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Pharmacokinetics of oral vitamin D_3 and calcifediol☆

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ABSTRACT

Aim: Long-term pharmacokinetics after supplementation with vitamin D_3 or calcifediol (the 25-hydroxyvitamin D_3 metabolite) is not well studied. Additionally, it is unclear whether bolus doses of vitamin D_3 or calcifediol lead to 25(OH)D_3 plasma concentrations considered desirable for fracture prevention (30 ng/mL). We therefore investigated plasma pharmacokinetics of 25(OH)D_3 during different vitamin D_3 and calcifediol supplementation regimens.

Methods: In this seven-arm, randomized, double-blind, controlled parallel-group study, 35 healthy females aged 50–70 years (5 per group) received 20 μg calcifediol or vitaminD_3 daily, 140 μg calcifediol or vitaminD_3 weekly, for 15 weeks, or a single bolus of either 140 μg calcifediol, or vitaminD_3, or both. 25(OH)D_3 plasma concentrations were quantified using LC–MS/MS in 14 clinical visits among all participants.

Results: For daily (weekly) dosing, the area under the concentration–time curve (AUC_{0-24h}), which is the measure for exposure, was 28% (67%) higher after the first dose of calcifediol than after the first dose of vitamin D_3. After 15 weeks, this difference was 123% (178%). All women in the daily and weekly calcifediol groups achieved 25(OH)D_3 concentrations >30 ng/mL (mean, 16.8 days), but only 70% in the vitamin D_3 daily or weekly groups reached this concentration (mean, 68.4 days). A single dose of 140 μg calcifediol led to 117% higher 25(OH)D_3 AUC_{0-96h} values than 140 μg vitamin D_3, while the simultaneous intake of both did not further increase exposure.

Conclusions: Calcifediol given daily, weekly, or as a single bolus is about 2–3 times more potent in increasing plasma 25(OH)D_3 concentrations than vitamin D_3. Plasma 25(OH)D_3 concentrations of 30 ng/mL were reached more rapidly and reliably with calcifediol.

Introduction

Recent recommendations by the US Endocrine Society Taskforce on Vitamin D [1], the International Osteoporosis Foundation (IOF) [2] and the Institute of Medicine (IOM) [3] agree that vitamin D supplementation is beneficial for bone health. While recommendations for desirable concentrations for bone health vary between 20 [3] and 30 [1] ng/mL (50 to 75 nmol/L), all guidelines agree that the goal of vitamin D supplementation is to correct vitamin D deficiency (<20 ng/ml) as soon and as reliable as possible.

Given the high prevalence of vitamin D deficiency [1,2,6,7] (25(OH)D_3 < 20 ng/ml), the evidence for the currently recommended dose of 800 IU vitamin D per day on the prevention of falls and fractures among seniors [4,5], an evaluation of well-defined supplementation strategies is warranted. The most common form of dietary supplementation used today is cholecalciferol or vitamin D_3. Most healthy adults reach 25(OH)D_3 plasma concentrations of about 20 ng/mL with 600 to 800 IU vitamin D per day [8–10]. However, vitamin D_3 doses to reach concentrations of at least 30 ng/mL are less well defined and may require 1600 IU to 4000 IU vitamin D_3 per day [8,9]. Among healthy postmenopausal white women, a multi-dose comparison suggested that a dose of 1600 IU per day may be sufficient for 97.5% of the study population to reach 30 ng/ml [9]. Notably, apart from standard dose comparison studies [9,10], detailed long-term pharmacokinetic data for the widely recommended dose of 800 IU vitamin D per day are lacking.

Abbreviations: AUC, area under the concentration vs time curve; C_{max}, maximum concentration; C_{last}, concentration quantified before the next study drug intake; GMR, geometric mean ratio; HPLC, high performance liquid chromatography; t_{max}, time to reach C_{max}; RCT, randomized controlled trial.

☆ Clinical trial registry: The trial was registered at clinicaltrials.gov (NCT00718276).

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Oral intake of the vitamin D metabolite calcifediol (25-hydroxyvitamin D_3) itself, commercially available in only a few countries has also been proposed as a means to increase "25(OH)D_3" concentrations [11–16]. Further research with this metabolite may serve to increase its availability. Compared to vitamin D_3, calcifediol is more hydrophilic, has a much shorter half-life, and causes a rapid and sustained increase in plasma 25(OH)D_3 concentrations [11,15]. A recent study showed that in a blood sample taken after 10 weeks of calcifediol intake, dose-normalized 25(OH)D_3 concentrations were 4.2- to 5-fold higher than after vitamin D_3 intake [17]. With respect to bone mineral density, small trials reported a benefit among groups of cardiac and kidney transplant patients, and senior hip fracture patients [18–20]; while the largest trial among 438 seniors with a lower dose of 15 μg per day did not fully confirm such a benefit [21].

The aim of this study was to investigate in detail the plasma pharmacokinetics of 25(OH)D_3 over 15 weeks during daily and weekly intakes of vitamin D_3 and calcifediol in doses equivalent to 20 μg per day. Additionally, we investigated pharmacokinetics after a single bolus dose of either drug, or their combination. The comparison of clinical effects between daily and weekly treatment arms with either vitamin D^3 or calcifediol has been described earlier [22].

Subjects and methods

Study participants and conduct

In this prospective, randomized, seven-arm, parallel group study, a total of 35 healthy, postmenopausal women (5 per group) were enrolled and attended 14 clinical visits. The study was performed at the Centre on Aging and Mobility at the University of Zurich, was approved by the Ethics Committee of the Canton of Zurich, Switzerland, and is registered with the Swiss national health authority, Swissmedic and the international trial registry clinicaltrials.gov (NCT00718276). All participants gave written informed consent before any study specific procedure was carried out. In a pre-study examination, eligibility was assessed. The participants had to be postmenopausal (no vaginal bleeding for at least 1 year) women age 50 to 70 years of age, have a body mass index between 18 and 29 kg/m^2, non-smoking, Caucasian, and in generally good health. Their plasma 25-hydroxyvitamin D_3 concentration had to be between 8 and 24 ng/mL at baseline. The study was carried out between January and July, 2008. Participants who planned a vacation in a sunny region during the study course were excluded.

The study had four different oral application groups (daily, weekly, bolus or combined bolus application) and was carried out in a double-blind, randomized fashion concerning treatment allocation (vitamin D_3 vs. calcifediol). For treatment regimen, the allocation was randomized but individuals knew if they were in the daily, weekly, or bolus regimen group. In all groups participants attended 14 clinical visits over the follow-up of 15 weeks. In the daily oral application groups, 5 women per group received 20 μg vitamin D_3 (800 IU) or 20 μg calcifediol daily together with breakfast for 15 weeks. In the weekly groups, 140 μg vitamin D_3 (5600 IU) or 140 μg calcifediol was taken orally once weekly together with breakfast for 15 weeks. In the three bolus application groups, volunteers received either 140 μg vitamin D_3 (5600 IU) or 140 μg calcifediol, or both (same doses as for single bolus), as a single dose at one occasion. The bolus administration was supervised by study personnel.

The blinded study drugs were supplied by Fisher Clinical Services, Allschwil, Switzerland, and contained vitamin D_3 or calcifediol manufactured by DSM Nutritional Products, Basel, Switzerland. The actual content of capsules was validated and control measurements were 790 IU vitamin D_3 (claim 800 IU), 5837 IU vitamin D_3 (claim 5600), 19.8 μg calcifediol (claim 20 μg calcifediol), 140 μg calcifediol (claim 140 μg calcifediol).

On the day of the first study drug intake (week 1) and of the last study drug intake (week 15), serial blood samples for pharmacokinetic profiling of 25(OH)D_3 plasma concentrations were collected. In addition, calcium, creatinine, and albumin in serum were quantified at the same time points: before, and 2, 4, 6, 8, 10, 12, and 24 h after the intake of the study drug. Urine samples were obtained before, and 4, 8, 12, and 24 h after the drug intake for quantification of urinary calcium and creatinine. In the three bolus groups, pharmacokinetic profiling was done only on day 1. Additionally, blood and urine samples were taken once daily in the morning before study drug intake on days 3 to 5 of the first trial week and at day 1 of the weeks 2, 3, 5, 7, 9, 11, 13, and 15 in all participants. Plasma for 25(OH)D_3 quantification was stored at −80 °C and shipped on dry ice for blinded analysis in the laboratories of DSM Nutritional Products in Kaiseraugst, Switzerland. All other biomarkers were analyzed at the Institute for Clinical Chemistry of the University Hospital Zurich, Switzerland.

Quantification of 25-hydroxyvitamin D_3 in plasma

Plasma 25(OH)D_3 concentration was assessed by means of a sensitive and selective assay based on liquid chromatography coupled to tandem mass spectrometry detection (HPLC–MS/MS) [23,24]. Inter-day precision expressed as %CV was between 4.2% and 6.0% (n = 43, 23 days), and accuracy was between 102% and 104%. The lower limit of quantification of 25(OH)D_3 was 2 ng/mL. Assay performance was confirmed at the winter 2010 exercise of the NIST/NIH Vitamin D Metabolites Quality Assurance Program [25].

Pharmacokinetic and statistical analysis

The maximum concentration (C_{max}), time to reach C_{max} (t_{max}), and the concentration quantified before the next study drug intake (C_{last}) were directly taken from the raw data, while the area under the concentration time curve during the first 24, or 96, hours, $AUC_{0–24}$, and $AUC_{0–96}$, respectively, was calculated using a compartment-free approach (Win Nonlin 5.2.1, Pharsight, Mountain View, CA, USA). Geometric means and geometric coefficients of variation are reported for concentration-dependent parameters. The primary comparison between daily and weekly treatments was based on the C_{last} 25(OH)D_3 plasma concentration on day 1 of week 15. Additionally, C_{max}, $AUC_{0–24}$, and C_{last} values were compared between groups for day 1 of week 1 and for day 1 of week 15, as well as between time points within groups. Between- and within-groups comparisons were handled as bioequivalence problems using ANOVA-based standard bioequivalence approaches taking the dosing regimen as random effect. A clinically relevant difference between groups, or within groups between time points, was accepted to be present if the 90% confidence interval around the geometric mean ratios of the pharmacokinetic parameter under investigation was entirely outside the 0.8–1.25 bioequivalence acceptance zone. The 90% confidence interval is justified by the assumption that bioinequivalence can either result in higher, or lower values, but not in both at the same time. Hence, a two-sided 90% confidence interval is the same as two one-sided 95% confidence intervals. Additionally, the time to reach target concentrations was calculated and compared between groups using Wilcoxon signed-rank tests. P-values <0.05 were considered statistically significant.

Sample size considerations

Based on the pharmacokinetic data of an earlier, published study, which compared different doses of vitamin D_3 and calcifediol for 8 and 4 weeks, respectively [26], a difference of at least factor 2 between the treatments can be anticipated. Considering the factor-2 difference, a target 25(OH)D_3 plasma concentration of about 25 ng/mL in the vitamin D_3 group and 48 ng/mL in the calcifediol group would be expected (in winter, when sunlight exposure is low). Furthermore, based on previously published work [26], the expected standard deviation would be 13.2 ng/mL for the calcifediol group (28% of the larger mean). In this case, with an alpha of 0.05 and a sample size of 5

per group, a power of 80% would be reached. Similar assumptions can be made for the other treatment groups.

Results

All 35 postmenopausal women who started the study medication intake attended all 14 clinical visits and completed the trial. Demographics and baseline characteristics are shown in Table 1.

Pharmacokinetics after daily administration of 20 μg vitamin D_3 or 20 μg calcifediol

After the first daily dose of 20 μg vitamin D_3 or 20 μg calcifediol, the area under the concentration time curve ($AUC_{0–24h}$), which is the measure of exposure, was 28% larger after calcifediol than after vitamin D_3 intake (Fig. 1a, Table 2). The peak and last concentrations before the next intake were also higher after calcifediol supplementation than after vitamin D_3, but these differences did not reach statistical significance (Table 2). In four of five participants taking vitamin D_3, an increase in plasma 25(OH)D_3 concentrations was visible during the first 24 h (i.e. the dose had no pharmacokinetic effect), while in all participants taking calcifediol, a maximum was reached approximately 10 h after the first dose, and concentrations declined thereafter.

Four months later, after the last dose, the plasma 25(OH)D_3 exposure ($AUC_{0–24h}$) and the C_{max} values after calcifediol supplementation were more than twice as high than with vitamin D_3 (Fig. 1a, Table 2). Similarly, the C_{max} values were 2.2-fold higher with calcifediol than with vitamin D_3. The last concentrations before the next intake also differed markedly: 31.2 ng/mL with vitamin D_3 (CV 13.8%) and 67.1 ng/mL with calcifediol (CV 17.0%). In other words, daily vitamin D3 had a relative apparent oral bioavailability of 44.8% (90% confidence interval (CI): 37.8–53.1%). Peak-trough fluctuation during the last administration interval was roughly comparable between the supplements: 12.1% for vitamin D_3 and 10.8% for calcifediol intake, respectively.

Pharmacokinetics after weekly administration of 140 μg vitamin D_3 or 140 μg calcifediol

After the first weekly dose of 140 μg vitamin D3 or 140 μg calcifediol, the $AUC_{0–24h}$ (exposure) was 67% larger after calcifediol intake (Fig. 1a, Table 3). An almost twice as high maximum plasma concentration during the first 24 h was observed after calcifediol than after vitamin D_3. In 2 of 5 participants in the weekly vitamin D_3 group, the maximum plasma concentrations were attained later than 24 h after dosing.

After the last weekly dose of the study medication, the $AUC_{0–24h}$ values were 2.8-fold higher with calcifediol than with vitamin D_3 supplementation (Table 3). Similarly, the C_{max} values were more than 3-fold higher with calcifediol than with vitamin D_3. The last blood sample was obtained 24 h after the last study medication intake: a 2.8-fold difference in plasma 25(OH)D_3 concentrations between calcifediol and vitamin D_3 intake was observed also at this time point. In other words, weekly vitamin D_3 had a relatively apparent oral bioavailability of 35.9% (90% CI: 30.3–42.6%). As expected, the peak–trough fluctuation was more pronounced with calcifediol (50.1%) than with vitamin D_3 (9.0%).

Comparison between weekly and daily supplementation regimens

No statistically significant difference in exposure ($AUC_{0–24h}$) was observed between weekly and daily supplementation with calcifediol for 15 weeks ($AUC_{0–24}$: geometric mean ratio (GMR) 1.18, 90% confidence interval: 0.99–1.40), while maximum concentrations were slightly, but significantly higher after weekly compared with daily administration of calcifediol (C_{max}: GMR 1.27, 90% confidence interval: 1.09–1.48).

Similarly, no statistically significant difference in exposure ($AUC_{0–24h}$) was observed between weekly and daily supplementation with vitamin D_3 for 15 weeks ($AUC_{0–24}$: GMR 0.95, 90% confidence interval: 0.79–1.12), and maximum concentrations were also not statistically significantly different (C_{max}: GMR 0.90, 90% confidence interval: 0.77–1.05).

Pharmacokinetics of a single oral bolus of calcifediol, vitamin D_3 or their combination

Three groups of five women each received an oral bolus of either 140 μg vitamin D_3, or 140 μg calcifediol, or both, on a single occasion. Individual concentration–time curves are shown in Fig. 1b, and pharmacokinetic characteristics are presented in Table 4. As for the weekly dosing groups, who received the same doses, a single calcifediol intake led to more than twofold higher exposures and maximum concentrations than with vitamin D_3 intake. In the group which received both drugs simultaneously on a single occasion, $AUC_{0–96}$ and C_{max} values were not significantly higher than after a single dose of calcifediol alone (mean values in Table 4, bioequivalence comparison results: $AUC_{0–96}$: GMR 1.11, 90% CI 0.90–1.36; C_{max}: GMR 1.04, 90% CI 0.86–1.26).

Time to reach plasma 25(OH)D_3 concentrations above 20 and 30 ng/mL

Concentrations above 20 ng/mL were reached by all participants: after a mean of 20.6 days with (daily or weekly) vitamin D_3 intake, and after a mean of 3.0 days with (daily or weekly) calcifediol. In the two groups of women with daily or weekly vitamin D_3 supplementation, only 7 of 10 participants reached plasma 25(OH)D_3 concentrations of at least 30 ng/mL during the 15 weeks study period, while in the two groups taking calcifediol, all women attained these concentrations. The mean time to reach 30 ng/mL was 68.4 days with (daily or weekly) vitamin D_3 supplementation, while it took only 16.8 days with (daily or weekly) calcifediol supplementation.

Discussion

To our knowledge, this is the first detailed and longer term pharmacokinetic study for oral supplementation with vitamin D_3 and calcifediol across 15 weeks of follow-up and assessed in 14 clinical visits. Our data support the higher efficacy and reliability in the extent and speed of change in plasma 25(OH)D_3 concentrations with calcifediol compared with vitamin D_3. Notably, after 15 weeks, the 25(OH)D_3 exposure (AUC) was more than twice as high with calcifediol than with vitamin D_3. Further, serum concentrations of at least 20 ng/mL were reached within less than a week with daily or weekly intake of calcifediol, while it took

Table 1
Demographics and baseline characteristics of the study participants (arithmetic means, standard deviation).

Characteristic	Treatment							P[a]
	20 μg D_3 daily	20 μg calcifediol daily	140 μg D_3 weekly	140 μg calcifediol weekly	140 μg D_3 bolus	140 μg calcifediol bolus	140 μg D_3 + 140 μg calcifediol bolus	
Age, years	61.69 (7.94)	54.59 (2.62)	65.21 (8.08)	64.37 (4.66)	62.19 (7.49)	63.89 (7.46)	59.69 (7.96)	0.25
BMI, kg/m^2	25.46 (4.47)	24.90 (3.20)	25.52 (2.37)	21.59 (2.49)	23.83 (2.20)	24.41 (3.36)	24.95 (2.69)	0.45
Serum calcium, mmol/l	2.24 (0.11)	2.21 (0.06)	2.29 (0.04)	2.30 (0.04)	2.26 (0.06)	2.27 (0.09)	2.25 (0.08)	0.49
Plasma 25(OH)D_3, ng/ml	12.08 (1.56)	13.06 (3.96)	16.28 (3.99)	11.50 (4.49)	8.59 (0.98)	13.59 (5.88)	12.71 (3.71)	0.13
Serum PTH, pg/ml	55.72 (9.72)	64.58 (19.75)	54.02 (12.71)	61.86 (14.44)	50.46 (16.24)	65.38 (16.47)	52.76 (8.17)	0.53

[a] P-values are based on one-way ANOVA model.

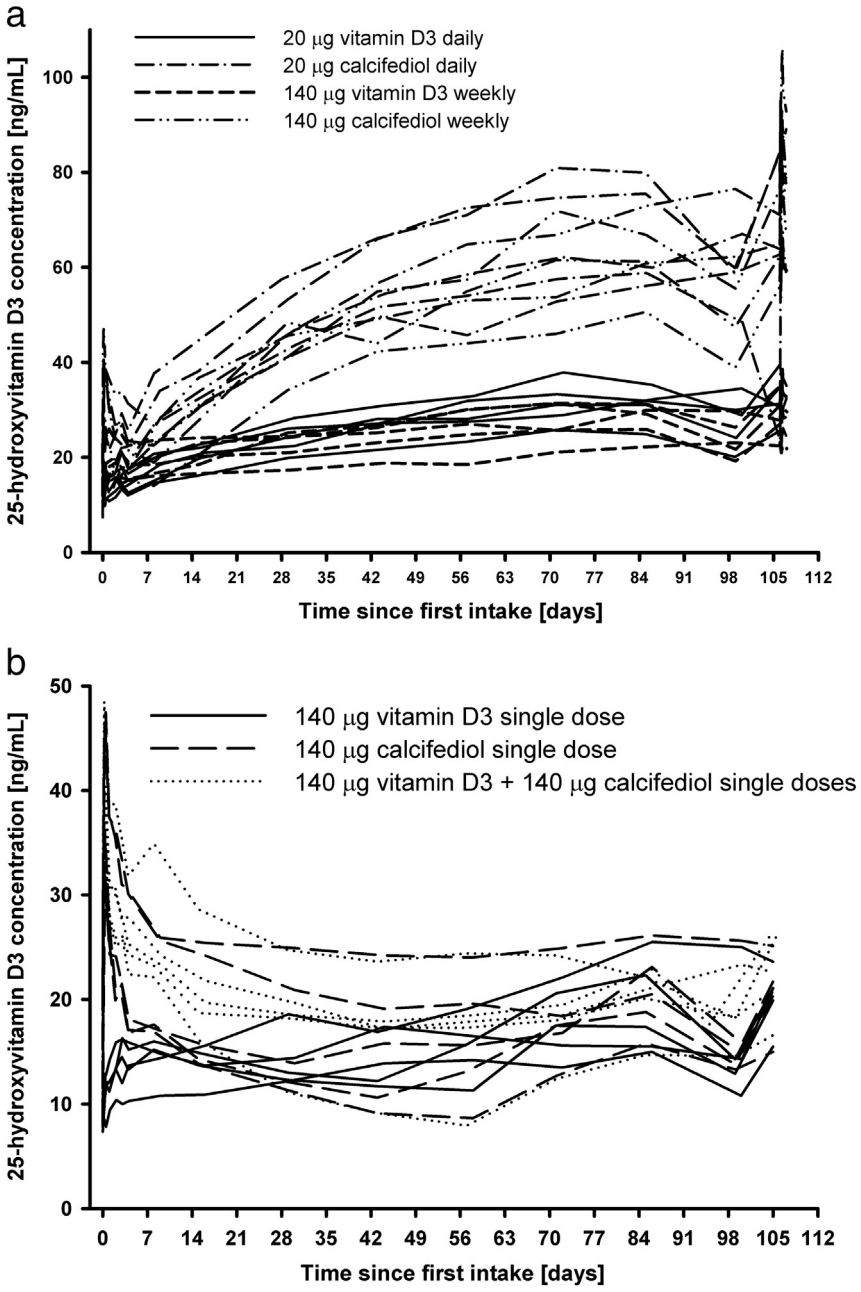

Fig. 1. Individual time–concentration plots of 25-hydroxyvitamin D_3 after oral administration of daily 20 μg vitamin D3 (solid line) or daily 20 μg calcifediol daily (dashes and one dot), or weekly 140 μg vitamin D_3 (short dashes) or weekly 140 μg calcifediol weekly (dashes and two dots) for 15 weeks (Fig. 1a). Individual time–concentration plots of 25-hydroxyvitamin D_3 after oral administration of a single dose of 140 μg vitamin D_3 (solid line) or 140 μg calcifediol (dashed line), or 140 μg vitamin D_3 + 140 μg calcifediol (dashes and dots) (Fig. 1b). Each group (daily, weekly, different bolus groups) included 5 healthy postmenopausal women attending 14 visits over the course of 4 month. Intense pharmacokinetic sampling was done on days 1 and 106 of the study, leading to the "peaks" in Fig. 1a. Please note the different lengths of the y-scales in Figs. 1a (daily and weekly groups) and b (bolus groups).

about 3 weeks to attain these concentrations with daily or weekly vitamin D_3. Likewise, serum concentrations of at least 30 ng/mL were reached with calcifediol within about 2 weeks with a daily or weekly intake of calcifediol, while it took about 9 weeks with a daily or weekly intake of vitamin D_3. Notably, all participants in the daily or weekly calcifediol group reached at least 30 ng/mL at 4 weeks, while only 7 of 10 participants in the daily or weekly vitamin D_3 group reached this goal within 15 weeks.

In clinical practice, the goal of vitamin D supplementation is to correct vitamin D deficiency as soon and as reliably as possible. In our study 25(OH)D_3 levels shifted to at least 20 ng/mL in less than 1 week and to at least 30 ng/mL in less than 4 weeks among all participants receiving daily or weekly calcifediol. In contrast, it took 3 weeks to reach 20 ng/ml in all participants in vitamin D_3 group, and even at the end of 15 weeks only 3 of 10 participants reached at least 30 ng/mL with vitamin D_3. This is in part explained by our finding that calcifediol is more than 2.5-fold more potent in increasing 25(OH)D_3 exposure compared with the same dose of vitamin D_3. In other words, the bioavailability of vitamin D_3 amounts only to 36–45% of the calcifediol bioavailability. The difference in bioavailability was already visible after the first dose with both daily and weekly regimens, and became more and more pronounced thereafter.

In another, recently published study which compared two single 25(OH)D_3 serum concentrations before and after 10 weeks of different supplementation regimens in 56 adults aged >50 years, a 4.2- to 5-fold higher potency of calcifediol in increasing 25(OH)D_3 concentrations was observed [17]. Whether this higher potency is related to different study designs, to the comparison between full 24-h concentration–time curves

Table 2
Pharmacokinetic parameters of 25-hydroxyvitamin D_3 plasma concentration after *daily* oral administration of 20 μg vitamin D_3 or 20 μg of calcifediol for 15 weeks.

Week 1, day 1		Geometric mean	Geometric CV [%]	Geometric mean ratio T/R	90% confidence interval
20 μg vitamin D_3 daily (R)	AUC_{0-24} [ng/mL*h]	315.6	13.8		
	C_{max} [ng/mL]	13.9	16.0		
	T_{max} [h][a]	22.2	26.9		
	C_{24h} [ng/mL]	13.8	18.1		
20 μg calcifediol daily (T)	AUC_{0-24} [ng/mL*h]	405.0	24.4	1.283	1.019–1.616*
	C_{max} [ng/mL]	17.6	25.6	1.261	0.984–1.616
	T_{max} [h][a]	10.6	24.4	n.a.	n.a.
	C_{24h} [ng/mL]	15.3	24.1	1.1085	0.880–1.397
Week 15, day 1		Geometric mean	Geometric CV [%]	Geometric mean ratio T/R	90% confidence interval
20 μg vitamin D_3 daily (R)	AUC_{0-24} [ng/mL*h]	763.6	17.0		
	C_{max} [ng/mL]	33.1	15.1		
	T_{max} [h][a]	10.8	79.4		
	C_{24h} [ng/mL]	31.2	13.8		
20 μg calcifediol daily (T)	AUC_{0-24} [ng/mL*h]	1704.4	18.1	2.232	1.818–2.741**
	C_{max} [ng/mL]	73.2	14.7	2.211	1.857–2.632**
	T_{max} [h][a]	9.9	2.0	n.a.	n.a.
	C_{24h} [ng/mL]	67.1	17.0	2.147	1.792–2.573**

[a] Arithmetic means and arithmetic coefficient of variation; n.a., not applicable; *, statistically significant difference; **, absence of bioequivalence, clinically relevant difference. Each group included 5 healthy postmenopausal women. Geometric mean ratios and 90% confidence intervals represent ANOVA-based comparisons between calcifediol over vitamin D_3. T, values after intake of calcifediol, R values after intake of vitamin D_3.

in our case versus two single concentrations in the cited study, or to other factors, is unknown, but a single blood concentration is generally more prone to sampling and processing errors than a full AUC comparison.

With respect to safety, at all time points and in all participants in our study, serum calcium concentrations were in the normal range, below 2.6 mmol/L. Also urinary calcium excretion did not differ significantly between participants who received calcifediol or vitamin D_3. Beyond its demonstrated safety, as discussed above, the uniform increase in 25(OH)D_3 exposure with calcifediol maybe be advantageous in clinical practice.

Relevant to clinical practice, there was no difference between daily and weekly administration of either vitamin D_3 or calcifediol in their effect on 25(OH)D_3 exposure across the 15 weeks of supplementation. Only maximum values were slightly (27%), but significantly higher after weekly calcifediol supplementation compared with daily calcifediol, which is explained by the higher dose administered once weekly and not of clinical relevance. Since 25(OH)D_3 exposure (AUC) did not differ significantly between daily or weekly application, either regimen, depending on the preference of the patient, may be used for supplementation with vitamin D_3 or calcifediol.

The pharmacokinetics after a single dose of 140 μg vitamin D_3, calcifediol, or both, confirmed the approximately 2-fold higher potency of calcifediol, already after the first dose. With the combination of calcifediol and vitamin D_3 in one bolus we did not see an improvement in 25(OH)D_3 exposure compared with the calcifediol only bolus, suggesting that their combination may not be relevant for the correction of vitamin D deficiency if compared with calcifediol alone.

A possible limitation is that our study was restricted to women aged 50 to 70 years, and therefore our findings may not be generalizable to men and younger adults. Although the number of participants per group was small and baseline plasma 25(OH)D concentrations varied between 11.5 and 16.3 ng/mL between groups, our study was sufficiently powered to determine the difference in potency of calcifediol over vitamin D_3. Adding to the quality of our study and the reliability of our findings is the high frequency of 14 clinical visits attended by all participants. Also, multiple repeated measurements compensate for a small sample size by reducing measurement error to some extent.

Our findings favor calcifediol, however, as we did not test an equivalent dose of calcifediol and vitamin D_3 with respect to 25(OH)D_3

Table 3
Pharmacokinetic parameters of 25-hydroxyvitamin D_3 plasma concentration after *weekly* oral administration of 140 μg vitamin D_3 or 140 μg of calcifediol for 15 weeks.

Week 1, day 1		Geometric mean	Geometric CV [%]	Geometric mean ratio T/R	90% confidence interval
140 μg vitamin D_3 weekly (R)	AUC_{0-24} [ng/mL*h]	467.4	22.4		
	C_{max} [ng/mL]	19.0	19.3		
	T_{max} [h][a]	21.1	42.8		
	C_{24h} [ng/mL]	18.6	21.7		
140 μg calcifediol weekly (T)	AUC_{0-24} [ng/mL*h]	780.8	17.9	1.671	1.319–2.115**
	C_{max} [ng/mL]	39.9	13.5	2.095	1.724–2.545**
	T_{max} [h][a]	4.8	25.3	n.a.	n.a.
	C_{24h} [ng/mL]	29.9	19.0	1.613	1.285–2.023**
Week 15, day 1		Geometric mean	Geometric CV [%]	Geometric mean ratio T/R	90% confidence interval
140 μg vitamin D_3 weekly (R)	AUC_{0-24} [ng/mL*h]	721.3	15.0		
	C_{max} [ng/mL]	29.7	15.3		
	T_{max} [h][a]	5.1	104.1		
	C_{24h} [ng/mL]	28.6	16.6		
140 μg calcifediol weekly (T)	AUC_{0-24} [ng/mL*h]	2007.2	11.2	2.783	2.383–3.249**
	C_{max} [ng/mL]	92.9	11.0	3.128	2.676–3.656**
	T_{max} [h][a]	6.0	51.9	n.a.	n.a.
	C_{24h} [ng/mL]	80.4	13.0	2.811	2.361–3.346**

[a] Arithmetic means and arithmetic coefficient of variation; n.a., not applicable; **, absence of bioequivalence, clinically relevant difference. Each group included 5 healthy postmenopausal women. Geometric mean ratios and 90% confidence intervals represent ANOVA-based comparisons between calcifediol over vitamin D_3. T, values after intake of calcifediol, R values after intake of vitamin D_3.

Table 4
Pharmacokinetic parameters of 25-hydroxyvitamin D_3 plasma concentrations after a *single* oral administration of 140 μg vitamin D_3, of 140 μg calcifediol, or both.

		Geometric mean	Geometric CV [%]	Geometric mean ratio T/R	90% confidence interval
140 μg vitamin D_3 single dose (R)	AUC_{0-96} [ng/mL*h]	1219.8	15.9		
	C_{max} [ng/mL]	14.0	18.5		
	T_{max} [h][a]	73.2	22.8		
	C_{96h} [ng/mL]	13.5	17.0		
140 μg calcifediol single dose (T1)	AUC_{0-96} [ng/mL*h]	2647.4	23.9	2.170	1.772–2.658**
	C_{max} [ng/mL]	38.3	19.4	2.726	2.253–3.298**
	T_{max} [h][a]	8.5	42.2	n.a.	n.a.
	C_{96h} [ng/mL]	21.7	31.4	1.600	1.253–2.043**
140 μg vitamin D_3 + 140 μg calcifediol single doses (T2)	AUC_{0-96} [ng/mL*h]	2929.2	12.8	2.401	1.961–2.941**
	C_{max} [ng/mL]	39.9	12.4	2.840	2.347–3.436**
	T_{max} [h][a]	7.2	50.6	n.a.	n.a.
	C_{96h} [ng/mL]	26.2	13.6	1.932	1.514–2.467**

[a] Arithmetic means and arithmetic coefficient of variation; n.a., not applicable; **, clinically relevant difference. Each group included 5 healthy postmenopausal women. Geometric mean ratios and 90% confidence intervals represent ANOVA-based comparisons between calcifediol intake (T1) over vitamin D_3 intake (R), and combined intake (T2) over vitamin D_3 intake (R), respectively.

level increase, benefits documented by calcifediol may likely be caused by its rapid increase in 25(OH)D_3 level and higher achieved 25(OH)D_3 level compared with the standard dose of vitamin D_3 tested. Alternatively, calcifediol may have additional benefits superior to vitamin D_3, which will need further investigation.

In conclusion, this study shows that in healthy, postmenopausal women aged 50–70 years, calcifediol supplementation is 2- to 3-fold more potent in raising 25(OH)D_3 exposures than vitamin D_3 supplementation. Further, 20 μg calcifediol given daily, or 140 μg given weekly, appears to correct vitamin D deficiency more rapidly and reliably than the same dose of daily or weekly vitamin D3. The higher bioavailability of calcifediol compared with vitamin D_3 documented in this trial of healthy postmenopausal women, may need further investigation in patients with malabsorption; i.e. chronic bowel inflammation.

Conflict of interest statement

ES and RG are employees of DSM Nutritional Products Ltd, Basel, Switzerland. None of the other authors have financial disclosures relevant to the content of the manuscript.

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