β-CTX (μg/L)[a] | 0.45 ± 0.19 | 0.45 ± 0.21 | 0.9008 | 0.47 ± 0.18 | 0.47 ± 0.22 | 0.8947 |
| P1NP (ng/mL)[a] | 54.8 ± 19.4 | 49.7 ± 22.2 | 0.1045 | 54.5 ± 21.4 | 53.6 ± 24.1 | 0.8051 |

*Note*: Values are expressed as mean ± SD of ITT population.
Abbreviations: iPTH, intact parathormone; P1NP, procollagen type 1N-terminal propeptide; β-CTX, β-isomerized C-terminal telopeptides.
[a]Assessed only in nonosteoporotic patients.

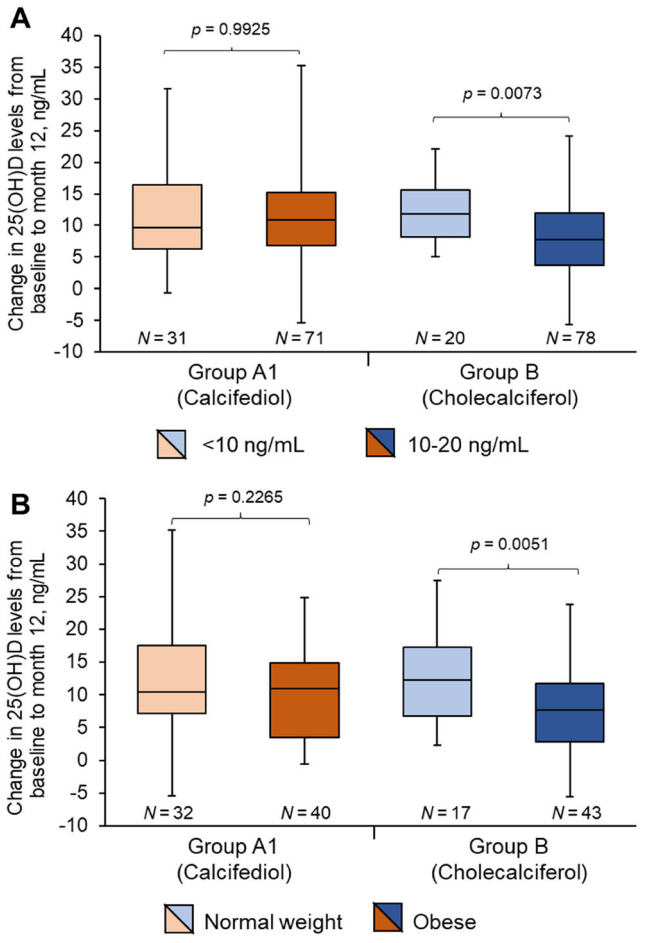


**Fig. 3.** Subgroup analysis (ITT population). Mean increase in 25(OH)D levels from baseline to month 12 as a function of (*A*) baseline levels of 25(OH)D and (*B*) BMI. Whiskers indicate maximum and minimum values.

25(OH)D stable levels were not observed, in contrast to this and other studies.[25,28,29] The fact that both calcifediol and cholecalciferol reached stable 25(OH)D levels by month 4 in this study suggests that the increments observed in the first months may determine the 25(OH)D levels that will be reached subsequently. A steady state with vitamin D supplements has been observed, usually after 11 to 14 weeks of treatment.[25]

This study also showed that when calcifediol treatment is withdrawn, levels quickly decrease to baseline. This observation agrees with the study performed by Graeff-Armas et al., which obtained similar results when daily doses of calcifediol or cholecalciferol were administered for 6 months and then treatment was discontinued.[25] This study showed that, regardless of initial dose, baseline concentrations were reached only 2 months after discontinuation. These results strongly suggest that treatment should be maintained in time to avoid the decrease of 25(OH)D levels. In addition, it suggests that cessation of treatment may not be advisable, as levels seem to remain fairly stable once a plateau is reached. In this regard, other authors have also previously recommended not stopping vitamin D supplementation during the summer months, for instance.[30] The clinical significance of maintaining optimal 25(OH)D levels in postmenopausal women, especially in osteoporotic ones, is that it aims to avoid an inadequate response to osteoporosis treatment.[31] Therefore, a rapid increase in 25(OH)D concentration to optimal levels could facilitate an adequate therapeutic response. This is especially important in cases of an imminent risk of fracture.[32]

When reporting mean 25(OH)D levels at month 8 for the PP population, the difference between active treatments was not significant, which was not the case for other 25(OH)D outcomes. This 8-month mean value for both groups was probably influenced by some patients who took the medication irregularly, as suggested by the fluctuation of their determinations, while achieving acceptable overall compliance. When the analysis was conducted in both groups without these patients, the difference was statistically significant.

Although most studies and analyses measure total 25(OH)D levels as vitamin D status biomarker, some authors suggest that

free 25(OH)D, 24,25$(OH)_2D_3$, or the vitamin D metabolite ratio (24,25$(OH)_2D_3$ to 25$(OH)D_3$) is a potential alternative.[33,34] In this regard, some studies have shown that free 25(OH)D presents a positive correlation with bone mineral density, unlike total 25(OH)D.[35-38] The interim analysis of this study after 4 months of treatment and other studies have shown a positive correlation between total and free 25(OH)D levels.[23,39] In line with this, results at month 12 of this trial showed that free 25(OH)D concentrations displayed a pattern similar to that of total 25(OH)D. Regarding the 24,25$(OH)_2D_3$ metabolite, a higher concentration was found in patients treated with calcifediol than with cholecalciferol. These results are congruent with the higher 25(OH)D levels in the calcifediol group. In fact, a strong correlation between both metabolites has been described.[33,40] The increase of 24,25$(OH)_2D_3$ at 12 months can be assessed in two ways, as a marker of vitamin D sufficiency or as a safety mechanism. The first of these is a marker for the increase of 25(OH)D since there is a strong positive correlation between 25$(OH)D_3$ and 24,25$(OH)_2D_3$.[41] When sufficient amounts of biologically active vitamin D are available, CYP24A1 is upregulated and more 24,25$(OH)_2D_3$ is formed. This may be attributed to 24 hydroxylation and the formation of the inactive metabolite, 24,25-dihydroxyvitamin D, instead of calcitriol (1,25 dihydroxycholecalciferol), a safety mechanism that prevents excess formation of the active metabolite.[42]

One of the consequences of vitamin D deficiency is secondary hyperparathyroidism. In this sense, calcifediol (with a different strength and in a daily dose) is approved in Europe and in the United States for the treatment of secondary hyperparathyroidism in stages 3 and 4 of chronic kidney disease in vitamin D-deficient patients.[43] In this study, no differences were found between iPTH levels in the groups treated with cholecalciferol or calcifediol after 12 months of treatment. However, the study population had mean baseline iPTH levels within normal range. Moreover, no differences were found between the cholecalciferol and calcifediol treatment groups in either the total serum calcium, phosphate, total alkaline phosphatase, β-CTX, or P1NP levels after 12 months of treatment.

When the subgroup analyses were performed by baseline 25 (OH)D levels, calcifediol, unlike cholecalciferol, showed similar increases independent of the patients' baseline level.[22] For BMI, calcifediol showed similar increases for both normal BMI and obese patients, whereas cholecalciferol showed a numerically lower increase in obese patients.[2,44] Additionally, in obese patients, calcifediol showed higher increases in 25(OH)D levels compared to cholecalciferol. Due to its reduced lipophilia, calcifediol presents lower accumulation in adipose tissue than cholecalciferol.[20,22,45] Also, low serum 25(OH)D in obese individuals can be a result of reduced hepatic 25-hydroxylation.[46] These analyses were conducted in a reduced sample size; therefore, further research would be required to thoroughly support these results.

In this study, no relevant safety issues were found in any of the treatment groups, indicating that both treatments represent safe alternatives to be administered in the long term. Furthermore, even in cases where unintended misuse of medication was reported, neither 25(OH)D toxic levels nor clinically relevant hypercalcemia cases were reported.

Several limitations of this study must be considered; these were also addressed in the previous publication.[23] The criteria for selecting the therapeutic regimes were based on the recommendations of clinical practice guidelines, the calcifediol SmPC, or prescribing information available at the time of study design, given the lack of international consensus on optimal treatment schemes. Moreover, at doses such as those used, calcifediol is approximately 3.2 times more potent than cholecalciferol.[22] In this case, the dose of cholecalciferol is 2.35 higher than calcifediol (0.625 mg/month versus 0.266 mg/month). These data balance both supplements at baseline. To our knowledge, a single clinical practice guideline and a position statement by the Italian Medicines Agency (96 note of the Agenzia Italiana del Farmaco, AIFA) for the treatment of vitamin D deficiency include calcifediol 0.266 mg soft capsules and cholecalciferol, in the doses used in this study for subjects with baseline 25(OH)D levels >10–12 ng/mL.[47,48] Another potential issue is the number of patients with protocol deviations, largely due to intake of less medication than planned (dosing errors) and patients' visits at month 12 out of the established window because of the COVID-19 pandemic. However, results were highly consistent between the ITT and PP populations. During the pandemic, a contingency plan was elaborated to manage potential risks, following recommendations by the competent authorities. By April 2, 2020, an estimated 6% of patient visits were pending (107 by month 12 or end of study visits). Remote patients' visits were coordinated to evaluate safety, and, whenever possible, blood samples were collected.

The main strengths of this study lie in its sample size (298 postmenopausal women), the homogeneity of the study population, the duration (12 months), and the centralization of laboratory analyses.

In conclusion, the results of this study demonstrate that calcifediol is superior to cholecalciferol in improving vitamin D deficiency in postmenopausal patients with and without osteoporosis, with a faster onset of action. Long-term treatment with calcifediol produces stable and sustained 25(OH)D concentrations, with no associated safety concerns. When discontinued, it has been proved detrimental, with a sharp decrease in levels previously obtained indicating the need of maintaining vitamin D supplementation.

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## Authors' Contributions

José Luis Pérez-Castrillón: conceptualization, investigation, writing—original draft. Antonio Dueñas-Laita: conceptualization, investigation, writing—original draft. Maria Luisa Brandi: investigation, writing—review and editing. Esteban Jódar: investigation, writing—review and editing. Javier del Pino-Montes: investigation, writing—review and editing. José Manuel Quesada-Gómez: conceptualization, investigation, writing—review and editing. Fernando Cereto Castro: investigation, writing—review and editing. Carlos Gómez-Alonso: investigation, writing—review and editing. Laura Gallego López: investigation, writing—review and editing. José Manuel Olmos Martínez: investigation, writing—review and editing. María Rosa Alhambra Expósito: investigation, writing—review and editing. Bernat Galarraga: investigation, writing—review and editing. Jesús González-Macías: conceptualization, writing—review and editing. José Luis Neyro: writing—review and editing. Roger Bouillon: conceptualization, writing—review and editing. Gonzalo Hernández: conceptualization, supervision, writing—review and editing. Nieves Fernández: conceptualization, project administration, supervision, writing—original draft. Sandra P. Chinchilla: visualization, writing—original draft.

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## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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