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ORIGINAL ARTICLE

Correction of vitamin D status by calcidiol: pharmacokinetic profile, safety, and biochemical effects on bone and mineral metabolism of daily and weekly dosage regimens

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Abstract
Summary Rationale: Calcidiol can be employed to correct vitamin D deficiency. Main results: Calcidiol administered at daily and weekly regimens over a period of 3 months was able to successfully raise 25-hydroxyvitamin D levels without altering other markers related to bone and mineral metabolism. Significance: Calcidiol supplementation is effective and safe.
Introduction The correction of vitamin D status is necessary to maintain an optimal mineral and skeletal homeostasis. Despite cholecalciferol (vitamin D_3) is the most commonly used drug for vitamin D supplementation, the more hydrophilic compound calcidiol (25-hydroxyvitamin D_3) can be employed at daily, weekly, and monthly regimens to reach in the short term the target levels of serum 25-hydroxyvitamin D [25(OH)D]. In the administration of different doses of calcidiol pharmacokinetic study (ADDI-D study), the efficacy and safety of daily and weekly dosages of calcidiol were tested.
Methods A total of 87 Caucasian, community-dwelling, postmenopausal women, aged 55 years or older, with vitamin D inadequacy (serum 25(OH)D levels <30 ng/ml, with mean 25(OH)D below 20 ng/ml, namely 16.5 ± 7.5 ng/ml) were randomized to receive three different dosages of calcidiol: 20 μg/day, 40 μg/day, and 125 μg/week for 3 months. The attained level of serum 25(OH)D was selected as primary endpoint to assess efficacy, while other parameters of mineral metabolism, (serum calcium, parathyroid hormone, phosphate, FGF23, urinary calcium, and markers of bone turnover) were assessed as secondary endpoints to establish safety.
Results In all the three groups, serum 25(OH)D values significantly and promptly rose and plateaued above the 30 ng/ml threshold remaining within safety interval after 14 days of treatment, with similar efficacy for the similar daily and weekly dose regimens. The different dosages were also equally effective in controlling secondary hyperparathyroidism. No significant changes in calcium and phosphate metabolism and in bone turnover markers were observed for any of the treatments, confirming the safety of this compound.
Conclusions The results of this study demonstrate the short- and mid-term efficacy and safety on core parameters of mineral metabolism of different daily or weekly dosages of calcidiol when used to treat vitamin D inadequacy or deficiency in postmenopausal women. Further studies are needed to assess falls as primary outcome of calcidiol supplementation.

Keywords Fracture · Hypovitaminosis D · Osteomalacia · Osteoporosis · Rickets · Vitamin D deficiency

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Introduction

The measurement of serum levels of 25-hydroxyvitamin D [25(OH)D], also referred to as calcidiol or calcifediol, still represents the preferred biomarker for the assessment of vitamin D status, reflecting the endogenous production and the exogenous exposure [1–3]. Calcidiol values are inversely

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correlated with serum PTH. However, owing to several means of calculating the point of maximal suppression of PTH by 25(OH)D, various thresholds (ranging from 12 to 50 ng/ml) have been suggested [4–7]. Alternatively, given the diurnal variation of serum PTH and dependency on recent calcium intake and level of mobility [8], a 25(OH)D threshold for bone health was assessed based on optimal hip bone mineral density [1] and reduction of fractures [9] pointing to a threshold of 24 to 30 ng/ml.

An optimal vitamin D status guarantees the maintenance of an appropriate calcium and phosphate homeostasis. Levels of 25(OH)D less than 20 ng/ml (i.e., 50 nmol/l) have been suggested as indicating vitamin D deficiency according to the majority of published guidelines [1, 6]. Vitamin D deficiency is very common worldwide, especially in seniors and osteoporotic patients, who are at increased risk of fracture. In adults, from a histological point of view, severe vitamin D deficiency (<10 ng/ml) has been associated with osteomalacia, which, due to impaired mineralization, can hamper bone strength and neutralize or diminish the effectiveness of anti-osteoporotic treatments [10]. Accumulated evidences indicate a threshold level range of serum 25(OH)D between 21 and 30 ng/ml (53 to 75 nmol/l) to be recommended in fragile seniors at high risk for falls and fracture [9–11], especially before commencing bone antiresorptive or anabolic therapy [1, 12].

Supplementation with vitamin D inactive precursors of the biologically active vitamin D metabolite (i.e., $1,25(OH)_2D$ or calcitriol) is an efficient way to correct a poor vitamin D status and restore an optimal mineral homeostasis [1, 13–17].

The most common form of vitamin D supplementation used today is cholecalciferol, also known as native or parental vitamin D_3. The recommended daily dosage regimens are usually not able to correct rapidly vitamin D inadequacy. A recent pharmacokinetic study suggested that it takes about 68 days with the daily dose of 800 IU of cholecalciferol to reach the optimal level plateau [15].

Therefore, high bolus doses (also referred to as mega doses) of cholecalciferol have been suggested and employed in order to achieve, in a relatively short time, the target levels of serum 25(OH)D necessary to begin osteoporosis treatment in subjects at increased risk of fracture [18]. Although the high doses of cholecalciferol (up to 10,000 IU per day) are safe as far as the classic side effects (hypercalciuria and hypercalcemia) are concerned [19, 20], unphysiologically high annual doses of vitamin D (300,000 to 500,000 IU) have been associated with an increased risk in falls and fractures [21, 22]. Notably, beyond the major endpoints such as falls and fractures, recent studies also suggested that bolus doses of 100,000 IU vitamin D_3 and above might increase bone resorption markers significantly and dose-dependently [23, 24].

Recently, a randomized controlled trial assessed the effect of two higher monthly doses of vitamin D (monthly 60,000 IU and 24,000 IU + 300 μg of calcidiol) in lowering the risk of functional decline and falls in community-dwelling men and women aged 70 or older with a prior fall event and compared with a monthly standard dose of 24,000 IU vitamin D (equivalent to 800 IU per day). Contrary to the expectations of the authors, the higher monthly bolus doses did not improve lower extremity function superior to the standard monthly dose and increase the odds of falling significantly despite raising 25(OH) levels more effectively [11]. These results have been confirmed by a study carried out in a population of vitamin D deficient elderly women (serum 25(OH)D less than 20 ng/ml) randomized to receive daily supplements of cholecalciferol at different dosages, with falls as primary outcome. In this group, while medium doses of vitamin D (i.e., daily dosages of 1600, 2400, and 3200 IU) were proven to prevent falls with respect to ineffective low doses (i.e., daily dosages of 400 and 800 IU), greater doses (i.e., daily dosages of 4000 and 4800 IU) leading to higher serum 25(OH)D concentrations (>40–45 ng/ml) increased fall rate [25].

Based on these data and the trials outlined earlier on the high annual doses, bolus applications of vitamin D administered intermittently are not warranted in seniors at increased risk of falling. Although no definitive conclusions can be drawn so far on this finding and no causal relationship has been demonstrated, it is nowadays not advisable to administer large doses of cholecalciferol at large intervals of time, according to most guidelines [1, 12]. Notably, higher daily doses of vitamin D need further exploration.

Calcidiol, the direct precursor of calcitriol, can represent an alternative strategy to enhance circulating serum 25(OH)D levels [15–17, 26, 27]. Several pharmacokinetic studies performed in the last four decades have demonstrated its hydrophilic properties, leading to higher solubility in organic solvents, lower trapping in the adipose tissues, smaller distribution volume, and shorter half-life (10–13 days), when compared to the parental compound cholecalciferol (30–45 days) [13, 26, 28]. The good predictability of achieved 25(OH)D levels in the short term, along with the effective PTH suppression and manageability in case of intoxication, may confirm the advantages of calcidiol supplementation versus cholecalciferol [14, 16]. Moreover, greater affinity of calcidiol for vitamin D binding protein (VDBP) allows a more efficient internalization in cells expressing the megalin-cubilin system of endocytic receptors, such as the parathyroids and the renal tissue [29].

The administration of calcidiol may be preferable in several conditions [13]. An example is offered by the alteration of liver cytochrome enzymes required for 25-hydroxylation [14, 30]. In the case of intestinal malabsorption, especially when associated with and steatorrhoea, calcidiol is better absorbed than cholecalciferol [31]. In addition, since a PTH-mediated inhibition of liver cytochrome isoforms has been shown in uremia, calcidiol utilization, instead of cholecalciferol, has been proposed for patients with chronic kidney disease [32].

The pharmacokinetics of calcidiol and cholecalciferol has been recently compared in a randomized, controlled parallel-group study [15]. Calcidiol administered daily, weekly, or in single bolus has been shown to be more effective and rapid in rising serum 25(OH)D levels with respect to cholecalciferol given at comparable doses daily (20 μg/day), weekly (140 μg/week for 15 weeks), or in single bolus (140 μg), with no risk of vitamin D intoxication [15]. Notably, the authors assessed also several clinical endpoints providing evidence that calcidiol given daily or weekly may be more effective in maintaining or improving lower extremity function and lowering systolic blood pressure among young postmenopausal women [16].

Calcidiol diluted in propylene glycol at a concentration of 150 μg/ml (5 μg calcidiol/drop) and administered as oral drops is registered and included in the European Pharmacopeia for the treatment of rickets due to vitamin D deficiency in children, as well as for vitamin D deficiency, osteomalacia, and spasmophilia in adults, plus additional indications in the various EU Countries [13].

The aim of this study has been to further analyze and describe the therapeutic regimens of calcidiol in terms of intervals of administration, and the mid-term effects on mineral and bone metabolism.

Methods

The administration of different doses of calcidiol study (ADDI-D study, EudraCT number: 2013-002648-10) is a multicenter, randomized, open label, three-arm, parallel group, and comparative phase III study. The study drug was calcidiol (Didrogyl®, solution, containing 1.5 mg of calcidiol and 10 ml propylene glycol in a dropper bottle, one drop = 5 μg calcidiol), to be taken in the morning in fasting state.

The primary objective of the study was to compare the effects of three different therapeutic regimens of calcidiol on the increase of circulating levels of serum 25(OH)D. Secondary objectives were to compare the effects of three different therapeutic regimens of calcidiol on changes of serum and urinary levels of mineral and bone biomarkers as compared at baseline.

The primary efficacy endpoint (primary variable) was represented by the attained circulating values of 25(OH)D at 3 months of treatment. Secondary efficacy endpoints (secondary variables) were the measurement of serum bone alkaline phosphatase (BALP), parathyroid hormone (PTH), $1,25(OH)_2D$, VDBP, and 24-h urine calcium over 3 months of treatment. Secondary safety endpoints were incidence of adverse events (AEs), serum calcium (corrected), ionized calcium, phosphate, creatinine, C-terminal telopeptides of type I collagen (CTX), fibroblast growth factor 23 (FGF 23), 24-h urinary calcium, and urinary deoxypiridinoline (DPD).

The study group was composed of Caucasian, community-dwelling, postmenopausal women, aged 55 years or older (years since menopause >2) with vitamin D inadequacy [i.e., serum 25(OH) D levels less than 30 ng/ml or 75 mmol/l], adequate calcium intake (1000 mg/day), and BMI < 30, consecutively recruited in two Italian referral centers for osteoporosis and metabolic bone diseases at the University Hospital of Florence, Florence, and University Hospital of Rome, Umberto I, respectively. The local internal review board of the two institutions approved the study. An informed consent was obtained from each participant.

Potentially eligible women were subjected to an assessment visit, consisting of medical history, clinical (vitals, weight, height, physical examination including blood pressure and pulse rate), and biochemical evaluations. An estimate of calcium intake was performed by administering a specific nutritional questionnaire [33].

Subjects were excluded on the basis of general criteria, as well as specific medical and therapeutic conditions (progressive major illness, severe malabsoption syndrome, IV stage chronic kidney disease, Paget's disease of bone, primary hyperparathyroidism/hypoparathyroidism, hypercalcemia, sarcoidosis, hypercalciuria, intolerance to calcidiol, treatments interfering with bone and mineral metabolism such as glucocorticoids, diuretics, lithium, immunosuppressants, antiretroviral therapy, and other drugs interfering with vitamin D absorption and catabolism). In particular, it was necessary for the candidates not to be exposed either to cholecalciferol (>400 IU/day in the previous month, doses >10,000 IU within the previous 3 months, or doses >50,000 IU within the previous 12 months) or to calcidiol and active vitamin D analogs during the 6 months prior to selection.

After the assessment visit, eligible subjects were randomized through a computer-based randomization system into three groups: group 1, receiving oral calcidiol 20 μg (Didrogyl® 4 drops) daily; group 2, receiving calcidiol 40 μg (Didrogyl® 8 drops) daily; and group 3, receiving 125 μg (Didrogyl® 25 drops) weekly. Thus, group 3 received on a weekly basis a similar dose as compared to group 1, taking calcidiol over 7 days. The 20 μg dosage was chosen because of the equivalence (in μg) to 800 IU of cholecalciferol, which is still considered the standard daily dose of vitamin D_3 [15–17]. It was decided not to exceed 125 μg for safety reason and possible side effects, since previous studies [15] showed that the administration of 140 μg/week in a single dose, although safe in terms of hypercalciuria and hypercalcemia, was followed by peaks in serum concentration of 25(OH)D nearly approaching 50 ng/ml possibly linked to non-classical side effects such as falls or increase in bone turnover markers [15, 23, 24]. Patients were evaluated at baseline, and 7, 14, 21, 28, 60, and 90 days, afterwards. Adverse events, compliance, and concomitant treatments were recorded at each time point.

Serum 25(OH)D, VDBP, calcium, phosphate, albumin, ionized calcium, creatinine, bone alkaline phosphatase, and CTX were assayed at each visit. Serum PTH, $1,25(OH)_2D$, intact FGF23, alkaline phosphatase (AP), albumin, total proteins, bilirubin, ASAT, ALAT, GGT, Na, Cl, and K were assessed at baseline and at 90 days. Urinary parameters such as 24-h urinary calcium, phosphate, and creatinine and DPD were assessed at each time point. Albumin and total proteins were determined for the calculation of corrected serum calcium, while AP, bilirubin, ASAT, ALAT, GGT, Na, Cl, K, urinary phosphate, and creatinine were assessed for safety issues. These latter parameters were not included in the endpoints of the study.

The determinations of serum 25(OH)D (vitamin D TOTAL Assay, DiaSorin USA, Stillwater, MN, USA, competitive one-step backfill chemiluminescence assay, with a measurement range of 4–150 ng/ml and functional sensitivity of 4.0 ng/ml or less; intra- and inter-assay precision of 8.9 and 12.8%, respectively, with reported cross-reactivity values of 100% for both 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3), intact FGF23 (ELISA, Immutopics, Inc., CA, USA; CV intra-assay 4.5%, CV inter-assay 11.5%), and VDBP (ELISA, DRG Instruments GmbH, Germany; CV intra-assay 5.5%, CV inter-assay: 12.5%) were performed on frozen samples and centralized at the University Hospital of Rome [for serum 25(OH)D samples, in a laboratory (no. 140) accredited to Vitamin D Eternal Quality Assessment Scheme, DEQAS] and University Hospital of Florence (for serum intact FGF23 and VDBP samples). The measurements of BALP (immunoassay, IDS-iSYS Multi-Discipline Automated System, UK), CTX (electrochemiluminescent assay, Cobas 601, Roche Diagnostics Gmb, Germany), DPD (chemiluminescent assay, Immulite, 2000, Siemens, UK), PTH (electrochemiluminescent assay, Cobas 601, Roche Diagnostics Gmb, Germany), and $1,25(OH)_2D$ (chemiluminescent assay, IDS-iSYS, Belgium) were carried out in central routine laboratory on frozen samples at University Hospital of Florence. All the other measurements, including ionized calcium, were carried out at local recruiting centers; random samples were assayed in a central laboratory for quality assurance.

All subjects were advised to maintain a systematic daily calcium intake of 1000 mg/day from dietary sources. When this was not achieved by diet only, calcium supplements (500 mg/day, to be taken at lunchtime) were also administered.

Based on the intention-to-treat principle, the full analysis set (FAS) corresponds to patients who have taken at least one dose of study treatment after randomization. The safety population consisted of all patients who received any study medication and was based on the treatment they actually received.

For the primary variable [serum 25(OH)D], the absolute change at the final visit in respect to baseline 25(OH)D has been estimated in the FAS population using analysis of variance (ANOVA). The model has included fixed effect terms for treatment. Considering that the central laboratory evaluated all main biochemical data and no evidence for center effect has been evidenced in preliminary analyses, final analyses do not take into account the center effect. Multiple comparison tests for main level effects in the model have been carried out using post hoc Bonferroni test. Sensitivity analyses on primary variable has been conducted applying analysis of co-variance (ANCOVA) on time profile using baseline data as co-variate in order, analysis of co-variance (ANCOVA) on time profile above the treatment response using baseline data as co-variate, dosage treatment, visit, and dose-visit interaction. Student *t* test has been used to evaluate intra-treatment change from baseline.

As far as secondary variables are concerned, the treatment effect has been estimated in the FAS population, and only biochemical relevant parameters have been processed with ANOVA for repeated measures. The model included dosage effect, visit, and dosage-visit interaction.

Safety analyses have been carried out on the safety population. Laboratory parameters have been described using the appropriate summary statistics at each visit stratified by treatment. A potentially clinically significant abnormal value has been evidenced.

Vital signs have been described by group of treatment. A potentially clinically significant abnormal value has been evidenced.

A sample size of 72 subjects was calculated to demonstrate a between-factor effect (Cohen's *d*) of 0.45 in an ANOVA repeated measure analysis, considering alpha and beta levels of 0.05, and the contrast between basic and final visit as primary end point. In order to be more conservative and taking into account around 15% of drop-outs during the study, the final sample size estimation was 84 subjects (28 in each group).

Results

A total of 87 women with vitamin D inadequacy were randomized to receive three different dosages of calcidiol. Three patients did not assume any treatment and then were therefore excluded from further analyses. Twenty-seven patients received calcidiol at the dosage of 20 μg (4 drops)/day (dosage 1), 28 patients received calcidiol at the dosage of 40 μg (8 drops)/day (dosage 2), and 29 patients received calcidiol at the dosage of 125 μg (25 drops) weekly (dosage 3).

The treatment groups were comparable for baseline demographic and clinical characteristics (age, age of menarche, age of menopause, height, weight, BMI, and body surface area), and calcium intake (including calcium as daily supplement for patients not reaching 1000 mg/day, as specified above), with no observed statistically significant difference for the different parameters (Table 1). Regarding vitamin D status, almost two thirds (62.1%) of the women showed levels of serum 25(OH)D less than 20 ng/ml, with a mean serum 25(OH)D

Table 1 Baseline characteristics of women in the three randomization groups

	Treatment group of calcidiol	*n*	Mean ± SD
Age (years)	20 μg/day	27	69.9 ± 8.0
	40 μg/day	28	64.4 ± 6.8
	125 μg/week	29	66.2 ± 7.8
Age of menarche	20 μg/day	27	12.6 ± 1.6
	40 μg/day	28	12.4 ± 1.5
	125 μg/week	29	13.1 ± 1.7
Age of menopause	20 μg/day	27	47.6 ± 5.8
	40 μg/day	28	49.3 ± 5.6
	125 μg/week	29	49.0 ± 5.3
Height (m)	20 μg/day	27	160.0 ± 7.4
	40 μg/day	28	160.9 ± 6.7
	125 μg/week	29	158.7 ± 6.3
Weight (Kg)	20 μg/day	27	63.9 ± 7.3
	40 μg/day	28	64.0 ± 8.6
	125 μg/week	29	62.9 ± 9.9
BMI (Kg/m^2)	20 μg/day	27	25.0 ± 3.0
	40 μg/day	28	24.7 ± 2.6
	125 μg/week	29	25.0 ± 3.8
BSA (m^2)	20 μg/day	27	1.7 ± 0.1
	40 μg/day	28	1.7 ± 0.1
	125 μg/week	29	1.7 ± 0.1
Calcium intake (mg/day)	20 μg/day	27	1035.7 ± 323.6
	40 μg/day	28	912.8 ± 386.4
	125 μg/week	29	964.6 ± 426.7

n number of subjects, *BMI* body mass index, *BSA* body surface area

levels of 16.5 ± 7.5 ng/ml and no significant difference between the treatment groups. Mean levels of serum 25(OH)D were comparable and below 20 ng/ml, namely 15.1 ± 7.4 ng/ml in group 1, 16.8 ± 6.6 ng/ml in group 2, and 16.4 ± 9.7 ng/ml in group 3 (Supplementary Table 1).

No alterations in vital signs or clinical abnormalities were recorded at the screening visit nor in the following examinations.

In Fig. 1, the profiles of serum 25(OH)D concentration attained with the three dose regimens of calcidiol are displayed. The ANOVA on absolute change of 25(OH)D levels from baseline (primary analysis) showed statistical difference between the final attained 25(OH) concentration at the end of the study period versus baseline levels in each of the three groups ($p < 0.0001$). Difference at day 90 in respect to baseline has been confirmed by paired Student *t* test for all treatment groups ($p < 0.0001$). Post hoc Bonferroni multiple comparison evidenced statistical difference between dosage 1 and 2 (estimated difference −23.44 with SE estimated 5.30 associated to probability corrected level equal to <0.0001) and dosage 2 and 3 (estimated difference 28.46 with SE estimated 5.20 associated to probability corrected level equal to <0.0001); no statistical difference has detected between dosage 1 and 3 (estimated difference 5.02 with SE estimated 5.30 associated to probability corrected level equal to 1.0000). Sensitivity analyses have confirmed results of the primary analysis. Analysis on time profile has evidenced a statistical significant difference in treatments response during time at the various time points for each treatment group (ANOVA for repeated measures: $p < 0.0001$ for each value versus respective baseline).

Thus, all dosage schemes enabled a significant increase in serum 25(OH)D levels at the end of treatment period with restrained variability, as demonstrated by 95% confidence limits. Considering two populations of women, those with baseline serum 25(OH)D levels below 20 ng/ml and those with serum 25(OH)D levels between 20 and 30 ng/ml, no differences were observed in terms of absolute percentual increase in serum 25(OH)D levels (final versus baseline) between the two categories of patients for each treatment group (+168.2 and +203.6% in group 1, +297.0 and 299.3% in group 2, 161.6 and 152.5% in group 3, respectively).

After 14 days of treatment, the majority (75%) of patients reached lower limit for serum 25(OH)D sufficiency (i.e., 20 ng/ml); then, a further increase was observed, maintaining the serum concentration in the classical safety window (30–100 ng/ml). While 25(OH)D levels were similar for the groups receiving 20 μg/day or 125 μg/week at each time point, with a final attained 25(OH)D of 49.3 ± 19.5 and 46.4 ± 15 ng/ml, respectively, they almost doubled in the group supplemented with 40 μg/day, reaching a mean 25(OH)D of 74.8 ± 22.5 ng/ml. Moreover, no difference was observed in serum 25(OH)D concentrations between 30 and 90 days of treatment, indicating a plateau phase during calcidiol administration in the short-to-medium term.

Figures 2, 3, 4, to 5 show the time course of different serum and urinary biochemical and hormonal markers (reported in detail in Supplementary Tables 1 and 2). Serum total and ionized calcium remained within the normal range for the duration of the study in the three groups of patients. As demonstrated by ANOVA for repeated measures, no statistical differences between the three treatment groups and versus baseline were observed for serum total calcium and ionized calcium, despite a statistical difference was shown for ionized calcium for visit term (*F* value = 5.99, Prob > *F* < 0.0001), which should be interpreted as consequence due to the relevant fluctuation of values at day 21 (Supplementary Table 1, Fig. 2a, b).

Serum phosphate, creatinine, and markers of bone turnover (BALP and CTX) stayed within the normal range in the three groups. No peak concentrations in bone turnover markers were observed. ANOVA for repeated measures confirmed no differences between treatments, despite significant fluctuation during the visits for serum phosphate (*F* value = 2.27, Prob > *F* = 0.0451), serum creatinine (*F* value = 1.27, Prob > *F* = 0.2825) and markers of bone turnover such as

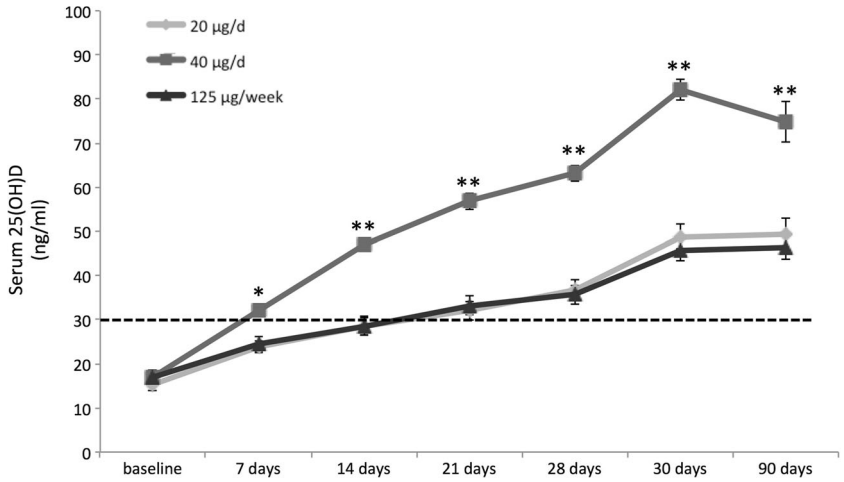

Fig. 1 Time profile of the response of serum 25(OH)D (ng/ml) to different dose regimens of calcidiol at various time points (mean ± SE); *the dotted lines* indicate serum levels of 25(OH)D vitamin D sufficiency/adequacy by different guidelines (i.e., 20 and 30 ng/ml) (*$p < 0.05$ and **$p < 0.001$ versus baseline for all treatment groups)

BALP (F value = 5.00, Prob > F = 0.0002) and CTX (F value = 2.91, Prob > F = 0.0128) (Supplementary Table 1, Fig. 2c, d, Fig. 3a, b).

Hormonal parameters were determined at baseline and final evaluation (Supplementary Table 2). Serum levels of PTH, $1,25(OH)_2D$ and intact FGF23 did not vary among the different treatments (ANOVA). While 1,25(OH)2D and intact FGF23 levels remained stable during calcidiol supplementation in the three groups of treatment (final versus baseline serum $1,25(OH)_2D$: $p = 0.44$, $p = 0.46$, and $p = 0.99$ for dosage 1, 2, and 3, respectively; final versus baseline serum FGF23: $p = 0.47$, $p = 0.40$, and $p = 0.43$ versus baseline for dosage 1, 2, and 3, respectively), PTH significantly decreased over the 90 days (final versus baseline serum PTH: $p < 0.0001$, $p < 0.0001$, and $p = 0.0005$ for dosage 1, 2, and 3, respectively), (Fig. 4a, b). Regarding VDBP (Supplementary Table 1, Fig. 4c), ANOVA for repeated measures showed a significant steady increase during the first 4 weeks of treatment (F value = 13.25, Prob > F < 0.0001) (Fig. 4c).

Urinary parameters have been evaluated for safety issues. ANOVA for repeated measures confirmed no differences between treatments despite significant fluctuation during the study

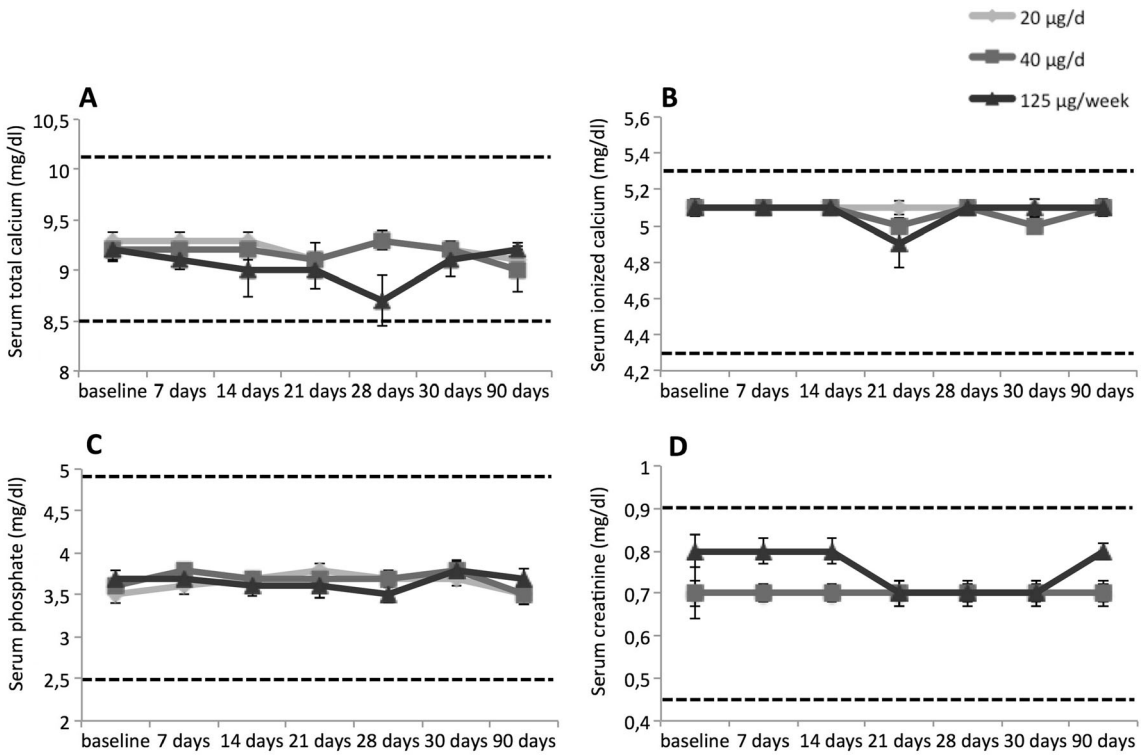

Fig. 2 Time profile of the values of serum calcium (mg/dl) (panel **a**), ionized calcium (mg/dl) (panel **b**), phosphate (mg/dl) (panel **c**), and creatinine (mg/dl) (panel **d**) under different dose regimens of calcidiol at various time points (mean ± SE); normal range is indicated between the *dotted lines*

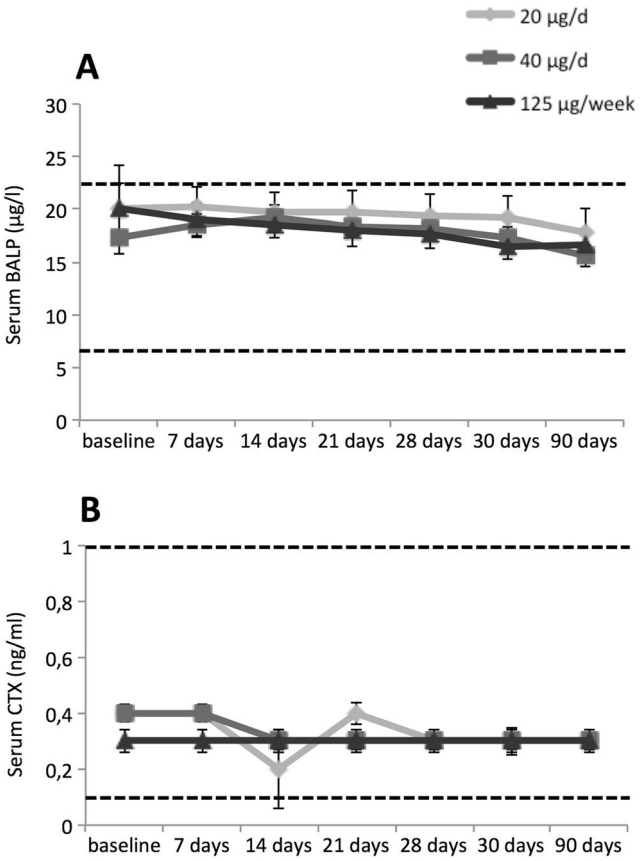

Fig. 3 Time profile of bone turnover markers [serum BALP (µg/l) (panel **a**) and CTX (ng/ml) (panel **b**) under different dose regimens of calcidiol at various time points (mean ± SE); normal range is indicated between the dotted lines

for 24-h urinary calcium (F value = 8.88, Prob > F < 0.0001) (Supplementary Table 1) (Fig. 5). Although a steady increase in urinary calcium was observed during the treatment with the three dosage regimens, there was no evidence of hypercalciuria (i.e., calcium >300 mg/24 h). Indeed, urinary calcium plateaued between 30 and 90 days of treatment. Twenty-four-hour urinary phosphate, 24-h urinary creatinine, and urinary DPD did not differ between the different dose regimens (Supplementary Table 1).

Other biochemical parameters have been evaluated both for efficacy and safety survey of patient status. No relevant changes were observed for serum alkaline phosphatase, albumin, total proteins, ASAT, ALAT, GGT, Na, Cl, and K (data not shown).

Throughout the study, only four events have been detected, one per patient: three at center 1 (patient no. 20, dosage 40 µg/day; patient no. 30, dosage 20 µg/day; patient no. 33, dosage 125 µg/day, and patient no. 34, dosage 125 µg/day) and one at center 2 (patient no. 41, dosage 125 µg/day). The three patients at center 1 reported a flu episode, while the patient at center 2 was found to be hypercalcemic in one occasion; this episode was considered "possibly" related to the treatment. All other events were not considered to be drug-related.

Discussion

The ADDI-D study extends previous observations on the efficacy of calcidiol when employed in vitamin D supplementation for vitamin D inadequacy in postmenopausal women with mean serum 25(OH)D levels below 20 ng/ml [15–17]. Moreover, for the first time, the effects of three different calcidiol regimens on core parameters of bone and mineral homeostasis in postmenopausal women with vitamin D inadequacy (i.e., serum 25(OH)D levels less than 30 ng/ml) have been assessed. The results confirm calcidiol's prompt efficacy in correcting vitamin D status and underline its safety, at least in the short/medium term, also in regard to some non-classical, unwanted adverse events (e.g., alterations in bone turnover markers or increased fall rate) observed during supplementation with native vitamin D [11, 21, 22, 25].

Calcidiol, which is also the circulating form of vitamin D as well as the best marker of vitamin D status, has been marketed in Europe for vitamin D supplementation in several conditions [13, 14]. Osteomalacia, the mineralization defect due to vitamin D deficiency, is its main therapeutic indication in adults. Especially in older individuals, this condition can coexist with an alteration of bone microarchitecture (namely osteoporosis). Calcidiol significantly decreases the osteoid volume and surface and greatly increases the front of calcification, normalizing biological parameters (serum calcium, phosphorus, AP) more efficiently than other vitamin D metabolites [13, 14].

When administering 25(OH)D, this metabolite enters the circulation and hence bypasses liver metabolism. This, together with peculiar chemical characteristics of calcidiol (greater polarity, unusual for a steroidal compound) and its consequent pharmacokinetic properties, make this drug preferable when a rapid replenishment of 25(OH)D reservoir and/or additional potency for attaining desired amounts of 25(OH)D are required.

These peculiar properties have made calcidiol widely used as vitamin D supplement, as demonstrated by the analysis of regional administrative pharmaceutical databases [34].

In this study, calcidiol has been administered at three different regimens: two daily treatments of 20 and 40 µg, respectively, and a weekly treatment of 125 µg, similar to the calculated cumulative dose of the lower daily dosage. In all the three groups, serum 25(OH)D values significantly and promptly rose above the 20 ng/ml threshold so that the majority of subjects were vitamin D repleted after just 14 days of treatment. This can be exploited particularly when vitamin D status has to be optimized in a short interval of time, for example when antifracture therapy has to be undertaken in subjects at high risk for fracture (e.g., after a major fragility fracture or when commencing glucocorticoid therapy). The rapidity in correcting profound vitamin D deficiency with overt or insidious osteomalacia makes calcidiol a good alternative to the mega doses of parental vitamin D_3, recently linked to increased falls and fractures [21, 22], since the non-

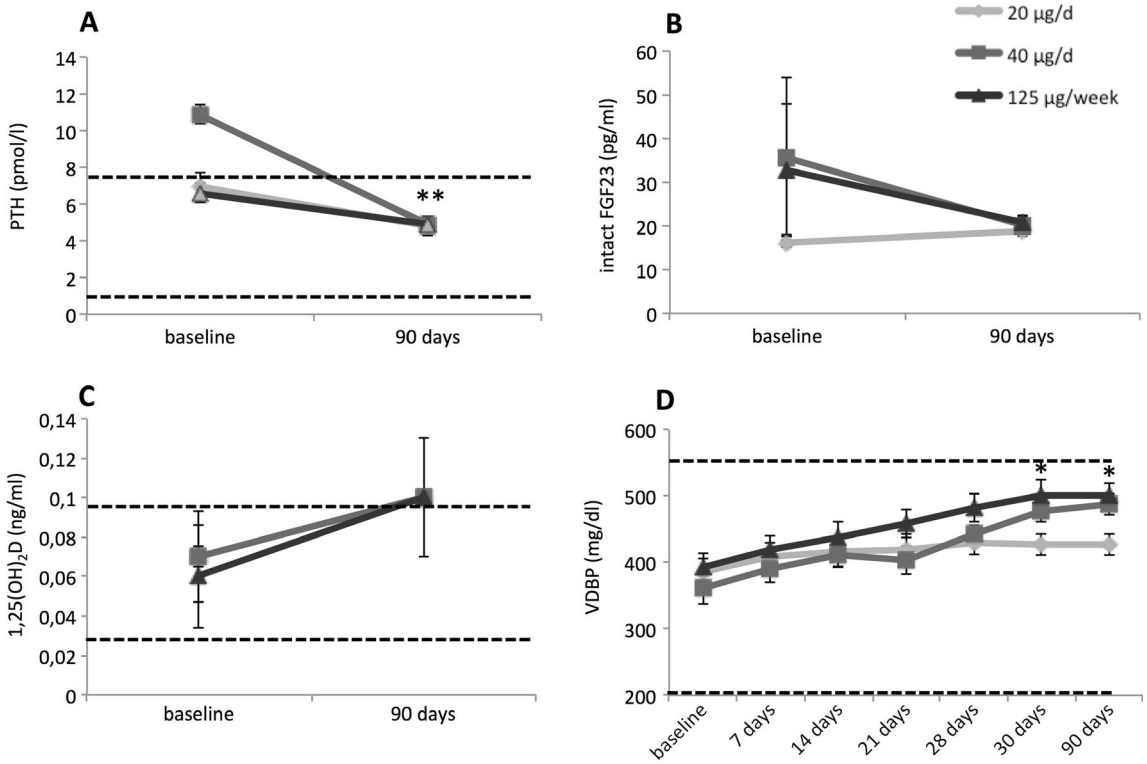

Fig. 4 Time profile of serum PTH (pmol/l) (panel **a**), intact FGF23 (pg/ml) (panel **b**), 1,25$(OH)_2$D (ng/ml) (panel **c**), and VDBP (mg/dl) (panel **d**), and to different dose regimens of calcidiol at various time points (mean ± SE); normal range is indicated between the *dotted lines* (*$p < 0.05$ and **$p < 0.001$ versus baseline for all treatment groups)

physiological, abnormal higher peaks in the concentration of 25(OH)D are avoided. Because calcidiol is more powerful in attaining the desired serum 25(OH)D level and due to its smaller volume of distribution, it can be proposed as a better, active vitamin D precursor for obese individuals, in whom massive doses of cholecalciferol must be employed to reach the threshold of 30 ng/ml [14, 35].

The baseline vitamin D status has a major influence on the percentual increase in serum 25(OH)D during cholecalciferol supplementation [33]. This does not seem to be true also for calcidiol, since, as demonstrated in the ADDI-D study, the increase in vitamin D levels obtained by calcidiol administration does not depend on initial vitamin D status. Indeed, the percentual gain in serum 25(OH)D levels comparable for women with baseline 25(OH)D less than 20 ng/ml and women with 25(OH)D comprised between 20 and 30 ng/ml. Nonetheless, this observation requires further studies.

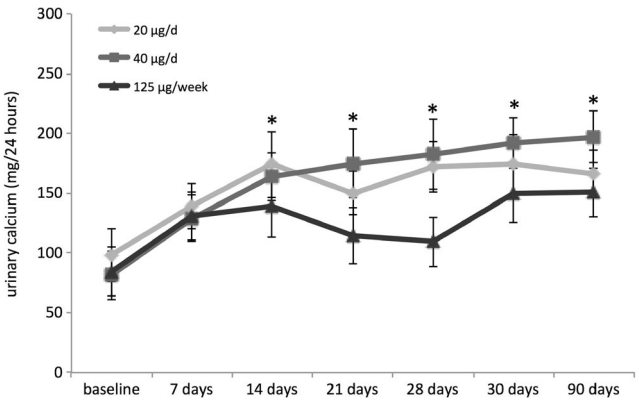

Fig. 5 Time profile of urinary calcium (mg/24 h) under different dose regimens of calcidiol at various time points (mean ± SE) (*$p < 0.05$ versus baseline for all treatment groups)

The similarity of efficacy of the daily and weekly dose regimens (i.e., 20 µg/day and 125 µg/week, respectively) in terms of attaining sufficient levels of 25(OH)D shown in this study confirm previous findings [15], indicating that administering calcidiol every day or administering an equal (140 µg) [15] or similar (125 µg) amount once a week or monthly [27] results in equal effectiveness with respect to attaining desired 25(OH)D levels. The efficacy of the lower dose regimens also demonstrates that there is no need to further increase the dosage in non-obese subjects to correct vitamin D status for optimizing mineral homeostasis. Nonetheless, the pharmacokinetic profile of the compound shows the attainment of a plateau of serum 25(OH)D levels in the long interval between 30 and 90 days of treatment, which is well below the upper limit of classical toxicity (i.e., hypercalciuria and hypercalcemia), even when administered at higher dose (40 µg/day). Nonetheless, given the increasing evidence of non-classical toxic effects (i.e., increased fall rate) for attained levels of serum 25(OH)D above 50 ng/ml [11, 25], the higher dosages

of calcidiol (i.e., 40 μg/day) should be possibly avoided, particularly in frail individuals at high risk of falling and related consequences [36].

The results of the ADDI-D study suggest a good safety and manageability of this compound at lower dosages in clinical practice, without the need of close monitoring serum 25(OH)D in the short-medium term. Indeed, levels of VDBP increased steadily within the first 30 days, before the plateau in 25(OH)D concentrations during daily administration of 40 μg and weekly administration of 125 μg. This may represent a buffering of the homeostatic vitamin D system itself to avoid dangerous increase in biologically active 25(OH)D.

In addition, a good predictability of achieved serum 25(OH)D levels has been observed, with increases by twofold though the administration of double doses of daily calcidiol.

Calcidiol has proven to be safe when administered at daily, weekly, monthly, or every other week [27] dose regimens. It is commonly used in clinical practice, without risk of vitamin D intoxication (i.e., hypercalciuria and hypercalcemia). Indeed, the attained 25(OH) levels are far below what is considered as the upper limit of normal values (i.e., 100 ng/ml) as indicated by most laboratories in order to avoid the vitamin D-related classic toxic effects, namely hypercalciuria and hypercalcemia. Total and ionized calcium levels were stable up to 90 days of treatment. Moreover, serum phosphate and creatinine remained within the normal range, along with urinary markers and routine biochemical tests, and no difference was observed between different dosages, beside expected physiological fluctuations within the study period.

Regarding safety issues, this study for the first time has also assessed the effect of calcidiol on several additional markers of mineral metabolism.

The increase of markers of bone turnover has been suggested as possible mechanisms to explain unwanted non-classic side effect of cholecalciferol [23, 24]. In this study, no increase of markers of bone turnover (such as serum BALP, CTX, and urinary DPD) was observed for any of the treatments with calcidiol within the whole interval of administration. Furthermore, in a previous study utilizing a monthly dose of 500 μg calcidiol, a decrease of bone alkaline phosphatase was even observed [27].

As expected, a decrease of serum PTH levels as well as an increase of 25(OH)D concentration was observed. Notably, however, the different dosages were equally effective in controlling secondary hyperparathyroidism. Alterations of FGF23, phosphate metabolism, and $1,25(OH)_2D$ have been indicated as possible mediators of non-classic toxic effects of cholecalciferol and/or possibly counteracting the effects of vitamin D [37, 38]. No differences in serum intact FGF23, phosphate, or $1,25(OH)_2D$ concentrations were observed during daily or weekly calcidiol administration, further underlining the safety of this compound regarding potential, non-classic unwanted events.

Overall, the results of this study confirm the efficacy and safety of calcidiol, the direct precursor of calcitriol, when used, at least in the short/medium term, to treat vitamin D inadequacy or deficiency in postmenopausal women.

Given the growing recent evidence of increased number of falls for higher dosages of cholecalciferol leading to serum 25(OH)D above 50–60 ng/ml [11, 25], caution has to be used before prescribing higher dosages of calcidiol (i.e., 40 μg/day), particularly in subjects at high risk of falling, even if an increased risk of falling has not still been demonstrated for calcidiol supplementation. Indeed, no data on falls are available during calcidiol supplementation, while a better muscular performance has been demonstrated [16, 39]. We acknowledge that not having included fall assessment in the safety analysis is a limitation of our study. In this respect, further mid- and long-term studies are necessary in this field, having falls as primary outcome, particularly in at-high-risk individuals.

Conclusions

The study hereby presented demonstrates, for the first time, the efficacy of calcidiol as well as its safety on multiple parameters related to mineral and bone metabolism in the short-medium term.

More studies are needed to further assess the calcidiol pharmacokinetics properties in the long term, along with unique properties of the compound that have not yet been fully recognized, such as musculoskeletal effects. Indeed, some randomized controlled trials have demonstrated increased muscle performance in postmenopausal women supplemented with calcidiol at usual doses with respect to cholecalciferol [16, 39], although the risk of falling during calcidiol supplementation has not been assessed yet as a primary outcome. These properties make calcidiol a good alternative to cholecalciferol in the treatment of the widespread vitamin D deficiency and related musculoskeletal consequences (osteomalacia, falls, and fractures), and even the supplement of choice when specific conditions hamper the efficacy of parental vitamin D.

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Compliance with ethical standards

Conflict of interest Luisella Cianferotti, Piergianni Biondi, Caterina Fossi, Francesco Franceschelli, Francesca Giusti, Gigliola Leoncini, and Jessica Pepe declare that they have no conflict of interest.

Salvatore Minisola served as speaker for Abiogen, Amgen, Bruno Farmaceutici, Diasorin, Eli Lilly, Italfarmaco, Fujii, and Merck Sharp & Dohme, Takeda. He served in advisory board of Amgen, Eli Lilly, and

Merck Sharp & Dohme and received paid consultancy from Bruno Farmaceutici.

Heike A. Bischoff-Ferrari contributed as invited speaker and on advisory boards for Roche, Pfizer, Sanofi, DSM Nutritional Products, Nestlé, and WILD. She received investigator initiated and independent funding from DSM Nutritional Products, Roche Diagnostics, Pfizer, and Nestlé.

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