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ORIGINAL ARTICLE

Vitamin D3 seems more appropriate than D2 to sustain adequate levels of 25OHD: a pharmacokinetic approach

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BACKGROUND/OBJECTIVES: The superiority of cholecalciferol (D_3) over ergocalciferol (D_2) in sustaining serum 25-hydroxy vitamin D (25OHD) levels is controversial. To compare D_2 with D_3 we performed a single-blind, placebo-controlled randomized trial spanning 11 weeks.

SUBJECTS/METHODS: Healthy volunteers ($n = 33$, aged 33.4 ± 6 years) were divided into three groups ($n = 11$, each): D_2, D_3 and placebo. Treatment started with a loading dose (100 000 IU) followed by 4800 IU/day (d) between d7 and d20 and follow-up until d77. Serum samples were obtained at baseline and at days 3, 7, 14, 21, 35, 49, 63 and 77.

RESULTS: Baseline 25OHD values in the D_2 group were lower than those in the D_3 and placebo groups ($P < 0.01$). Placebo 25OHD levels never changed. As after the loading dose both D_2 and D_3 groups had reached similar 25OHD levels, we tested equivalence of the area under the concentration × time curve (AUC) between d7 and d77. The AUC was 28.6% higher for D_3 compared with D_2, and both were higher with respect to placebo. At d77, D_2 25OHD levels were higher than those at baseline, but similar to placebo; both were lower than D_3 ($P < 0.04$). According to raw data, the elimination half-life of 25OHD was 84 and 111 days under D_2 and D_3 supplementation, respectively; after subtracting the placebo values, the corresponding figures were 33 and 82 days.

CONCLUSIONS: D_2 and D_3 were equally effective in elevating 25OHD levels after a loading dose. In the long term, D_3 seems more appropriate for sustaining 25OHD, which could be relevant for classic and non-classic effects of vitamin D.

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INTRODUCTION

Vitamin D deficiency is highly prevalent worldwide[1,2] and increases the risk for several medical conditions, including osteoporosis, falls, cancer, diabetes, hypertension, autoimmune diseases so on.[3,4] This led to suppose that maintenance of adequate 25-hydroxy vitamin D (25OHD) levels in the blood is required for vitamin D regulation of a large number of physiologic functions beyond the classic actions on bone mineral metabolism.

Different regimens for prevention and correction of vitamin D deficiency have been proposed, but there is no universally accepted dose, frequency, route or type of vitamin D to use or to prescribe.[5–8]

Two chemically distinct natural forms of vitamin D exist: ergocalciferol or vitamin D_2 (D_2) and cholecalciferol or vitamin D_3 (D_3). D_3 is produced from 7-dehydrocholesterol in human skin exposed to UVB radiation and is present in a few foods (oily fish and egg yolk). D_2 is sourced from UV irradiation of ergosterol, which is mainly found in fungi. Food and/or supplement intake may provide either D_2 or D_3. Both D_2 and D_3 function as prohormones through metabolism, first in the liver to 25OHD and later in the kidney, where 1α-hydroxylase converts 25OHD in the active metabolite 1, 25-dihydroxy vitamin D. This process is under homeostatic control.[9]

Serum 25OHD levels, including both $25OHD_2$ and $25OHD_3$, are the chief circulating forms of the vitamin and are representative of vitamin D status, reflecting D_2 and D_3 intake and cutaneous synthesis of D_3. To date, the equivalence of D_2 and D_3 as well as their corresponding doses and administration route remains controversial.

Several reports, but not all, using highly different dosing regimens found D_3 to be more effective than D_2 in increasing or maintaining serum 25OHD levels.[10–21] These reports used mainly only a type of supplementation: daily doses, between 1000 and 4000 IU,[10,11,15,17,21] a single bolus or intermittent weekly or monthly high doses, with a range between 50 000 and 300 000 IU.[12,14,16,19] They had a variable follow-up (from 4 weeks to 1 year). A few of them involved a pharmacokinetic analysis[12,14,19] and were controlled with placebo.[15,20]

Taking into account such difficulties, and considering the importance of maintaining adequate 25OHD levels both for classic and for non-classic actions, and the heterogeneity of the published protocols, we designed the present pharmacokinetic study, including two sequential periods of administering D_2, D_3 and placebo as one single large loading dose, followed by maintenance daily doses, and later an elimination phase without vitamin D supplementation.

SUBJECTS AND METHODS

Study participants

Thirty-three healthy volunteers, 24–46 years old, either hospital employees or physicians, were recruited. None of them had a history of liver, kidney, malabsortive or granulomatous diseases, nor had they received corticosteroids, anticonvulsants or vitamin D supplements. All subjects were from Buenos Aires City (latitude 34°S) and had limited sun exposure (< 8 h/week). This protocol was approved by the Institutional Review Board of the Hospital de Clínicas José de San Martin (the teaching hospital of our university) and, according to current regulations, after approval it was

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communicated to Argentina's National Drug Regulatory Agency; all volunteers gave written informed consent. This placebo-controlled, parallel-group, randomized interventional study was designed to compare pharmacokinetic characteristics of the two main vitamin D natural forms, and not specific drug products.

Pharmacokinetic design

Participants were randomly assigned with a computer-generated code in a single-blind design to receive placebo (PLA), D_2 or D_3 in oral drops in the schedule presented in Figure 1: first, to boost vitamin D concentration, one single loading dose of 100 000 IU at the beginning of the study (baseline), followed by a daily dose of 4800 IU from days 7 to 20, and finally no vitamin D from days 21 to 77, to evaluate mainly the elimination phase. All subjects received an oral dose of 500 mg calcium (one calcium carbonate tablet/day) during the whole study. Fasting blood samples were collected at baseline and at days (d) 3, 7, 14, 21, 35, 49, 63 and 77. Two-hour urine fasting samples were collected at baseline and at d7, d21 and d77. Serum and urine samples were stored at − 20 ° C until processed.

Serum calcium and urine calcium and creatinine (Cr) were measured at baseline, d7 and d21 to evaluate the safety of bolus and daily doses. Hypercalcemia was defined as serum calcium >10.5 mg/dl and hypercalciuria as a ratio urine calcium/urine Cr >0.37 mgCa/mgCr.[22] The trial ran from September to December 2010.

Procedure

The loading dose was administered by the physicians. Compliance to the vitamin D administration schedule was determined by measuring the residual volume (as number of drops) in the returned vials at day 21. D_2 and D_3 concentrations of the batches used, analyzed blindly by an independent laboratory using High Performance Liquid Chromatography with UV detection, confirmed the label values. The intake of vitamin D was determined by a nutritionist using a food frequency questionnaire.[23]

Analytical methods

Serum levels of 25OHD were determined by radioimmunoassay (DiaSorin, Stillwater, MN, USA). All serum 25OHD concentrations for a given individual were determined in a single assay to minimize variability. Intra-assay and inter-assay coefficients of variations were 6% and 8%, respectively. The quality and accuracy of 25OHD analysis were assured by a periodical participation in the Vitamin D External Quality Assessment Scheme (DEQAS). Levels of 25OHD <20 ng/ml were considered as deficient.[24–26]

Total and bone alkaline phosphatase (measured by agglutination with wheat germ lectin), intact parathormone, serum phosphorus and Cr were measured at baseline and at d77. Calcium, phosphorus, Cr and alkaline phosphatase were determined using standard methods[21] and intact parathormone by electrochemical luminescence (ECLIA) (Roche Diagnostics, Indianapolis, IN, USA).

Pharmacokinetic and statistical analyses

Determination of pharmacokinetic parameters (C_{max}, area under the concentration × time curve (AUC) and elimination half-life) and their statistical analysis were performed using WinNonlin Professional Software version 5.0 (Pharsight Corporation, Mountain View, CA, USA). Applying a noncompartmental model to the log-transformed plasma concentration of 25OHD, pharmacokinetic parameters (C_{max}, AUC_{7-77}) were compared by analysis of variance for a parallel design.[27,28] The Anderson and Hauck test were used to examine equivalence.[29] The ratios and 90% confidence intervals of C_{max} and AUC_{7-77} were calculated for D3/D2 and PLA/D2, and two one-sided *t*-tests[30] were employed to evaluate whether the 90% confidence intervals met the criterion for bioequivalence (80–125%).[28,31] Other statistical analyses were nonparametric tests (Mann–Whitney and Wilcoxon) and analysis of variance (ANOVA), performed, as appropriate, with SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Significance level was 0.05.

RESULTS

As shown in Table 1, the baseline anthropometric characteristics of the subjects of the three groups were similar, with the only exception being the body mass index in the PLA group, which was higher than the corresponding values in the D_2 and D_3 groups ($P<0.01$). The three groups had similarly low vitamin D intake from diet: 13 out of the 33 volunteers (39%) presented vitamin D deficiency. The D_2 group presented baseline 25OHD values lower than the D_3 and placebo groups ($P<0.01$) and with a higher number of subjects with vitamin D deficiency. There were no significant differences among groups with respect to any of the other biochemical parameters evaluated at baseline. Most subjects were adherent to protocol: the mean rates of compliance with treatment, on the basis of drop counts, was 88 ± 10%, 92 ± 7% and 85 ± 9% for D_3, D_2 and PLA, respectively. Figure 2 presents the time course of 25OHD levels during treatment. The PLA group had no significant change in their 25OHD levels during the 77 days of follow-up. After the loading dose of 100 000 IU the 25OHD levels of both vitamin D groups had a rapid and similar increment at d3, which persisted at d7. The absolute increment over baseline was 20.3 ± 10.5 ng/ml in D_2 and 16.7 ± 6.4 ng/ml in D_3 (P = not significant), reaching similar 25OHD values (D_2, 36.6. ± 11.0, and D_3, 41.0 ± 4.9 ng/ml; P = not significant). No patient who received the

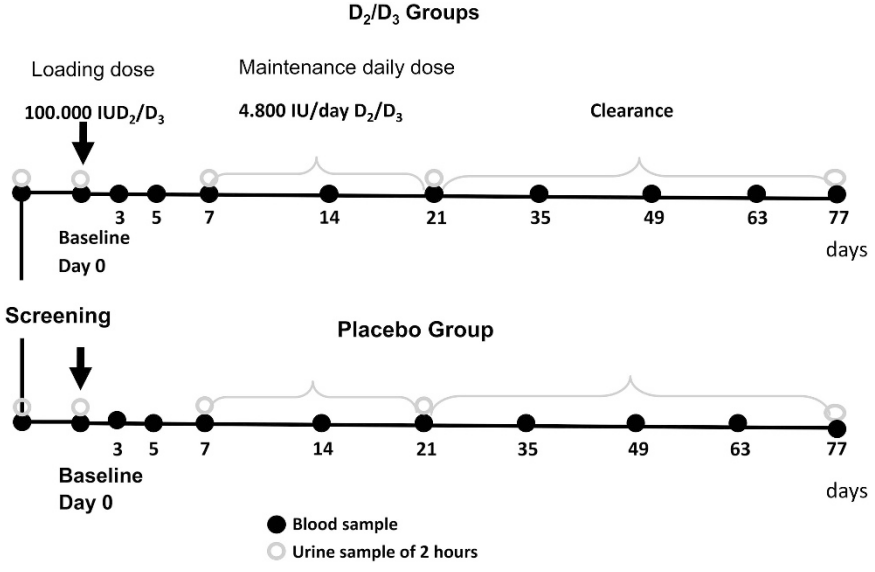

Figure 1. Design of the study. Three groups of healthy subjects received a loading dose of vitamin D (D_2 or D_3) or placebo, followed by a maintenance dose for 2 weeks and a periodic control until d77 of follow-up.

Table 1. Baseline anthropometric and nutritional characteristics of the study participants

	Placebo	*Vitamin D_2*	*Vitamin D_3*
Number of subjects (*n*)	11	11	11
Age in years (range)	34.0 ± 5.0 (26–41)	32.2 ± 5.0 (25–41)	33.5 ± 7.0 (24–46)
Sex (female/male)	(8/3)	(9/2)	(8/3)
BMI (kg/m^2)	24.5 ± 2.0$^{\#}$	21.3 ± 2.0	21.8 ± 2.0
Vitamin D intake (μg/d)[a]	3.2 ± 2.0	3.4 ± 2.0	4.3 ± 2.0
25OHD (ng/ml)	22.6 ± 7.6	16.3 ± 7.3*	24.3 ± 6.6
25OHD level (n)			
< 20 ng/ml	3	7	5
20–30 ng/ml	7	4	4
⩾ 30 ng/ml	1	0	2

Abbreviations: BMI, body mass index; 25OHD, 25-hydroxy vitamin D. Data are expressed as mean ± s.d., except for sex and number (*n*) of subjects with different vitamin D status. [a]RDA for vitamin D in adults < 70 years: 15 μg/day (600 IU/day). *$P < 0.01$ vs G3 and placebo. $^{\#}P < 0.01$ vs G2 yG3.

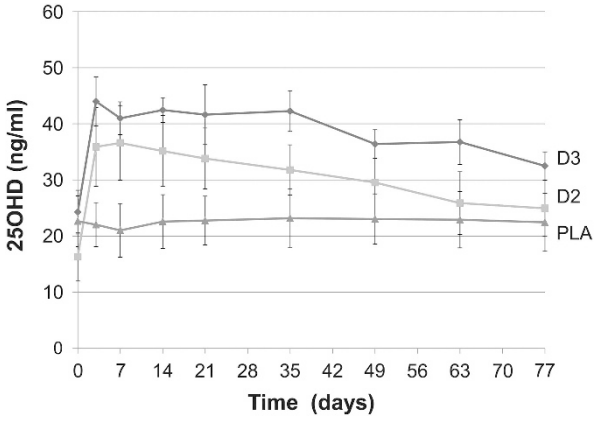

Figure 2. Time course of 25-hydroxy vitamin D (25OHD) levels (geometric mean) during the protocol (from baseline to day 77).

vitamin D loading dose (either D_2 or D_3) had 25OHD values in the deficiency range. The percentage increment between baseline and d7 was higher in subjects with basal 25OHD values < 20 ng/ml than in those with baseline values ⩾ 20 ng/ml (179 ± 68% vs 62 ± 36%, $P < 0.0001$). At d21, after the maintenance dose period, D_2 and D_3 25OHD levels were similar (33.8 ± 9.2 and 41.9 ± 8.6 ng/ml; not significant).

Pharmacokinetic analysis

We analyzed the geometric mean of C_{max} during the course of the study, whereas for AUC we used AUC_{7-77} days, as at d7 both D_2 and D_3 groups had reached similar 25OHD levels after the loading dose administered at baseline. We performed an equivalence analysis to test whether the declined rate after stopping vitamin D supplementation was similar between groups, which is shown in Table 2, for C_{max} and AUC, respectively. According to criteria commonly applied for equivalence, C_{max} obtained by the loading dose of D_2 and D_3 was equivalent. In contrast, the C_{max} for PLA (26.3 ng/ml) was lower than that for both D_2 and D_3, with a power of 76%. On the other hand, AUCs were not equivalent: neither the D_3/D_2 ratio nor the PLA/D_2 ratio complied with criteria usually employed to check equivalence. The D_3 AUC was 28.6% higher than the D_2 AUC, and the PLA/D_2 ratio was 71.84%, with a power of 71% in both cases (Table 2). D_2 and D_3 AUCs were higher than the PLA AUC. At the end of the protocol (d77), after 56 days without vitamin D supplementation, D_2 25OHD levels were higher than at baseline, but similar to PLA, and both were lower than D_3 ($P < 0.04$; Figures 2 and 3). With the same software, their elimination half-lives were calculated with two different approaches: the direct value and after subtraction of the values corresponding to PLA.

The first approach resulted in a geometric mean for the elimination half-life of 25OHD of 84 and 111 days for 25OHD under D_2 and D_3 supplementation. The second approach (aiming to take into account the presence of vitamin D independent of the loading dose) resulted in values of 33 and 82 days, respectively, implying a shorter half-life for 25OHD_2 than for 25OHD_3.

Safety

No subject complained of treatment-related adverse events or experienced hypercalciuria or hypercalcemia at any of the measured points. Serum calcium and urine calcium/urine Cr did not differ in any group and no between-group differences were observed during the period of vitamin D administration (Table 3). The highest individual levels of 25OHD after D_2 or D_3 supplementation were lower than those associated with vitamin D intoxication. In every group, no difference between baseline and final levels was observed for serum calcium, serum phosphorus, serum Cr, alkaline phosphatase, bone alkaline phosphatase, intact parathormone and urine calcium/urine Cr (data not shown).

DISCUSSION

Herein we have shown that under these experimental conditions vitamin D_2 was as effective as vitamin D_3 in increasing 25OHD levels with a loading dose. On the other hand, after a period with the same daily doses of vitamin D_2 and D_3, the 25OHD levels in the group that received vitamin D_2 declined faster than the levels in the vitamin D_3 group, reaching similar levels as the placebo group at the final point. This finding is consistent with and expands previous reports, providing additional elements to the current debate about vitamin D requirements and supplementation strategies, if required.

A central point for framing the discussion about the best supplementation is the understanding about the continuous or discontinuous need of some level of vitamin D in blood. In addition to the well-known effect on bone, vitamin D possesses pleiotropic actions on the immune and endocrine systems, and on common cell functions, such as proliferation and differentiation.[4,32] Most of these non-classic effects depend upon the tissue-specific regulation of $1,25(OH)_2D$, which requires adequate blood levels of 25OHD as substrate, suggesting that prolonged or continuous level of 25OHD could be worthwhile.

Many studies have analyzed the difference between vitamins D_2 and D_3, as well as the effect of dose, route and schedule of administration, over a wide range of ages and follow-up periods. In a recent meta-analysis[33] cholecalciferol obtained a higher response than ergocalciferol in terms of increase and maintenance of 25OHD level. Our approach was to compare both calciferols in terms of concentration-time profiles after a loading dose and during a maintenance dose in the order recommended to sustain adequate levels in young adults, as well as in the elimination phase after withdrawal of maintenance.

Unexpectedly, the blind analysis of 25OHD of volunteers after randomization found a lower value in the D_2 group, which prompted us to divide the analysis into two steps: the response to the loading dose and separately, after achieving similar levels of 25OHD, the comparison of both forms of vitamin D in the maintenance and elimination phases. The study began at the end of the Southern winter, and as previously reported many subjects (almost 40%) presented vitamin D deficiency, probably due to low vitamin D intake: only 20–25% of the RDA for adults[24] and lower UV radiation in winter at this latitude.[34,35] The influence of solar radiation and spontaneous intake of vitamin D in the follow-up were probably limited, as the 25OHD levels almost did not change

Table 2. Equivalence analysis of the C_{max} and AUC $_{(7–77\ days)}$

Groups	*Geometric mean*	*90% CI*	*Comparison*	*Ratio of geometric means (90% CI)*	*Anderson y Hauck (probability < 80 > 125%)*	*Equivalent*	*Power*
C_{max} (ng/ml)							
Placebo	26.31	23.11–31.51	Placebo/D_2	56.1% (47.9–65.6)	0.99	No	0.76
Vitamin D_2	46.92	42.92–52.68	D_3/D_2	102.2% (87.25–119.33)	0.01	Yes	0.76
Vitamin D_3	47.87	45.30–50.93					
AUC $_{7–77\ days}$							
Placebo	1669.7	1446.43–2066.51	Placebo/D_2	71.84 (60.3–85.5)	0.60	No	0.71
Vitamin D_2	2324.1	2114.71–2627.81	D_3/D_2	128.6 (108.0–153.1)	0.85	No	0.71
Vitamin D_3	2988.2	2836.99–3167.98					

Abbreviations: AUC, area under the concentration × time curve; CI, confidence interval.

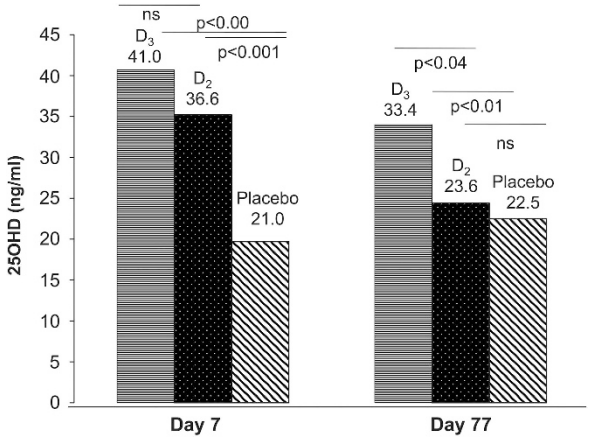

Figure 3. Comparison of the D_2, D_3 and placebo groups' serum 25-hydroxy vitamin D levels (25OHD) on day 7 (1 week after the vitamin D loading dose) and day 77 (end of follow-up).

in the PLA group over the course of the study. Subjects were equally adherent to protocol and no safety problems were detected. As expected, the loading dose of either vitamin D quickly and markedly increased 25OHD levels at d3 and maintained them in the first week. Some of the reports, with follow-up up to a week after the loading dose, found persistence with both vitamins[19,20] and others only with vitamin D_3.[12,14] Such discrepancies could involve several factors, including the size of the groups, intra-individual variation or non-registered vitamin D supplementation.[6,20] As a trend, the increase was higher in cases whose baseline values were lower. Further studies should determine whether compensatory mechanisms to accelerate vitamin D hydroxylation are involved. On the other hand, after reaching optimal values of 25OHD, maintenance doses of each vitamin D (4800 IU) effectively sustained those levels during the 14 days of supplementation. The dose used is very close to the daily utilization rate (4000 IU) reported for young adults at levels in the order of 32 ng/ml.[36] 25OHD plasma profiles after supplementation with either D_2 or D_3 are controversial: two reports, using the same dose level as us, found higher concentration values with vitamin D_3, independently of the baseline value (whether deficient or not). In contrast, a report using 1,000 IU/day did not find any difference between D_2 and D_3.[15] In our study, D_3 AUC (a good measure of drug exposure after systemic administration) was 28% higher than D_2 AUC. This finding was consistent, although lower, with previous reports with different loading doses and follow-up: all found higher AUC for D_3 (between 56 and 340%).[12,14,19] Consistent with previous reports, our main finding is the faster decrease in 25OHD serum concentration for D_2 supplementation with respect to D_3, resulting in a longer elimination half-life for $25OHD_3$. Such a difference can result from differences in the volume of distribution or in the clearance: both have been described for D_2 and D_3. The pharmacokinetics of vitamin D is highly complex and method limitation can add further complexity: for instance, RIA detects several 25-hydroxylated derivatives of vitamin D, such as $1,25(OH)_2D$, which, being relatively low and with a fast elimination, can marginally affect the values we measured. RIA does not distinguish between $25OHD_2$ and $25OHD_3$, although the sharp increase after the loading dose can logically be ascribed to the vitamin D (either D_2 or D_3) used. Moreover, as the time periods involved are long, many regulatory mechanisms can be in function. As to the volume of distribution (which is directly proportional to the elimination half-life) vitamin D Binding Protein depicts higher affinity for $25OHD_3$ than for $25OHD_2$.[37–39] In healthy adults, supplementation with 50 000 IU/week of D_3 produced 2 to 3-fold greater storage of vitamin D in subcutaneous fat biopsies than did equimolar D_2.[19] 25OHD clearance is essentially determined by a highly complex network of metabolic transformations held in many different tissues. The main component[40] is 1α-hydroxylation by CYP27B1, whose expression is upregulated by PTH and downregulated by its product $1,25(OH)_2D$. This active metabolite is short-lived, in part due to its high ability to induce CYP24A1, which is also detected in many organs. This enzyme can also metabolize 25OHD, although with an affinity that is one order lower. Hypothetically, such a lower affinity can become relevant at higher concentrations of substrate. Thus, differences in the metabolic rate by different enzymes, together with changes in the level of these enzymes, can be involved in our findings. Further studies are required to elucidate the relative contribution of each mechanism *in vivo*, at different dose levels of vitamins D.

A different AUC among vitamin D-supplemented subjects could be clinically relevant if adequate levels of 25OHD were continuously required. If this were the case, vitamin D_3 use could be a better option. The rationale for a need of continuous 25OHD is currently insufficient, although some evidence suggests that such levels are worthwhile, in particular for non-classic vitamin D effects, which seem mediated via localized autocrine or paracrine synthesis of $1,25(OH)_2D$ depending on the adequacy of 25OHD levels.[4,19,32,41] A lower tissue exposure to 25OHD after D_2 supplementation should result in a proportionally lower synthesis of $1,25(OH)_2D$, in comparison with a similar dose of D_3. However, further determinants can be also relevant; i.e. besides 25OHD level, the concentration of intact vitamin D delivered to tissues is important for $1,25(OH)_2D$ synthesis, either for classic or for non-classic vitamin D effects.[42]

In summary, under these experimental conditions, loading doses of D_2 or D_3 were similar with respect to increasing 25OHD.

Table 3. Calciuria/creatininuria ratio and calcemia during the supplementation period

	Placebo		*Vitamin D_2*		*Vitamin D_3*		*Inter-groups*
	sCa	*uCa/uCr*	*sCa*	*uCa/uCr*	*sCa*	*uCa/uCr*	P
Basal	9.5 ± 0.3	0.08 ± 0.04	9.5 ± 0.4	0.09 ± 0.03	9.5 ± 0.3	0.08 ± 0.10	NS
d7	9.6 ± 0.4	0.10 ± 0.07	9.5 ± 0.2	0.12 ± 0.06	9.5 ± 0.4	0.07 ± 0.02	NS
d21	9.6 ± 0.2	0.08 ± 0.04	9.6 ± 0.4	0.10 ± 0.04	9.7 ± 0.3	0.08 ± 0.06	NS
P	NS	NS	NS	NS	NS	NS	

Abbreviations: NS, not significant; sCa, serum calcium; uCa, urine calcium; uCr, urine creatinine.

After a maintenance period with the same daily doses, in the D2-supplemented group 25OHD levels declined faster than in the D3 group, reaching similar 25OHD levels than placebo at the final point. In the long term, vitamin D_3 seems more appropriate to sustain adequate levels of 25OHD, which could be relevant for classic and non-classic effects of vitamin D.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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