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ORIGINAL CONTRIBUTION

Is a daily supplementation with 40 microgram vitamin D_3 sufficient? A randomised controlled trial

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Abstract
Purpose The effect of 40 μg (1,600 IU) per day of vitamin D_3 on serum 25-hydroxyvitamin D (25(OH)D) and markers of bone and mineral metabolism was evaluated.
Methods This intervention study was designed as a double-blind randomised controlled trial. Forty-five community-dwelling subjects (32 females), age 55–84 years, at 58° North latitude were supplemented for 1 year with 40 μg vitamin D_3 plus 1,000 mg calcium per day, or with 1,000 mg calcium per day for controls. Safety parameters and 25(OH)D, intact parathyroid hormone (PTH), ionized calcium, bone-specific alkaline phosphatase (BALP), and tartrate-resistant acid phosphatase isoform 5b (TRACP5b) were measured over the study period.
Results All subjects supplemented with vitamin D_3 reached a 25(OH)D level above 50 nmol/L. Mean (SD) serum 25(OH)D increased from 50.4 (13.5) nmol/L to 84.2 (17.5) nmol/L, range 55.0–125.0 nmol/L in the vitamin D_3 supplemented group and the corresponding levels for the control group were 47.3 (14.1) nmol/L and 45.7 (13.4) nmol/L, range 26.0–73.0 nmol/L. No serious adverse event was recorded and the highest 25(OH)D level reached, 125.0 nmol/L, is well below toxic levels. BALP and TRACP5b did not change significantly over the study period.
Conclusions This trial suggests that a daily supplementation with 40 μg vitamin D_3 is sufficient to secure a 25(OH)D level of 50 nmol/L. No side effects were observed in the study group.

Keywords 25-Hydroxyvitamin D · Vitamin D insufficiency · Hyperparathyroidism · Bone turnover · Calcium · Parathyroid hormone

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Introduction

The quest for the optimal dose of vitamin D for prevention or treatment of disease caused by vitamin D deficiency or insufficiency has a long history that is far from ended. Hundreds of studies were performed mainly in rats on rachitogenic diet as well as in children in the search for prevention of rickets. In 1927, UV-irradiation of ergosterol from yeast was found to yield vitamin D_2, which in 1929 was standardised by the Steenbock rat unit based on its healing power in rachitic rats [1]. Various doses were tried in infants with prevention of rickets as the outcome variable [2]. Simultaneously, vitamin D_3 was identified in cod-liver oil, which came into common use. Two hundred IU or 5 μg of vitamin D, roughly corresponding to a teaspoon of cod-liver oil, became the recommended daily intake (RDI) of vitamin D in 1942 for the prevention of rickets. The occurrence of rickets fell dramatically in the United States and other countries [2]. The RDI for adults was set to

10 μg, while FAO/WHO and the United Kingdom had an RDI of only 2.5 μg for many years.

The advent of methods for measuring serum concentrations of vitamin D metabolites and parathyroid hormone (PTH) in the 1970s and 1980s raised new possibilities for dose finding studies and assessment of clinical effects in humans. Serum 25-hydroxyvitamin D (25(OH)D) was identified as the main circulating metabolite of vitamin D, and in 1997 the Food and Nutrition Board confirmed that the serum concentration of 25(OH)D was the best indicator of vitamin D status. Rickets and osteomalacia are usually associated with serum 25(OH)D levels below 30 nmol/L [3]. Serum 25(OH)D levels may also be of importance for the intestinal calcium absorption [4, 5]. Several cross-sectional studies have shown an inverse correlation between 25(OH)D and PTH continuously throughout a wide range of 25(OH)D levels. Different 25(OH)D threshold levels above which serum PTH is plateauing has been suggested in different studies from 15 nmol/L to 150 nmol/L, clustering between 50 and 75 nmol/L [6–8]. Supplementation with vitamin D, with or without calcium, has in some but not all studies resulted in suppression of serum PTH [9, 10].

In addition to bone and mineral metabolism, other putative targets of vitamin D actions have recently been studied. Balance, muscle function and risk of falling have been reported to improve by vitamin D supplementation [11]. The tolerable upper intake level (UL; the highest daily intake of the nutrient that is likely to pose no risk) was for many years kept at 50 μg (2,000 IU) per day due to the concern about potential vitamin D toxicity [12]. However, the report recently published by the Institute of Medicine (IOM) adjusted the UL to 100 μg (4,000 IU) per day [13]. The recommended dietary allowance of vitamin D for maintaining an optimal 25(OH)D level above 50 nmol/L (20 ng/mL) (which cover the requirements of at least 97.5% of the population) according to the IOM report was set to 15 μg (600 IU) for individuals 1–70 years, and 20 μg (800 IU) for ages 71 years and above [13].

This double-blind randomised controlled intervention trial was designed to evaluate the effect of one-year supplementation with 40 μg (1,600 IU) vitamin D_3 plus 1,000 mg calcium per day, or with 1,000 mg calcium per day for controls with serum 25(OH)D as a primary endpoint. Intact PTH, ionized calcium, bone-specific alkaline phosphatase (BALP), and tartrate-resistant acid phosphatase isoform 5b (TRACP5b) were measured as safety parameters over the study period.

Subjects and methods

Subjects

Recruitment started with advertising in the local press and the first interview was carried out via telephone. Subjects within the age group 55–85 years at 58° North latitude, with no exclusion criteria but with circumstances suggesting a risk for vitamin D insufficiency, were invited for a screening visit at the Department of Endocrinology, Linköping University Hospital, Sweden. Written patient information was added to the invitation letter and informed consent was received at the beginning of the appointment. The local research ethics committee of Linköping University, Sweden, approved this study.

Exclusion criteria were ongoing vitamin D supplementation, diseases or drugs that may influence vitamin D status or other study endpoints. Subjects with serious medical illness, hypercalcemia, urolithiasis, disease and drugs known to influence vitamin D or calcium levels, renal insufficiency, hepatic disease or mental inability to fulfil the study protocol were thus excluded. Subjects planning to travel to sunny countries were also excluded.

Urinary and venous blood samples were taken at the screening visit for assessment of serum 25(OH)D, alanine aminotransferase (ALAT), albumin, alkaline phosphatase (ALP), calcium, creatinine, gamma-glutamyltransferase (GGT), ionized calcium, phosphate, thyroid-stimulating hormone (TSH), and urinary calcium. Subjects with 25(OH)D below 10 nmol/L or above 70 nmol/L, serum creatinine more than 130 μmol/L, ALP more than 50% above the upper reference interval limit, ALAT more than 100% above the upper reference interval limit, or albumin below 32 g/L were excluded.

Fifty-six subjects (40 females), age 55–84 years, were found eligible for the study and were invited to visit number 1 within 2 months after the screening visit during February 2007 through December 2007. Forty-five subjects (32 females), age (mean ± SD) 70 ± 8 years, weight 71 ± 11 kg, and height 167 ± 8 cm at inclusion, completed the study per protocol. Body mass index (BMI) ranged from 19.2 to 39.7 with three subjects above 30 kg/m^2. The vitamin D_3 supplemented group and control group did not differ significantly with respect to age, weight, height or initial serum 25(OH)D concentrations (data not shown).

Design and clinical assessment

This study was designed as a double-blind randomised controlled intervention trial to evaluate the effect of 1 year supplementation with 40 μg (1,600 IU) vitamin D_3 plus 1,000 mg calcium per day, or with 1,000 mg calcium per day for controls. Allocation of subjects to treatment groups was done by randomisation using the method of randomly permuted blocks of six. The number of subjects allocated to each of the two treatments was equal. A randomisation list with subject numbers with corresponding sealed envelopes (for emergency at site) was prepared by Pharma Consulting Group AB (Uppsala, Sweden). Compliance was

monitored by tablet counting performed at each visit. Medical history and physical examination were performed at the baseline visit and at termination, and blood pressure was measured with the patient in sitting position. The timed-up-and-go test (TUG-test) [14, 15] was assessed by the study nurse at baseline and after 3, 6, 9, and 12 months, and the number of falls since last visit was documented after 3, 6, 9, and 12 months. Fasting venous blood samples were taken at baseline, 3, 6, 9, and 12 months, between 08.00 and 10.00 a.m., except for BALP and TRACP5b, which were sampled at baseline, 6 and 12 months.

Biochemical determinations

Serum albumin, calcium, ionized calcium, creatinine, phosphate, ALP, ALAT, GGT, TSH, and fasting urinary calcium/creatinine were assessed by accredited clinical chemistry routine methods. Serum 25(OH)D was assessed at the laboratory of Women's Clinic, HUCH and HUSLAB, Helsinki, Finland, by high-performance liquid chromatography (HPLC) [16]. Serum intact PTH was analysed by the Elecsys two-site (1-37 and 38-84), sandwich-direct electrochemiluminescence immunoassay on a Modular Analytics E170 (Roche Diagnostics Scandinavia AB, Bromma, Sweden) [17]. The serum BALP activity was determined by enzyme-linked immunosorbent assay (Quidel Corp., San Diego, CA, USA) [18], and serum TRACP5b was determined by a solid-phase immunofixed enzyme activity assay (IDS Ltd., Boldon, UK) [19].

Statistical analysis

The sample size calculation was based on a 5% two-sided Sattertwaite t-test with the assumptions of serum 25(OH)D mean (SD) of 50 (20) and 70 (25) nmol/l in the calcium alone group and vitamin D_3 plus calcium group, respectively. A sample size of 22 evaluable subjects in each group will have 80% power to detect this difference. With an estimated dropout rate of 20% it was decided to include 28 subjects per treatment group. Serum 25(OH)D, BALP, TRACP5b, intact PTH and TUG were analysed with analysis of covariance (ANCOVA) where the models included baseline value as a covariate where appropriate. Least square mean based on the model in each group and the difference between groups was presented along with the corresponding 95% confidence interval and *p*-value. The categorical TUG and number of falls variables were analysed with proportional odds models. In the safety analysis no formal statistical inference was performed.

Results

Adverse events and drop-outs

Forty-five subjects completed the study per protocol. Eleven subjects had some protocol deviation of which five withdrew due to adverse events (cardiac insufficiency, exanthema, migraine, gastroenteritis), three had insufficient compliance (i.e. less than 80%), two began thiazide treatment interacting with serum calcium, and one withdrew on her own wish. The occurrence of adverse events did not differ between the groups, and there was no adverse event suspected to be related to the supplemented amount of 40 μg vitamin D_3. Constipation was reported by 5 subjects in each group. Blood pressure did not change in any of the groups. Serum calcium, ionized calcium and fasting urinary calcium/creatinine excretion did not change over the study period and did not differ between groups.

Effects of 40 μg vitamin D_3 supplementation

Baseline values for markers of bone and mineral metabolism are given in Table 1. The mean serum 25(OH)D was 49.0 nmol/L at baseline in the combined group of all subjects ($n = 46$). One patient was an obvious outlier with

Table 1 Biochemical markers of bone and mineral metabolism

	Baseline		6 months		12 months	
	Control	Vitamin D	Control	Vitamin D	Control	Vitamin D
25(OH)D (nmol/L)	47.3 ± 14.1	50.4 ± 13.5	47.0 ± 12.9	85.8 ± 18.6*,**	45.7 ± 13.4	84.2 ± 17.5*,**
PTH (ng/L)	50 ± 15	55 ± 21	49 ± 14	52 ± 24	46 ± 14	49 ± 20
Ionized calcium (mmol/L)	1.24 ± 0.04	1.25 ± 0.04	1.24 ± 0.04	1.24 ± 0.03	1.25 ± 0.04	1.25 ± 0.05
BALP (U/L)	25.8 ± 7.2	29.3 ± 9.3	24.0 ± 6.0	26.4 ± 6.6	23.8 ± 7.4	27.2 ± 8.8
TRACP5b (U/L)	3.1 ± 1.0	3.3 ± 1.0	2.8 ± 1.2	3.0 ± 0.8	2.7 ± 1.1	3.1 ± 1.0

Values are the mean ± SD. Vitamin D group, $n = 22$; Control group, $n = 23$

* $p < 0.0001$ represent the significance level within the vitamin D group in comparison with baseline

** $p < 0.0001$ represent the significance level between the vitamin D group versus the control group at each time point

inappropriately high serum PTH of 110 ng/L at baseline and elevated levels throughout the study but with normal serum calcium. When this subject was excluded from the calculation, the correlation coefficient between baseline 25(OH)D and PTH was $r = -0.30$, $p < 0.05$.

Serum 25(OH)D in the vitamin D group ($n = 22$) increased from a mean value of 50.4 nmol/L at baseline to 84.2 nmol/L (range 55.0–125.0) after 12 months. Serum 25(OH)D increment per kilo body-weight was on average 0.44 nmol/L/kg (range 0–1.1 nmol/L/kg). The increment was not correlated to BMI. The steady state 25(OH)D level was reached within 3 months, mean 81.8 nmol/L, $p < 0.0001$. The serum 25(OH)D values did not exceeded 125.0 nmol/L in any subject during the entire study period. Only two subjects had a value above 100 nmol/L after 12 months, while six subjects remained below 75 nmol/L. Serum 25(OH)D did not change in the control group during the study period (Table 1; Fig. 1). The 25(OH)D increment was inversely correlated to baseline serum 25(OH)D, $r = -0.38$, $p < 0.05$. The individual increment from baseline to 12 months differed from zero to 75 nmol/L (mean 34 nmol/L); however, the individual fluctuations from 3 months to 12 months were only −16 to 32 nmol/L (mean 8 nmol/L). Serum PTH did not change (although there was a falling trend from baseline to 12 months, 50–46 ng/L, and 55–49 ng/L in the control and vitamin D groups, respectively), and no significant change was observed between the groups. Neither fasting urinary calcium/creatinine nor serum BALP or TRACP5b changed significantly over the study period (Table 1).

Six falls were documented in the control group and four falls in the vitamin D group (non-significant) over the entire study period. No change was observed between the groups over the study period in the TUG-test; 9.8–10.5 s in controls; and 8.1–8.6 s in the vitamin D group.

Discussion

The investigated supplementation dose of 40 μg vitamin D_3 was sufficient to raise serum 25(OH)D in these community-dwelling subjects above the recommended target level of 50 nmol/L [13] and most of them rose above 75 nmol/L, which has been suggested to be an optimal level by some expert consensus groups [7, 20]. Previous reports show that the effect on serum 25(OH)D, of a defined oral dose of vitamin D, differ widely between studies (Table 2). The increment of serum 25(OH)D has been reported to be in the range 0.58–3.50 nmol/L per given microgram per day. The reason for these, approximately 6-fold, discrepancies is largely unknown. The serum 25(OH)D increment is usually inversely correlated to the baseline 25(OH)D level in a biphasic mode [21, 22]

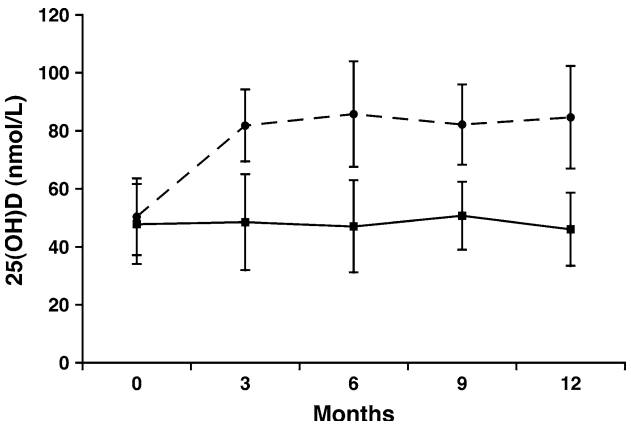

Fig. 1 Serum 25(OH)D concentrations (mean ± SD) during daily supplementation with 40 μg vitamin D_3 plus 1,000 mg calcium (hatched line) and with 1,000 mg calcium for controls (black line). A significant difference was found between the vitamin D group and the control group at 3, 6, 9 and 12 months, $p < 0.0001$

Table 2 Comparison of the effect on serum 25(OH)D of various doses of vitamin D_3 in selected studies sorted according to the daily dose given

Study author	Daily dose (μg)	Age (years), Mean or median	Number of subjects	Study duration (months)	Basal 25(OH)D, Mean ± SD (nmol/L)	Final 25(OH)D, Mean ± SD (nmol/L)	Increment per given μg/day	Method[b]
Ooms et al. [35]	10	80	135	12	27 (19–36)[a]	62 (52–70)[a]	3.50	HPLC, CPB
Meyer et al. [36]	10	85	34	12	47 ± 26	64 ± 21	1.70	HPLC
Bischoff-Ferrari et al. [11]	17.5	71	121	36	70 ± 33	104 ± 42	1.94	CBP
Chapuy et al. [37]	20	84	877	12	40 ± 28	105 ± 22	3.25	CPB
Pekkarinen et al. [38]	20	74	20	12	54.0	70.4	0.82	HPLC
Vieth et al. [28]	25	42	33	3	43.3 ± 16.8	68.7 ± 16.9	1.02	RIA (Diasorin)
Present study	40	70	22	12	50.4 ± 13.5	84.2 ± 17.5	0.85	HPLC
Schleithoff et al. [39]	50	57	42	12	36	103	1.34	RIA (Diasorin)
Vieth et al. [28]	100	40	28	5	37.9 ± 13.4	96.4 ± 14.6	0.58	RIA (Diasorin)
Mocanu et al. [40]	125	71	45	12	28.5	125.6	0.78	RIA (Diasorin)

[a] Median and interquartile range

[b] *HPLC* High-performance liquid chromatography, *RIA* Radioimmunoassay, *CPB* Competitive protein binding assay

and so even in the present study. The mildness of vitamin D insufficiency in our study group may thus contribute to the modest effect on serum 25(OH)D.

Vitamin D deficiency may develop from intestinal malabsorption, however, vitamin D deficiency is not common in diseases with malabsorption, e.g. celiac disease [23]. Obesity is associated with reduced bioavailability of vitamin D [24]; however, only three of the participants in this study were obese (BMI > 30) and none was underweight. Genetic determinants of vitamin D deficiency have recently been reported in a genome-wide association study [25] and further studies will hopefully elucidate whether differences reside in the absorption, synthesis or elimination processes. Drugs such as phenytoin and carbimazole may accelerate the elimination of vitamin D and its metabolites but subjects using such drugs were excluded from the present study. Compliance in the present study was monitored by tablet counting. Another general concern in clinical vitamin D research are the methodological problems in standardising 25(OH)D assay methods, which has only recently made progress [26]. HPLC was used for the measurement of serum 25(OH)D in the present study [16], which is regarded as a highly accurate assay in comparison with most immunoassays. Both serum $25(OH)D_3$ and $25(OH)D_2$ were included in the result obtained from the HPLC analysis; however, vitamin D_2 is currently not used as food fortification in Sweden and no participant took vitamin D_2 supplements during the study period.

Some previous studies have been limited to part of the year which may result in bias or lost information due to seasonal variations in circulating 25(OH)D [21, 22, 27, 28]. The study protocol was, therefore, designed to comprise 12 months with continuous vitamin D_3 supplementation in order to control for the known effects of seasonal variation. A main determinant for the vitamin D status is the exposure of UV-B radiation, which in turn is dependent on latitude, weather, clothing and outdoor habits. This study was performed at 58° North latitude where the UV-B radiation reach sufficient levels (to synthesise 25(OH)D) only from April through August. Milk is fortified with 15 IU vitamin D_3/dl and margarine with 7.5 μg/100 g. Although that 95% of all patients are reported to reach a 25(OH)D level above 50 nmol/L when supplemented with 20 μg vitamin D per day [13], non-responders may be difficult to predict, and this raise the question whether a control measurement would be of clinical value some time after initiating vitamin D supplementation.

The lower level of vitamin D sufficiency is debated. Serum 25(OH)D above 30 nmol/L seem to be sufficient to abolish rickets and osteomalacia [3], while Need et al. [29] found no reduction of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) or calcium absorption unless 25(OH)D was below 10 nmol/L. On the basis of serum PTH in cross-sectional studies, the lower limit of vitamin D sufficiency has been suggested to be 50 nmol/L or 75 nmol/L of 25(OH)D [7, 11, 20, 30]. In the present study, although serum PTH at baseline was inversely correlated to serum 25(OH)D and a falling trend was observed during the study, serum PTH did not fall more in the vitamin D group than in the control group. Previous studies have demonstrated that serum PTH is independently associated with the calcium intake as well as serum 25(OH)D [31]. The small number of patients in our study, with mean baseline 25(OH)D levels at 50.4 nmol/L in the vitamin D_3 supplemented group, may explain the absence of an effect on serum PTH levels.

No effect was observed for the markers of bone turnover BALP and TRACP in this study, which is in accordance with previous studies (Table 2). This might be related to the absence of significant changes in serum PTH and may possibly suggest that the baseline level of 25(OH)D was sufficient to maintain bone health.

Even the highest serum 25(OH)D value obtained throughout the study (i.e. 125 nmol/L) was well below reported levels of toxicity, i.e. 200–500 nmol/L [12], which is in accordance with previous studies (Table 2). No difference in adverse events was found between groups, however, the study groups were small and larger studies are needed to address this aspect thoroughly. Mean values of serum calcium, ionized calcium and urinary calcium did not change during the present study. The study of Narang et al. [32] where elevated serum calcium levels were found in patients with tuberculosis receiving vitamin D, has attracted lots of attention when deciding the UL. It should be noted that granulomatous diseases such as sarcoidosis and tuberculosis sometimes are associated with hypercalcemia, suggested to be caused by unregulated activation of 25(OH)D to $1,25(OH)_2D$ in the activated macrophages [33]. Of interest in this context is the recent study by Sanders et al. [34] where community-dwelling subjects with a mean age of 76 years and baseline median serum 25(OH)D of 53 nmol/L were treated with 500.000 IU vitamin D_3 orally once a year, corresponding to 34 μg/day, which resulted in an elevated incidence of falls and fractures but not in hypercalcemia or hypercalciuria.

The main strength of the present study is that it is a double-blind randomised controlled intervention trial continuing over 12 months and that a higher supplemental dose of vitamin D_3 than usual was evaluated. The main limitation is the small number of subjects included. Nevertheless, these results support the view that 40 μg per day of vitamin D_3 is sufficient for obtaining circulating 25(OH)D levels above 50 nmol/L in community-dwelling elderly people in Sweden. No side effects were observed in the study group.

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Conflict of interest The authors declare no conflict of interest.

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