VU VRIJE UNIVERSITEIT AMSTERDAM

VU Research Portal

Prevention of Bone Loss by Vitamin-D Supplementation in Elderly Women - A Randomized Double-Blind Trial

Ooms, M.E.; Roos, J.C.; Bezemer, P.D.; Vandervijgh, W.J.F.; Bouter, L.M.; Lips, P.T.A.M.

published in
Journal of Clinical Endocrinology and Metabolism
1995

DOI (link to publisher)
10.1210/jc.80.4.1052

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Ooms, M. E., Roos, J. C., Bezemer, P. D., Vandervijgh, W. J. F., Bouter, L. M., & Lips, P. T. A. M. (1995). Prevention of Bone Loss by Vitamin-D Supplementation in Elderly Women - A Randomized Double-Blind Trial. *Journal of Clinical Endocrinology and Metabolism*, *80*(4), 1052-1058. https://doi.org/10.1210/jc.80.4.1052

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 29. Mar. 2024

0021-972X/95/$03.00/0
Journal of Clinical Endocrinology and Metabolism
Copyright © 1995 by The Endocrine Society
Vol. 80, No. 4
Printed in U.S.A.

Prevention of Bone Loss by Vitamin D Supplementation in Elderly Women: A Randomized Double-Blind Trial*

MARCEL E. OOMS, JAN C. ROOS, P. DICK BEZEMER, WIM J.F. VAN DER VIJGH, LEX M. BOUTER, AND PAUL LIPS

Institute for Research in Extramural Medicine (EMGO-Institute) (M.E.O., P.D.B., L.M.B., P.L.) and Department of Epidemiology and Biostatistics (P.D.B., L.M.B.), Vrije Universiteit; Departments of Nuclear Medicine (J.C.R.) and Endocrinology (W.J.F.V., P.L.), Free University Hospital, Amsterdam, The Netherlands

ABSTRACT

The purpose of the study was to determine the effect of vitamin D supplementation on bone turnover and bone loss in elderly women. Three hundred forty-eight women, ages 70 yr and older, were randomized to receive 400 IU vitamin D_3 per day (n = 177) or placebo (n = 171), double-blind, for a period of 2 yr. Main outcome measures were bone mineral density of both hips (femoral neck and trochanter) and the distal radius, as well as biochemical markers of bone turnover. The effect of vitamin D supplementation was expressed as the difference in mean (percentage) change between the placebo group and the vitamin D group.

The measurements were repeated in 283 women after 1 yr and in 248 women after 2 yr. Vitamin D supplementation significantly increased serum 25-hydroxyvitamin D (25OHD) (+35 nmol/L) and 1,25-dehydroxyvitamin D [1,25-$(OH)_2$D] (+7.0 pmol/L) levels and urinary calcium/creatinine ratios (+0.5%) and significantly decreased PTH(1–84) secretion (−0.74 pmol/L) after 1 yr. No effect was found for the parameters of bone turnover. The effect on the bone mineral density of the left femoral neck was +1.8% in the first yr, +0.2% in the second yr, and +1.9% during the whole period (95% confidence interval 0.4, 3.4%). At the right femoral neck the effects were +1.5%, +1.1%, and +2.6% (confidence interval 1.1, 4.0%), respectively. No effect was found at the femoral trochanter and the distal radius.

Supplementation with 400 IU vitamin D_3 daily in elderly women slightly decreases PTH secretion and increases bone mineral density at the femoral neck. (*J Clin Endocrinol Metab* **80:** 1052–1058, 1995)

VITAMIN D deficiency is common in elderly people because of lesser exposure to sunlight, decreased efficiency of the skin in producing vitamin D, and deficient nutrition (1, 2). It may be an important risk factor for hip fractures, which are a major cause of morbidity and mortality in the elderly. One yr after a fracture, mobility is restored in less than half of the patients, and about 20% of the patients will have died (3). Although true osteomalacia is rare, a suboptimal vitamin D status impairs the production of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), which is essential for active intestinal calcium absorption. The ensuing secondary hyperparathyroidism will result in increased bone turnover and predominantly cortical bone loss, thus increasing the risk of fractures (4). Patients with a hip fracture often have lower vitamin D levels than do controls of similar age (5, 6). Moreover, positive correlations of vitamin D levels with bone mineral density (BMD) of the proximal femur and vertebrae have been found in elderly groups and among younger women with a poor vitamin D status (7, 8).

Vitamin D supplementation of 400 IU daily in elderly people has little or no side effects and improves vitamin D status. In elderly people who are 25-hydroxyvitamin D (25OHD)-deficient, supplementation increases serum 1,25-$(OH)_2$D levels and intestinal calcium absorption and decreases the secretion of PTH (9, 10). This suggests that vitamin D supplementation may reduce bone turnover and bone loss and consequently may lower fracture incidence. However, experimental data from randomized, double-blind trials are scarce. Dawson-Hughes *et al.* (11) observed that vitamin D supplementation reduced wintertime bone loss from the spine in a group of postmenopausal women, whereas in another study (12), no effect of a vitamin D supplement on appendicular bone loss was found in men. There is also some evidence that an annual injection of vitamin D protects against fractures (13). In a recent study (14), a daily supplement of 800 IU vitamin D_3 and 1200 mg calcium reduced bone loss and the incidence of hip fractures in elderly women, but to what extent vitamin D caused this effect cannot be determined.

Inasmuch as vitamin D supplementation may offer a safe and substantial contribution to the prevention of osteoporosis and hip fractures in the elderly, we studied the effect of a daily supplement of 400 IU vitamin D_3 during a 2-yr period on bone turnover and bone loss from the hip and distal radius, in a double-blind, placebo-controlled trial in women ages 70 yr and older.

Materials and Methods

Study population

Elderly people ages 70 yr and older were asked to participate in a clinical trial on the effect of vitamin D supplementation on the incidence of hip fractures (15). Exclusion criteria were hip fracture in the past, total hip prosthesis, and recent history of urolithiasis, hypercalcemia, or sarcoidosis. Female residents of homes for the elderly and apartments for

Received September 27, 1994. Revision received January 5, 1995. Accepted January 12, 1995.

Address all correspondence and requests for reprints to: M.E. Ooms, M.D., Ph.D., EMGO-Institute, Medical Faculty of the Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

* This study was supported by the Praeventiefonds, The Hague, Grant 28–1112-1.

Downloaded from jcem.endojournals.org on February 17, 2005

the elderly were asked to participate in an additional study involving BMD and biochemical measurements. The women had to be reasonably mobile in order to be able to visit the hospital for BMD measurements, which were performed at baseline (t_0), after 1 yr (t_1), and after 2 yr (t_2). The protocol was approved by the hospital Ethical Committee, and all participants gave informed consent.

Intervention, randomization, and compliance

The subjects were randomized to receive either 400 IU vitamin D_3 or placebo, 1 tablet daily, during a 2-yr study. Randomization was performed by the hospital pharmacy, and double-blinding was assured. Compliance was established by questionnaire, by pill counting, and by measuring serum 25OHD levels in blood at t_0 and t_1. If participants were suspected of poor compliance resulting from memory problems, the nursing staff were asked to supervise the taking of the trial medication or to administer it.

BMD measurements

BMD was measured at t_0, t_1, and t_2 by single-photon absorptiometry (SPA, Norland OsteoAnalyzer, Norland Corp., Fort Atkinson, WI) at the distal radius of the dominant forearm. The long-term coefficient of variation (CV) in a group of 27 volunteers and osteoporotic patients was 3.1% (unpublished local data). BMD of both hips was measured using dual-energy x-ray absorptiometry (DXA, Norland XR-26) at the femoral neck and trochanter. After collecting the data, all DXA scans were reanalyzed with the most recent Norland software version 2.3.0, optimizing the localization and angle of the regions of interest in subsequent scans of the subjects in order to enhance the intraindividual precision. The long-term CV in a group of 50 volunteers and osteoporotic patients was 2.1% for the femoral neck and 2.4% for the femoral trochanter (unpublished local data). During the study period, the drift did not exceed 0.5%, as measured daily using a spine phantom.

Questionnaires

Height and body weight were measured while the participants wore indoor clothes and no shoes. Height was measured using a stadiometer. Participants were asked to bring their current medication. They were also asked whether preparations containing vitamin D had been used at any time during the previous 12 months. Current calcium intake was estimated at t_0, t_1, and t_2 using a questionnaire restricted to dairy products, which underestimated calcium intake by 200–300 mg/day (16).

Biochemical measurements

Blood samples and 2-h fasting urine (17) were obtained from fasting subjects at t_0 and t_1. Osteocalcin was measured using an RIA kit from the Incstar Corporation (Stillwater, MN). Serum intact PTH [PTH(1–84)] was measured in plasma using a two-step immunochemical method involving amino-terminal immuno-extraction followed by a midregion immunoassay. The interassay CV of this method is 10.2% (18). Serum calcitonin was measured in 200 women at t_0 and t_2 using an RIA kit from the Nichols Institute (San Juan Capistrano, CA) to measure native calcitonin (1–32). The serum 25OHD and 1,25-$(OH)_2$D measurements at t_0 and t_1 were performed in the same run after storage at −20 C using a competitive protein-binding assay after purification by acid phase extraction and gradient high performance liquid chromatography. The intraassay and interassay CVs are 5% and 6% for 25OHD and 6% and 15% for 1,25-$(OH)_2$D, respectively (19). Serum bone-specific alkaline phosphatase was determined using a wheat germ lectin-precipitation method, with an interassay CV of 6.8% (20). Measurement of serum sex-hormone binding globulin (SHBG) at t_0 was performed using an immunoradiometric assay kit from Farmos Diagnostica (Oulunsalo, Finland) with an interassay CV of 5.3%. Urinary hydroxyproline was measured by high performance liquid chromatography with an interassay CV below 3.2% (21) and expressed as hydroxyproline/creatinine ratio. Serum alkaline phosphatase, calcium, phosphate, albumin, and creatinine were measured using standard laboratory methods. Serum calcium was corrected for serum albumin using the following equation: corrected calcium = calcium + [40 − albumin (g/L)] × 0.02 mmol/L.

Statistical analysis

Statistical analysis was performed using SPSS-PC. The data on the treatment assignment were joined to the other data immediately before the statistical analyses and were made anonymous in order to maintain blinding. Differences in drop-out rates were analyzed with χ^2 tests with Yates correction or Fisher's Exact Tests when appropriate. The effect of vitamin D supplementation on the biochemical variables was defined as the difference in mean change of biochemical measurements between the vitamin D group and the placebo group and was analyzed with analysis of variance. The change in BMD during the whole period was analyzed with analysis of variance for repeated measurements. For each participant, bone loss was calculated as percentage change. The effect of vitamin D supplementation was defined as the difference in mean change of BMD (%) between the vitamin D group and the placebo group during the period at issue; a positive effect indicated a relative gain in the vitamin D group. The effect of vitamin D supplementation at t_1 and t_2, and modification of this effect, was analyzed with linear multiple regression analysis, checking for linearity of the relation, normal distributions, and constancy of variance of the residuals. The calcium intake was averaged for the study periods at issue. Other significant determinants of bone loss were added to the model to enhance precision and to correct for potential confounding variables. When necessary, logarithmic transformations were performed to normalize variance for parametric tests. Serum 25OHD values were corrected for season according to a multivariate model (22). All reported P values are two-sided.

Results

A total of 348 women were randomized to vitamin D (n = 177) or placebo treatment (n = 171). The baseline characteristics are presented in Table 1. There were no substantial differences in these characteristics between the groups. One woman who appeared to have hypercalcemia at baseline was excluded from further participation. One participant in the vitamin D group reported a rash that disappeared after discontinuing the trial medication. At the second and third visit, 6 and 5 women in the placebo group and 7 and 2 women in the vitamin D group, respectively, seemed to be taking a supplement containing vitamin D, and these were excluded from the analysis. In 283 and 248 women, respectively, the measurements were repeated at t_1 and t_2. The reasons for not having a follow-up measurement are presented in Table 2. The drop-out rate was 19% in the first yr, 11% in the second yr, and 29% for the whole study period. The drop-out rate was somewhat greater in the placebo group, mainly because of a higher death rate ($P = 0.12$ in the first yr, $P = 0.08$ for the total period), although none of the differences were significant.

Compliance

The compliance in the women who had repeated measurements was good. According to the yearly questionnaire, 85% used one tablet daily, and 14% used between three and six tablets weekly at t_1 as well as at t_2. The analysis of the remaining tablets showed a slightly better compliance in the second study yr. In the first yr, 63% had used between six and seven tablets weekly, and 4% had used less than three weekly; in the second yr, these compliance rates were 78% and 1%, respectively. Of the women receiving the vitamin D supplement, only 5 participants (3%) did not achieve a serum 25OHD level higher than 30 nmol/L at t_1, whereas 68.4% of the participants in the placebo group had serum levels below 30 nmol/L.

Downloaded from jcem.endojournals.org on February 17, 2005

TABLE 1. Characteristics of 348 participants at baseline by intervention group

	Vitamin D (n = 177)		Placebo (n = 171)	
	Mean	SD	Mean	SD
Variable				
Age (yr)	80.1	5.6	80.6	5.5
Years since menopause	32.6	7.5	32.3	6.5
Body weight (kg)	70.6	10.9	71.5	11.7
BMI (kg/cm^2)	28.1	4.1	28.6	4.0
BMD, left femoral neck (g/cm^2)	0.697	0.111	0.707	0.106
BMD, right femoral neck (g/cm^2)	0.700	0.124	0.698	0.105
BMD, left femoral trochanter (g/cm^2)	0.614	0.113	0.630	0.111
BMD, right femoral trochanter (g/cm^2)	0.608	0.109	0.617	0.107
BMD, distal radius (g/cm^2)	0.316	0.078	0.318	0.083
Skewed variable[a]	median	percentiles	median	percentiles
25OHD (nmol/L)	27.0	19–36	25.0	19–37
1,25-$(OH)_2$D (pmol/L)	110	89–138	111	90–131
Calcium intake (mg/day)[b]	876	638–1101	859	644–1099
SHBG (pmol/L)	59	41–79	55	39–72

BMI, body mass index; BMD, bone mineral density; SHBG, serum sex-hormone binding globulin.
[a] For skewed variables, median values and the 25th and 75th percentiles are given.
[b] From dairy products.

TABLE 2. Number of drop-outs by treatment category and follow-up year (Pl = placebo, D_3 = vitamin D) and significance of the difference during the whole period

Reason for drop-out	$t_0 \rightarrow t_1$		$t_1 \rightarrow t_2$		$t_0 \rightarrow t_2$
	Pl	D_3	Pl	D_3	*P* value
Deceased	12	5	9	6	0.08
Reported side effect	0	1	0	0	1.00
Unable to visit hospital	5[a]	1	3	1	0.17
Other health problems	5	9[a]	3	8	0.23
Not motivated	12	12	2	6	0.65
PHP	0	0	0	1	1.00
Hypercalcemia	0	1	0	0	1.00
Use of bisphosphonate	2	0	0	0	0.29
Total	36	29	17	22	0.75
Remaining in study	283		248		

PHP, primary hyperparathyroidism.
[a] Of whom 2 agreed to BMD measurements at t_2, which are included in the results (total of 4).

Biochemical results

The biochemical measurements at t_0 and t_1 are presented in Table 3. Vitamin D supplementation significantly increased serum 25OHD (+35 nmol/L) and 1,25-$(OH)_2$D (+7.0 pmol/L) levels at t_1. The median serum PTH(1–84) value decreased in the vitamin D group, whereas there was a slight increase in the placebo group, the effect at t_1 (-0.74 pmol/L)) being statistically significant. Serum calcitonin increased in the group treated with vitamin D, but there was a similar increase in the placebo group. Although serum osteocalcin, alkaline phosphatase, bone alkaline phosphatase, and urinary hydroxyproline/creatinine demonstrated a slight relative decrease at t_1, this effect of vitamin D supplementation was not significant. A significant relative increase was observed for the urinary calcium/creatinine ratio (+0.5%).

Bone mineral measurements

Bone loss at t_1 and t_2 was expressed as the difference in mean change in BMD (%), as presented in Table 4 and Fig. 1. To enhance precision, weight loss was used as a covariate for all measurement sites, and serum SHBG at baseline was used for the trochanter and distal radius, as these were significantly related to bone loss at these measurement sites. Adding the covariates to the model did not markedly alter the results. The effect of vitamin D supplementation at t_2 was an increase in BMD of 1.9% for the left and 2.6% for the right femoral neck. During the whole period (t_1 and t_2), a statistically significant gain was observed at the left and right femoral neck ($P = 0.01$ and $P = 0.002$, respectively, analysis of variance for repeated measurements), and no significant effects were observed for the other measurement sites. In multiple regression analysis, the effects seemed to be most prominent in the first study yr (t_0 to t_1), in which the differences in change between the groups were 1.5% and 1.8% for the left and right femoral neck, respectively. In the second study yr (t_1 to t_2), minor positive effects were observed as well, but these were not significant. No significant effects were found at the left and right trochanter and the distal radius.

Modification

Baseline serum 25OHD, serum SHBG, and calcium intake were tested as potential modifiers of the effect of vitamin D on bone loss during the first yr. The effect of vitamin D supplementation seemed to be independent of baseline serum 25OHD as well as of serum 25OHD corrected for season (all $P > 0.20$). A greater effect in favor of the vitamin D group was found at higher serum SHBG levels at all measurement sites. This modification was significant at the left femoral neck ($P = 0.01$) and right femoral neck ($P = 0.05$), and of borderline significance at the left trochanter ($P = 0.08$), but not at the other measurement sites. At the left trochanter, a greater effect of vitamin D supplementation was found at lower calcium intakes ($P = 0.004$). In contrast, the effect of vitamin D supplementation was greater at higher calcium intakes at the right femoral neck ($P = 0.03$).

Downloaded from jcem.endojournals.org on February 17, 2005

TABLE 3. Mean and standard deviation (SD) or median and 25th and 75th percentiles for biochemical measurements at baseline (t_0) and after 1 yr intervention (t_1) with placebo (Pl) or vitamin D (D_3) in 270 participants[a]

		t_0		t_1		$P_{Pl/D3}$
		Mean	SD	Mean	SD	
Serum concentration						
Calcium (corrected) (mmol/L)	Pl	2.4	0.1	2.4	0.1	0.90
	D_3	2.4	0.1	2.4	0.1	
Phosphate (mmol/L)	Pl	1.0	0.1	1.1	0.2	0.83
	D_3	1.1	0.1	1.1	0.1	
Serum concentration (skewed)		median	percentiles	median	percentiles	
25OHD (nmol/L)	Pl	26.0	19–37	23.0	17–31	0.001
	D_3	27.0	19–36	62.0	52–70	
1,25-$(OH)_2$D (pmol/L)	Pl	114	90–135	110	84–130	0.03
	D_3	111	93–138	115	94–133	
PTH(1–84) (pmol/L)	Pl	3.5	2.7–5.0	3.7	2.5–5.8	0.005
	D_3	3.3	2.4–4.6	3.1	2.3–4.1	
Calcitonin (pg/mL)[b]	Pl	5.2	2.8–9.5	6.0	2.8–11.5	0.38
	D_3	4.0	1.5–7.9	5.6	3.2–8.0	
Osteocalcin (μg/L)	Pl	3.4	2.7–4.5	3.2	2.4–4.2	0.35
	D_3	3.7	2.7–4.9	3.3	2.4–4.4	
Alkaline phosphatase (U/L)	Pl	66	55–78	66	54–76	0.41
	D_3	63	53–78	62	53–74	
Bone alkaline phosphatase (U/L)[c]	Pl	28	22–34	33	24–35	0.50
	D_3	27	22–32	28	22–34	
Creatinine (μmol/L)	Pl	83	76–94	82	74–93	0.83
	D_3	82	74–94	83	75–96	
Hydroxyproline/creatinine (μmol/mmol)[d]	Pl	20	17–26	19	16–24	0.68
	D_3	21	16–26	19	16–24	
Calcium/creatinine (mmol/mmol)[e]	Pl	0.31	0.17–0.47	0.29	0.16–0.42	0.03
	D_3	0.27	0.16–0.41	0.27	0.18–0.46	

The P value ($P_{Pl/D3}$) refers to the statistical significance of the difference in mean change between the groups.
[a] Participants using vitamin D supplements at t_1 are excluded (n = 13).
[b] Measured in 192 women at t_0 and t_2.
[c] Measured in 158 patients.
[d] Hydroxyproline/creatinine ratio in fasting urine.
[e] Calcium/creatinine ratio in fasting urine.

TABLE 4. Mean change (%) in the first yr (n = 270), second yr (n = 241), and total period in the placebo (Pl) and vitamin D (D_3) group, the difference in mean change between the groups and 95% confidence interval, and statistical significance over the whole period

		$t_0 \rightarrow t_1$		$t_1 \rightarrow t_2$		$t_0 \rightarrow t_2$		$t_0 \rightarrow t_1 \rightarrow t_2$
		Pl	D_3	Pl	D_3	Pl	D_3	P^a
Left femoral neck		−1.5	0.3	1.2	1.3	−0.3	1.6	
	diff	1.8 (0.6, 3.0)		0.2 (−1.4, 1.7)		1.9 (0.4, 3.4)		0.01
Right femoral neck		−0.9	0.5	−0.34	0.7	−1.4	1.2	
	diff	1.5 (0.4, 2.6)		1.1 (−0.2, 2.3)		2.6 (1.1, 4.0)		0.001
Left femoral trochanter		−1.7	−1.0	0.9	0.4	−0.9	−1.1	
	diff	0.7 (−0.6, 1.9)		−0.5 (−2.2, 1.3)		−0.2 (−1.9, 1.5)		0.90
Right femoral trochanter		−1.1	−1.5	0.3	0.8	−0.9	−0.9	
	diff	−0.4 (−1.7, 0.9)		0.5 (−1.2, 2.3)		−0.0 (−1.7, 1.7)		0.52
Distal radius		−1.5	−3.8	1.5	3.1	−2.4	−2.7	
	diff	−2.4 (−5.5, 0.8)		1.6 (−3.3, 6.4)		−0.3 (−4.9, 4.3)		0.25

[a] Analysis of variance for repeated measurements.

Discussion

We found that supplementation with 400 IU vitamin D_3 daily in elderly women improved the vitamin D status to that of the young adult range (23), slightly increased serum 1,25-$(OH)_2$D levels, slightly decreased PTH secretion, and increased BMD at the femoral neck. No effect on the parameters of bone turnover was observed, except for a small increase in the urinary calcium/creatinine ratio. Higher serum levels of 1,25-$(OH)_2$D may increase calcitonin secretion in elderly women (24), but this was not confirmed by our results.

The increase in BMD at the femoral neck was most apparent in the first yr (between 1.5 and 1.8%), whereas during the second yr there was still some additional gain (0.2 and 1.1%), although it was not statistically significant. Indeed, some of the effects of vitamin D supplementation can be expected to be confined to the first yr (4, 25). Vitamin D supplementation may increase calcium availability, which may promote the mineralization of previously undermineralized bone and the rate at which new bone is mineralized (26). The lower serum PTH levels will reduce bone turnover, thus decreasing the remodeling space and the quantity of new bone with lower mineral content (25). However, this could not be confirmed by a decrease in the markers of bone turnover. Although all were lower in the vitamin D group,

Downloaded from jcem.endojournals.org on February 17, 2005

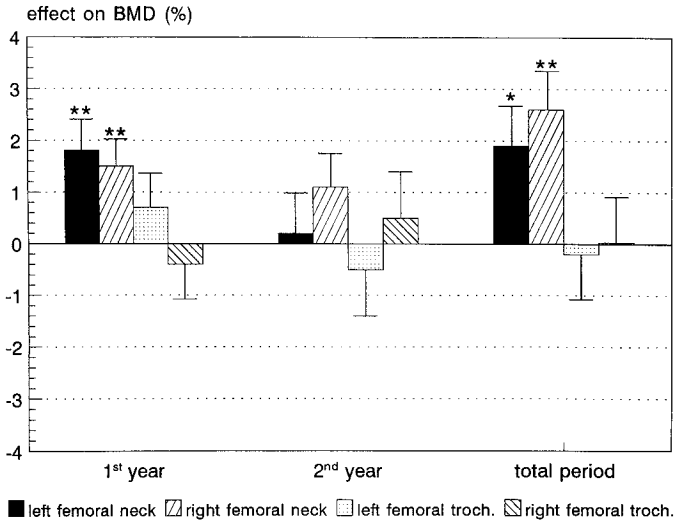


FIG. 1. Effect of vitamin D supplementation on bone loss by study yr. The standard error is indicated by *error bars*. $^{*}P < 0.05$, $^{**}P < 0.01$.

none of the observed effects were statistically significant. 1,25-$(OH)_2$D is known to stimulate osteocalcin and alkaline phosphatase production in the osteoblasts, which may counteract the effect of lowering turnover (26). When the balance between bone resorption and formation is negative, a decrease in bone turnover will lower the rate of age-related bone loss (27). This effect will persist for as long as vitamin D supplementation is continued. The small, nonsignificant benefit during the second yr suggests a decrease of the age-related bone loss, but a longer follow-up period would have been necessary to confirm this more subtle effect.

Dawson-Hughes *et al.* (11) observed a reduction of wintertime bone loss from the lumbar spine as a result of vitamin D supplementation in younger women, and no effect on whole-body BMD. Eighty percent of the skeleton is cortical bone. Therefore, they suggested that trabecular bone is more sensitive to vitamin D deficiency than cortical bone is. Heikinheimo *et al.* (13) found that vitamin D supplementation greatly reduced the incidence of upper limb fractures, whereas a much smaller and nonsignificant reduction in the incidence of lower limb fractures was observed. This suggests that the effect of vitamin D supplementation depends on the skeletal region rather than on the ratio of cortical to trabecular bone. In our study, the increase in BMD was confined to the femoral neck, an area with a relatively high content of cortical bone, and no effect of vitamin D supplementation was found at the femoral trochanter and distal radius, which are predominantly trabecular areas. We did not measure BMD at the lumbar spine, as this measurement has been found to be inaccurate because of the high prevalence of spinal osteoarthritis in elderly people (27). In a population of elderly women, Chapuy *et al.* (14) observed a greater effect of a combination of 800 IU vitamin D and 1200 mg calcium daily on BMD of the proximal femur and serum PTH than was found in our study. It is unlikely that the higher dose of vitamin D is responsible for the difference in the results. The additional increase in serum 25OHD when using a 800-IU dose is negligible (9), which is confirmed by the similar increase of serum 25OHD in both studies. Although mean baseline serum 25OHD and age were similar, the dietary calcium intake was much lower in the Chapuy study, which may account for the higher baseline serum PTH levels and the subsequently greater reduction after supplementation. This suggests that the calcium supplement contributed substantially to the observed effects in this study.

We expected participants with serum 25OHD concentrations below 30 nmol/L to benefit more from vitamin D supplementation than participants with higher levels. In a cross-sectional analysis of the baseline data in this group (22), we found a clear distinction between vitamin D-deficient and vitamin D-replete participants at a serum 25OHD of approximately 30 nmol/L. Higher serum PTH and osteocalcin and lower BMD values were observed only in participants with values below this threshold. Surprisingly, the effect of vitamin D supplementation was not significantly greater in participants with low baseline serum 25OHD levels than in those with higher levels. When the serum 25OHD levels were corrected for the season of measurement, the results were similar. Only about 30% of the participants had baseline serum 25OHD levels higher than 30 nmol/L, and this group may be too small to detect modification of the effect of vitamin D supplementation. At the left and right femoral neck and left trochanter, the effect of vitamin D supplementation was greater in participants with high serum SHBG than in those with lower serum levels. Inasmuch as serum SHBG has been found to be the main determinant of the levels of free estrogen in elderly women, it can be used as an inverse marker of remaining estrogen activity (28–30). High serum SHBG, *i.e.* low estrogen activity, may be associated with lower calcium absorption because of decreased sensitivity of the gut to 1,25-$(OH)_2$D or to a direct effect of estrogen on the gut (31, 32). Indeed, higher serum PTH levels were observed at baseline among participants in whom low serum 25OHD was combined with high serum SHBG (22). In this group, higher serum 1,25-$(OH)_2$D levels will be required to maintain adequate absorption, which will be possible only when the serum 25OHD levels are sufficient. The active metabolite 1,25-$(OH)_2$D is essential for active intestinal calcium absorption. When calcium intake is high, the contribution of passive calcium diffusion to intestinal calcium absorption may be higher. The effect of vitamin D supplementation may be less in these subjects. This could only be confirmed at the left trochanter with high statistical significance, whereas (surprisingly) the opposite was found at the right femoral neck.

It may be expected that an increase in BMD at the femoral neck will reduce the risk of hip fracture. However, quantification of this reduction is difficult and may be different for femoral neck and trochanteric fractures. Using data from Cummings *et al.* (33) on the relation between BMD and fracture risk, the effect of vitamin D supplementation on BMD in this study would correspond with a reduction in the incidence of femoral neck fractures by 12–17%. The risk of trochanteric fractures may be unchanged or even decreased inasmuch as BMD of the femoral neck is a predictor of trochanteric fractures as well.

Downloaded from jcem.endojournals.org on February 17, 2005

As could be expected in an elderly population, the dropout rate was substantial. The total number of deaths was almost twice as high in the placebo group, which may bias the results. However, a higher number of frail elderly, who are more prone to bone loss, may have remained in the vitamin D supplemented group, so the true effect of vitamin D supplementation may be even greater rather than smaller.

We conclude that vitamin D supplementation with 400 IU daily slightly increases serum 1,25-$(OH)_2$D and lowers PTH secretion without side effects. Bone loss is prevented at the femoral neck but not at the femoral trochanter and distal radius. The effect is independent of the baseline vitamin D status, but our data suggest that elderly women with little remaining estrogen activity benefit more than women with greater remaining estrogen activity. Therefore vitamin D supplementation may be a safe method for the prevention of hip fractures in the elderly.

Acknowledgments

We thank Simon Velt and Rob Barto for the laborious task of measuring the vitamin D metabolites; Corrie Popp for measuring SHBG; Karin ten Kroode and colleagues from the department of Nuclear Medicine for the BMD measurements; Professor Roger Bouillon (LEGENDO, University of Leuven, Belgium) for measuring serum calcitonin; Wil Hackeng (formerly of the Laboratory for Endocrinological Chemistry, Bergwegziekenhuis, Rotterdam) for the measurements of PTH and osteocalcin; the Laboratory of Clinical Chemistry for other biochemical estimations; and Els Lommerse, Nel van de Kreeke, and Saskia van Bennekom for their invaluable contributions. This study would not have been possible without the help of the Unie van Vrijwilligers volunteers who arranged transportation and guided and assisted the participants. The trial medication was kindly provided by Duphar Nederland BV, Amsterdam, The Netherlands.

References

1. **Lips P, van Ginkel FC, Jongen MJM, Rubertus F, van der Vijgh WJF, Netelenbos JC.** 1987 Determinants of vitamin D status in patients with hip fracture and elderly control subjects. Am J Clin Nutr. 46:1005–1010.
2. **Lester E, Skinner RK, Mills MR.** 1977 Seasonal variation in serum 25-hydroxyvitamin D in the elderly in Britain. Lancet. I:979–980.
3. **Sernbo I, Johnell O.** 1993 Consequences of hip fracture: a prospective study over 1 year. Osteoporosis Int. 3:148–153.
4. **Parfitt AM, Gallagher JC, Heaney RP, Johnston CC, Neer R, Whedon GD.** 1982 Vitamin D and bone health in the elderly. Am J Clin Nutr. 36:1014–1031.
5. **Pun KK, Wong FHW, Wang C, et al.** 1990 Vitamin D status among patients with fractures neck of femur in Hong Kong. Bone. 11:365–368.
6. **Lips P, Netelenbos JC, Jongen MJM, et al.** 1982 Histomorphometric profile and vitamin D status in patients with femoral neck fracture. Metab Bone Dis Rel Res. 4:85–93.
7. **Villareal DT, Civitelli R, Chines A, Avioli LV.** 1991 Subclinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. J Clin Endocrinol Metab. 72:628–634.
8. **Khaw KT, Sneyd MJ, Compston J.** 1992 Bone density, parathyroid hormone and 25-hydroxyvitamin D concentrations in middle aged women. Br Med J. 305:273–277.
9. **Lips P, Wiersinga A, van Ginkel FC, et al.** 1988 The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. J Clin Endocrinol Metab. 67:644–650.
10. **Francis RM, Peacock M, Storer JH, Davies AEJ, Brown WB, Nordin BEC.** 1983 Calcium malabsorption in the elderly: the effect of treatment with oral 25-hydroxyvitamin D_3. Eur J Clin Invest. 13:391–396.
11. **Dawson-Hughes B, Dallal GE, Krall EA, Harris S, Sokoll LJ, Falconer G.** 1991 Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. Ann Intern Med. 115:505–512.
12. **Orwoll ES, McClung MR, Oviatt SK, Recker RR, Weigel RM.** 1989 Histomorphometric effects of calcium or calcium plus 25-hydroxyvitamin D_3 therapy in senile osteoporosis. J Bone Miner Res. 4:81–88.
13. **Heikinheimo RJ, Inkovaara JA, Harju EJ, et al.** 1992 Annual injection of vitamin D and fractures of aged bones. Calcif Tissue Int. 51:105–110.
14. **Chapuy MC, Arlot ME, Duboef F, et al.** 1992 Vitamin D_3 and calcium to prevent fractures in elderly women. N Engl J Med. 327: 1637–1642.
15. **Lips P, Ooms ME, Ter Schegget RM.** 1990 Prevention of hip fractures in the elderly by vitamin D supplementation. In: Christiansen C, Overgaard K, eds. Osteoporosis 1990. Kobenhavn Denmark: Osteopress Aps; 604–606.
16. **Elders PJM, Netelenbos JC, Lips P, et al.** 1989 Perimenopausal bone mass and risk factors. Bone Miner. 7:289–299.
17. **Lips P, van Ginkel FC, Netelenbos JC, Wiersinga A, van der Vijgh WJF.** 1990 Lower mobility and markers of bone resorption in the elderly. Bone Miner. 9:49–57.
18. **Hackeng WHL, Lips P, Netelenbos JC, Lips CJM.** 1986 Clinical implications of estimation of intact parathyroid hormone (PTH) *versus* total immunoreactivity PTH in normal subjects and hyperparathyroid subjects. J Clin Endocrinol Metab. 63:447–453.
19. **Jongen MJM, Kuipers S, van der Vijgh WJF, Lips P, Netelenbos JC.** 1988 Improvements in the simultaneous determination of calcidiol and calcitriol in human serum or plasma. J Clin Chem Clin Biochem. 26:25–28.
20. **Brixen K, Nielsen HK, Eriksen EF, Charles P, Mosekilde L.** 1989 Efficacy of wheat germ lectin-precipitated alkaline phosphatase in serum as an estimator of bone mineralization rate: comparison to serum total alkaline phosphatase and serum bone gla-protein. Calcif Tissue Int. 44:93–98.
21. **Teerlink T, Tavenier P, Netelenbos JC.** 1989 Selective determination of hydroxyproline in urine by high-performance liquid chromatography using precolumn derivatization. Clin Chem Acta. 183: 309–316.
22. **Ooms ME, Lips P, Roos J, van der Vijgh WJF, Bezemer PD, Bouter LM.** 1993 Bone mineral density of the hip and bone turnover are related to vitamin D status in elderly women. In: Christiansen C, Riis B, eds. Proceedings 1993, Fourth International Symposium on Osteoporosis, Hong Kong. Denmark: Rødovre; 160–161.
23. **Netelenbos JC, Jongen MJM, van der Vijgh WJF, Lips P, van Ginkel FC.** 1985 Vitamin D status in urinary calcium stone formation. Arch Intern Med. 145:681–684.
24. **Quesada JM, Mateo A, Jans I, Rodriguez M, Bouillon R.** 1993 Calcitriol corrects deficient calcitonin secretion in the vitamin D-deficient elderly. J Bone Miner Res. 9:53–57.
25. **Parfitt AM.** 1980 Morphologic basis of bone mineral measurements: transient and steady state effects of the treatment in osteoporosis. Miner Electrolyte Metab. 273–287.
26. **Reichel H, Koeffler HP, Norman AW.** 1989 The role of the vitamin D endocrine system in health and disease. N Engl J Med. 320:980–991.
27. **Burger H, van Daele PLA, Algra D, et al.** 1994 The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone Miner. 25:1–13.
28. **Selby C.** 1990 Sex hormone binding globulin: origin, function and clinical significance. Ann Clin Biochem. 27:532–541.
29. **van Hemert AM, Birkenhäger JC, de Jong FH, Vandenbroucke JP, Valkenburg HA.** 1989 Sex hormone binding globulin in elderly women: a predictor of osteoporosis superior to endogenous oestrogens. Clin Endocrinol. 31:499–509.
30. **Davidson BJ, Ross RK, Paganini-Hill A, et al.** 1982 Total and free estrogens and androgens in postmenopausal women with hip fractures. J Clin Endocrinol Metab. 54:115–120.

Downloaded from jcem.endojournals.org on February 17, 2005

31. **Gennari C, Agnusdei D, Nardi P, Civitelli R.** 1990 Estrogen preserves a normal intestinal response to 1,25-dihydroxyvitamin D_3 in oophorectomized women. J Clin Endocrinol Metab. 71:1288–1293.
32. **Arjmandi BH, Salih MA, Herbert DC, Sims SH, Kalu DN.** 1993 Evidence for estrogen receptor-linked calcium transport in the intestine. Bone Miner. 23:63–74.
33. **Cummings SR, Black DM, Nevitt MC, et al.** 1993 Bone density at various sites for prediction of hip fractures. Lancet. 341:72–75.

Downloaded from jcem.endojournals.org on February 17, 2005