Annals of Internal Medicine | ORIGINAL RESEARCH

Dose Response to Vitamin D Supplementation in Postmenopausal Women

A Randomized Trial

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Background: Serum 25-hydroxyvitamin D (25-[OH]D) is considered the best biomarker of clinical vitamin D status.

Objective: To determine the effect of increasing oral doses of vitamin D_3 on serum 25-(OH)D and serum parathyroid hormone (PTH) levels in postmenopausal white women with vitamin D insufficiency (defined as a 25-[OH]D level ≤50 nmol/L) in the presence of adequate calcium intake. These results can be used as a guide to estimate the Recommended Dietary Allowance (RDA) (defined as meeting the needs of 97.5% of the population) for vitamin D_3.

Design: Randomized, placebo-controlled trial. (ClinicalTrials.gov registration number: NCT00472823)

Setting: Creighton University Medical Center, Omaha, Nebraska.

Participants: 163 healthy postmenopausal white women with vitamin D insufficiency enrolled in the winter or spring of 2007 to 2008 and followed for 1 year.

Intervention: Participants were randomly assigned to receive placebo or vitamin D_3, 400, 800, 1600, 2400, 3200, 4000, or 4800 IU once daily. Daily calcium supplements were provided to increase the total daily calcium intake to 1200 to 1400 mg.

Measurements: The primary outcomes were 25-(OH)D and PTH levels at 6 and 12 months.

Results: The mean baseline 25-(OH)D level was 39 nmol/L. The dose response was curvilinear and tended to plateau at approximately 112 nmol/L in patients receiving more than 3200 IU/d of vitamin D_3. The RDA of vitamin D_3 to achieve a 25-(OH)D level greater than 50 nmol/L was 800 IU/d. A mixed-effects model predicted that 600 IU of vitamin D_3 daily could also meet this goal. Compared with participants with a normal body mass index (<25 kg/m^2), obese women (≥30 kg/m^2) had a 25-(OH)D level that was 17.8 nmol/L lower. Parathyroid hormone levels at 12 months decreased with an increasing dose of vitamin D_3 ($P = 0.012$). Depending on the criteria used, hypercalcemia occurred in 2.8% to 9.0% and hypercalciuria in 12.0% to 33.0% of participants; events were unrelated to dose.

Limitation: Findings may not be generalizable to other age groups or persons with substantial comorbid conditions.

Conclusion: A vitamin D_3 dosage of 800 IU/d increased serum 25-(OH)D levels to greater than 50 nmol/L in 97.5% of women; however, a model predicted the same response with a vitamin D_3 dosage of 600 IU/d. These results can be used as a guide for the RDA of vitamin D_3, but prospective trials are needed to confirm the clinical significance of these results.

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Aside from the classic actions of vitamin D on bone metabolism and calcium homeostasis, experts postulate that it may play an important role in cellular proliferation and differentiation and survival of cells in disorders of immunity (1), as well as in cancer (2). Serum 25-hydroxyvitamin D (25-[OH]D) is considered the best biomarker of vitamin D status (3). Vitamin D is a unique nutrient because its requirement can be met by both endogenous production from sunlight and dietary sources, which complicates determining the body's daily nutritional requirements.

To better quantify requirements for intake of nutrients, including vitamin D, the Institute of Medicine (IOM) and the U.S. National Academy of Science developed a system known as Dietary Reference Intakes (3). This system provides an Estimated Average Requirement (EAR) of a nutrient that meets the needs of 50% of the population and the Recommended Dietary Allowance (RDA), which is the level of a given nutrient that meets the needs of 97.5% of the population. In addition, the tolerable upper intake level is as the highest average daily intake of a nutrient that is likely to pose no risk for adverse health effects for nearly all persons in the general population—it is not a recommended intake (**Table 1**).

The IOM attempted to determine the RDA for vitamin D and found no comprehensive studies on the relationship between doses of vitamin D on serum 25-(OH)D; a lack of intervention trials that could establish an RDA for 25-(OH)D linked to clinical outcomes; and that most studies reported combined, not separate, calcium and vitamin D levels (4). Further complicating the determination of an RDA for vitamin D is that its supply depends on other factors, including sun exposure (5), body mass index (BMI) (6), and skin color (7, 8).

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Context

Vitamin D supplementation is widely recommended to patients, but the optimal dose is debated.

Contribution

Postmenopausal white women with vitamin D insufficiency received either placebo or increasing doses of vitamin D_3, as well as calcium supplements. A dosage of vitamin D_3, 800 IU/d, achieved a serum 25-hydroxyvitamin D level greater than 50 nmol/L, which is the Recommended Dietary Allowance for vitamin D_3 recently recommended by the Institute of Medicine.

Caution

This study did not assess clinical outcomes. A dosage of 600 IU/d of vitamin D_3 was not studied but might have been comparable to 800 IU/d.

Implication

Relatively modest doses of vitamin D_3 can achieve the current Recommended Dietary Allowance for vitamin D.

—The Editors

The IOM updated its guidelines on Dietary Reference Intakes in 2011. A serum 25-(OH)D level greater than 50 nmol/L was selected to indicate efficacy, mainly on the basis of fracture studies (4, 9). An analysis of data from the literature was used to correlate total vitamin D intake with 25-(OH)D on the basis of studies performed in winter to minimize the effects of the sun. On the basis of these findings, the new IOM guidelines estimated the RDA for vitamin D as 600 IU/d for adults aged 19 to 70 years and 800 IU/d for adults older than 70 years and defined the EAR for vitamin D_3 as 400 IU/d. In a separate analysis, the tolerable upper intake level was set at 4000 IU/d (4, 9).

The main objective of this randomized clinical trial was to study the effect of increasing doses of vitamin D_3 on serum 25-(OH)D and serum parathyroid hormone (PTH) levels in postmenopausal white women with vitamin D insufficiency, defined as a 25-(OH)D level of 50 nmol/L or less by the World Health Organization in 2003, in the presence of sufficient calcium intake (10). This differs from vitamin D deficiency, generally defined as a 25-(OH)D level less than 25 nmol/L (11). Our overall goal was to determine the RDA and EAR of vitamin D_3 based on the response of serum 25-(OH)D to various doses of vitamin D_3. Our results can be used to guide future trials of clinical outcomes, such as fractures, cancer, and heart disease.

METHODS

Design Overview

Our study was a 1-year randomized, prospective, placebo-controlled clinical trial, VIDOS (Vitamin D Supplementation in Older Subjects), aimed at establishing the dose of vitamin D_3 required to increase serum 25-(OH)D levels to 75 nmol/L and normalize serum PTH levels in participants with a starting 25-(OH)D level of 50 nmol/L or less and sufficient intake of calcium. The trial was stratified by race (white and African Americans) and screening 25-(OH)D levels less than 30 nmol/L versus those 30 nmol/L or higher for African American participants and less than 37.5 nmol/L versus those 37.5 nmol/L or higher for white participants.

This article reports only on the stratum of white women. Participants were equally allocated to a placebo group and 7 dose groups. We used vitamin D_3 as the study supplement because it is the physiologic form of vitamin D.

The institutional review board at Creighton University, Omaha, Nebraska, approved the study protocol, and all participants signed and received a copy of an informed consent form. A data safety and monitoring board was established before the study started. Because of slower recruitment of African American participants, we requested extending recruitment for an additional year and reducing the treatment groups from 8 to 5; the data safety and monitoring board and institutional review board approved these changes for protocol amendment. No other important changes were made to the protocol during the study.

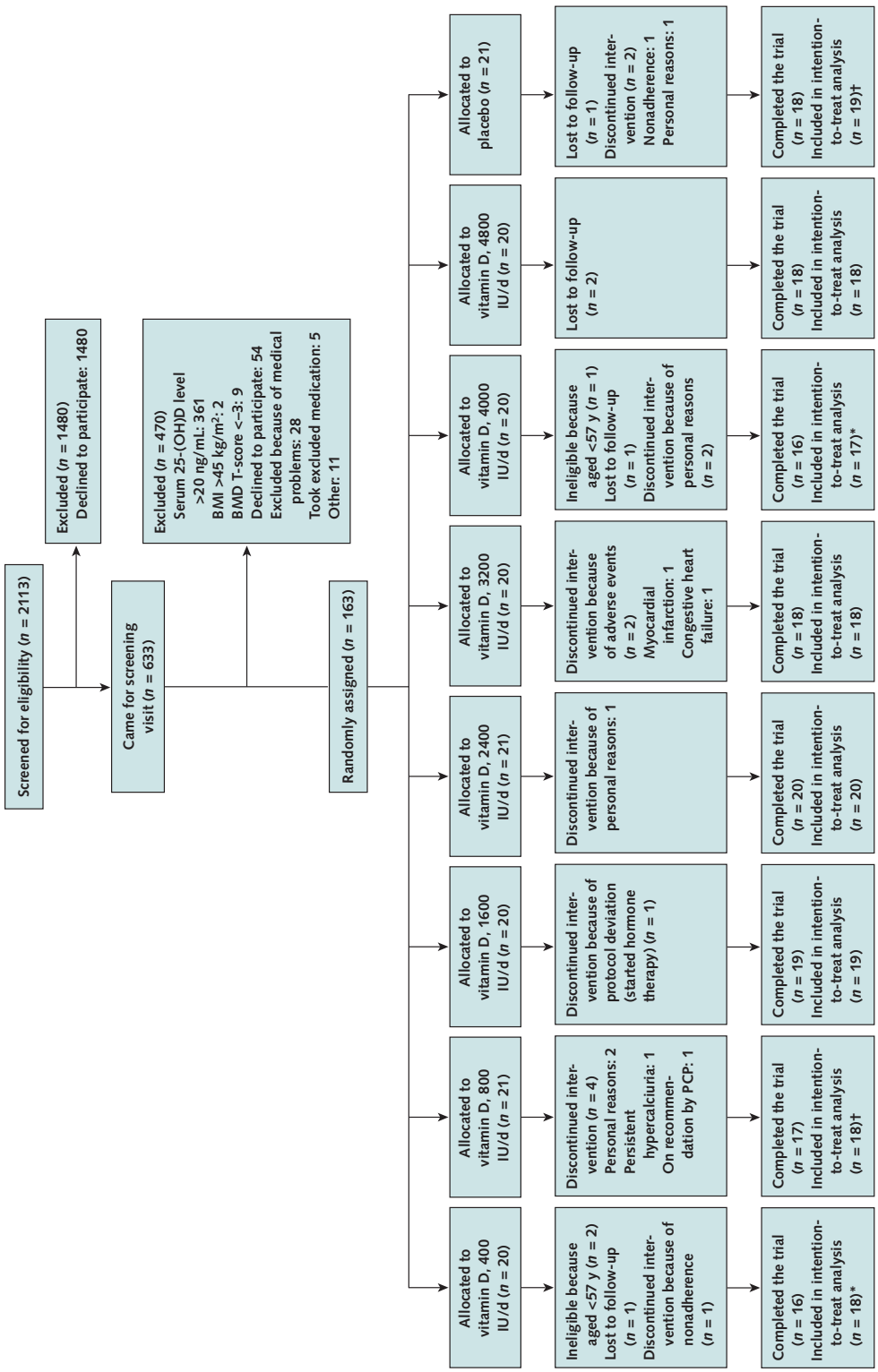
Setting and Participants

We enrolled 163 healthy, white, postmenopausal women aged 57 to 90 years who were at least 7 years postmenopausal (determined from the history of their last menstrual period) with vitamin D insufficiency. Volunteers were recruited by advertisements in local newspapers and church bulletins with a toll-free telephone number. The recruiter then contacted respondents and interviewed them about exclusion and inclusion criteria (**Figure 1**). All laboratory tests and participant examinations were done in a

Table 1. **Terms Used to Quantify Requirements for Nutrient Intake**

Term	Definition
Dietary Reference Intakes	A system developed by the Institute of Medicine and the U.S. National Academy of Science to quantify nutrient intake; it includes the Estimated Average Requirement, Recommended Dietary Allowance, and tolerable upper intake level
Estimated Average Requirement	The average daily nutrient intake level that is estimated to meet the requirements of 50% of the healthy persons in a particular life stage and sex group
Recommended Dietary Allowance	The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97.5%) healthy persons in a particular life stage and sex group
Tolerable upper intake level	The highest average daily intake of a nutrient that is likely to pose no risk for adverse health effects for nearly all persons in the general population; it is not a *recommended* intake

Figure 1. **Study flow diagram.**



25-(OH)D = 25-hydroxyvitamin D; BMD = bone mineral density; BMI = body mass index; PCP = primary care physician.
* Participants who discontinued the study who came in for the final visit.
† Participants who were ineligible because of age criteria were included in the intention-to-treat analysis; there were no crossovers from assigned groups.

Table 2. **Exclusion Criteria**

Substantial comorbid conditions
Any history of cancer (except skin cancer) within the past 10 y
Terminal illness
Previous hip fracture
Hemiplegia
Uncontrolled diabetes with or without significant proteinuria or a fasting blood glucose level <7.8 mmol/L (<140 mg/dL) in persons with type 2 diabetes
Active kidney stone disease or a history of kidney stones more than twice in a lifetime
Chronic renal failure*
Evidence of chronic liver disease, including alcoholism
Physical conditions, such as rheumatoid arthritis, osteoarthritis, and heart failure, severe enough to prevent reasonable physical activity
Unwillingness to discontinue therapy with vitamin D supplements after entering the study
25-(OH)D level <13 nmol/L or >50 nmol/L
BMI >45 kg/m^2
Serum calcium level >2.57 mmol/L (>10.3 mg/dL) on 2 baseline tests
24-hour urinary calcium level >7.3 mmol/d (>290 mg/d) on 2 baseline tests
Bone mineral density T-score less than −3 at the spine or hip
Current use of bisphosphonates or prior use for >3 mo
Use of fluoride, PTH, or PTH derivatives (e.g., teriparatide) in the past 6 mo
Use of calcitonin or estrogen in the past 6 mo
Use of a corticosteroid, >10 mg/d, for more than 6 mo
Current use of phenytoin or phenobarbital, high-dose thiazide therapy†, or any drugs interfering with vitamin D metabolism
Inability to give informed consent

25-(OH)D = 25-hydroxyvitamin D; BMI = body mass index; PTH = parathyroid hormone.
* Defined as a serum creatinine level >124 μmol/L (>1.4 mg/dL).
† Defined as a thiazide dosage >37.5 mg/d.

research study center at Creighton University Medical Center.

At the initial telephone interview, participants were instructed to stop taking multivitamins containing vitamin D before the screening visit; there was no defined washout period. **Table 2** shows exclusion criteria.

Randomization and Interventions

White women who met the eligibility criteria signed informed consent forms and were randomly assigned to 1 of 7 vitamin D_3 doses (400, 800, 1600, 2400, 3200, 4000, and 4800 IU/d) or placebo for 1 year. The study statistician generated the randomization list by using the letters A through H, with SAS software, version 9.2 (SAS Institute, Cary, North Carolina). The randomization method was randomly assigned blocks of 8 and 16, stratified by screening 25-(OH)D levels less than 37.5 nmol/L versus 37.5 nmol/L or more.

The research coordinators dispensed study medication to the participants and managed the allocation record. Each bottle had a label with one of the letters A to H, the study number, and the date dispensed. The information was simultaneously entered in each participant's medication log. Participants, researchers, and all staff involved in the study were blinded to treatment assignment throughout the study with administration of matching placebos. Only the statistician had access to the randomization code. The blinding was removed in case of a serious adverse event or some other compelling reason. The drug company provided the supplement in appropriately labeled bottles and the dose code to the statisticians but had no further role in the study.

Participants were screened in late winter and early spring to minimize seasonal effects. The first phase was primarily between April and May 2007, and the second phase was from January to May 2008. Random assignment occurred an average of 5 weeks after initial screening for 25-(OH)D levels.

Vitamin D_3 in 400-, 800-, 1600-, 2400-, 3200-, 4000-, and 4800-IU capsules and matching placebo capsules were custom-manufactured for the study (Douglas Laboratories, Pittsburgh, Pennsylvania). The actual vitamin D_3 concentrations in the capsules were measured independently in a laboratory at the University of Wisconsin, Madison, Wisconsin, every 6 months for 3 years. No significant change in potency occurred during this time. The average of 6 analyses of the vitamin D_3 capsules for each dose group was 503 IU for the 400-IU capsules, 910 IU for the 800-IU capsules, 1532 IU for the 1600-IU capsules, 2592 IU for the 2400-IU capsules, 2947 IU for the 3200-IU capsules, 4209 IU for the 4000-IU capsules, and 4937 IU for the 4800-IU capsules. The capsules were stored in dark bottles at room temperature in a locked, temperature-monitored storage room.

Every participant received 1 vitamin D_3 capsule in the morning. Citracal calcium supplements (Bayer HealthCare, Morristown, New Jersey) were administered to maintain a total calcium intake of 1200 to 1400 mg/d, based on a baseline 7-day food diary. Participants were advised to take calcium tablets twice daily.

A central medication log of all study drugs administered to the participants was maintained. At 3-, 6-, 9-, and 12-month follow-ups, adherence was calculated by counting the pills; new bottles of vitamin D_3 and calcium were supplied at these visits.

Participants underwent a comprehensive medical history at baseline. Questionnaires included smoking history, alcohol use, caffeine intake, depression scale, sun exposure, physical activity, and fall and fracture history or incidence, as was done in our previous studies (12). Fasting blood samples were collected at all visits (baseline and at 3, 6, 9, and 12 months) between 7:00 and 10:00 a.m., were allowed to clot, and then were centrifuged at 4 °C for 15 minutes at 2056*g* to separate serum. All samples were stored frozen at −70 °C until analysis.

A comprehensive panel that included serum electrolytes, calcium, creatinine, blood glucose, urea, liver enzyme, bilirubin, and protein levels was done at baseline and at 12 months. A basic panel, including sodium, chloride, potassium, blood glucose, urea, creatinine, and calcium levels, was done at 3, 6, and 9 months. Serum levels of 25-(OH)D and PTH were measured at baseline and at 6 and 12 months. In addition, 24-hour urine samples were

collected at baseline and at 3, 6, 9, and 12 months to measure calcium and creatinine levels. All serum and urine chemistries were measured at Creighton University Clinical Chemistry Laboratory by standard autoanalyzer methods. The laboratory is approved by the Clinical Laboratory Improvement Amendment and certified by the College of American Pathologists.

In the Bone Metabolism Laboratory at Creighton University, serum 25-(OH)D was measured by using radioimmunoassay kits (Diasorin, Stillwater, Minnesota). The minimum detection range reported from Diasorin and in Creighton University's laboratory is 12.5 nmol/L. Over 3 years, the interassay variation in our laboratory standards was 10.3% for 32.5 ng/mL, 12.7% for 70 ng/mL, and 8.9% for 125 ng/mL. The laboratory participates in the Vitamin D External Quality Assessment Scheme, which is an international program for monitoring the accuracy and precision of 25-(OH)D assays; our results were within 1 SD of the all-laboratory trimmed mean (13). Serum intact PTH levels were measured by immunoradiometric assay (Diasorin). The interassay variation for standards was 10.1% for 25 pg/mL and 15% for 51 pg/mL.

Dietary intake of calcium, vitamin D, protein, fat, carbohydrates, phosphorus, caffeine, and other components was collected from 7-day food diaries by a trained dietitian using the Food Processor II Plus, version 5.1 (ESHA Research, Salem, Oregon), nutrition and diet analysis system. This was done at baseline and at the end of the study. Plastic food models (Nasco, Fort Atkinson, Wisconsin) were used to help participants better estimate the quantities consumed, as was done in our previous studies (12).

Outcomes and Follow-up

Primary outcomes were serum 25-(OH)D and PTH levels after 6 months and 1 year. The dose–response effect of vitamin D_3, 400, 800, 1600, 2400, 3200, 4000, and 4800 IU/d, plus calcium were compared with a matching placebo vitamin D plus calcium control group. Serum levels of 25-(OH)D and PTH were measured at baseline and at 6 and 12 months. Secondary outcomes of the study were levels of serum 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$), serum calcium and creatinine, 24-hour urinary calcium and creatinine, and urine bone markers; bone mineral density; calcium absorption; incidence of falls; pulmonary function (FEV_1); physical performance tests; blood pressure; and cellular studies. Only the results of primary outcomes are presented here.

Data on harms were collected at each visit. An adverse event was defined as any adverse effect that occurred during the trial. Adverse events were subdivided into serious and nonserious categories; they were coded by level of intensity (that is, mild, moderate, severe, and life-threatening) and qualified by the relationship to the study drug, action taken on the drug, and clinical outcome, similar to forms used in guidelines from the National Institute on Aging (14).

Before enrollment, participants were informed about the possibility and medical significance of adverse effects from the study medication. At the time of the initial administration of the study drug, each participant was advised to call the study coordinator to report any new symptoms. When a participant called about symptoms between regularly scheduled visits, the study coordinator reviewed the symptoms with the physician, who determined their relationship to the study drug and the action to be taken.

At each regularly scheduled visit, the study coordinator reviewed the participants' medical progress since the previous visit. To monitor all medical events, the study coordinator consistently asked general, direct questions, such as, "Since your last visit: Have you had any illnesses?"; "Have you visited your physician?"; "Have you been hospitalized?"; and "Have you started taking any new medications or experienced a change in any medications?" All adverse events since the last visit were recorded on a form; after the participant signed a Health Insurance Portability and Accountability Act release form, information was obtained from medical records that was adequate to determine the outcome and whether the medical event met the criteria for a serious adverse event.

At each visit, vital signs were measured and forms were filled out on current medications, falls, and sun exposure at 6 and 12 months. Participants also completed forms on physical activity and quality of life. An internal monitor reviewed all records on an ongoing basis.

Hypercalcemia was defined as a fasting serum calcium level greater than 2.65 mmol/L (>10.6 mg/dL) or more than 0.075 mmol/L (>0.3 mg/dL) above the upper limit of normal at baseline and at 3, 6, 9, and 12 months. If hypercalcemia was noted, then fasting serum calcium levels were measured again within 2 weeks. If the repeated value was confirmed as high, calcium supplements were withdrawn and serum calcium level was measured again within 1 week. If calcium levels remained high, then the study drug was withdrawn.

Hypercalciuria was defined as a 24-hour urinary calcium level greater than 10 mmol/d (>400 mg/d) at any of the follow-up visits. If hypercalciuria developed during the treatment period, the 24-hour urinary calcium level was measured again within 2 weeks. If hypercalciuria persisted, then calcium supplements were withdrawn and dietary calcium levels were rechecked. The 24-hour urinary calcium levels were measured again in another 2 weeks; if the elevation persisted, then the study drug was withdrawn.

Statistical Analysis

The study was powered to detect differences among the dose groups for 12-month serum 25-(OH)D levels; a sample size calculation was not performed for PTH. On the basis of our previous studies, the placebo group was conservatively estimated to have an average 12-month 25-(OH)D level of 39 nmol/L (SD, 8.2). We used results of vitamin D supplementation studies in North America and

Table 3. **Baseline Characteristics***

Characteristic	Study Group		
	All Participants (n = 163)	Vitamin D, 400 IU/d (n = 20)	Vitamin D, 800 IU/d (n = 21)
Age, *y*	67 (7.3)	68 (8.6)	68 (8.1)
Weight, *kg*	79 (16)	78 (14)	75 (17)
BMI, kg/m^2	30.2 (5.7)	30.3 (5.4)	28.2 (6.1)
Smoking status, *n (%)*			
Current	17 (10)	2 (10)	1 (5)
Former	60 (37)	7 (35)	7 (33)
Never	86 (53)	11 (55)	13 (62)
Alcohol use, *n (%)*			
No	72 (44)	13 (65)	9 (43)
Yes	91 (56)	7 (35)	12 (57)
Serum calcium level			
mmol/L	2.37 (0.075)	2.40 (0.075)	2.35 (0.05)
mg/dL	9.5 (0.3)	9.6 (0.3)	9.4 (0.2)
Urinary calcium level			
mmol/d	3.62 (2)	3.25 (2.1)	3.87 (1.9)
mg/d	145 (80)	130 (84)	155 (76)
Serum creatinine level			
μmol/L	71 (9)	71 (18)	71 (9)
mg/dL	0.8 (0.1)	0.8 (0.2)	0.8 (0.1)
Serum ALP level, *μkat/L*	1.3 (0.3)	1.3 (0.3)	1.2 (0.2)
Blood glucose level			
mmol/L	5.6 (0.6)	5.6 (1.0)	5.6 (1.0)
mg/dL	101.0 (18.0)	100.0 (17.6)	100.0 (18.6)
Serum AST level, *U/L*	19.2 (4.3)	19.0 (4.1)	19.3 (4.0)
Serum ALT level, *U/L*	19.0 (7.6)	18.6 (7.3)	18.1 (6.1)
Serum 25-(OH)D level, *nmol/L*	38.2 (9.4)	37.8 (10.8)	39.0 (9.5)
Serum PTH level, *ng/L*	36.1 (14.0)	38.3 (16.7)	33.0 (10.3)
Calcium intake, *mg/d*†	685 (259)	606 (212)	741 (247)
Vitamin D intake, *IU/d*†	114 (69)	98 (58)	135 (70)
Medication use, *n (%)*			
Thiazide diuretic	38 (23)	3 (15)	2 (10)
Loop diuretic	11 (7)	3 (15)	2 (10)

25-(OH)D = 25-hydroxyvitamin D; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; PTH = parathyroid hormone.
* Values are reported as means (SDs), unless otherwise noted.
† Derived from 7-d food diary.

Europe to create a predictive model by using linear regression to estimate the final 25-(OH)D level at each of the chosen dose levels (31–52). From this model, the estimated 12-month 25-(OH)D levels were 38.9 nmol/L for placebo, 60.9 nmol/L for the 400-IU/d dosage, 74.4 nmol/L for the 800-IU/d dosage, 87.9 nmol/L for the 1600-IU/d dosage, 95.8 nmol/L for the 2400-IU/d dosage, 101.3 nmol/L for the 3200-IU/d dosage, 105.8 nmol/L for the 4000-IU/d dosage, and 109.3 nmol/L for the 4800-IU/d dosage.

We assumed that the within-group SD was 37.5 nmol/L, with a significance level of 0.05 and a uniform withdrawal rate of 10% across dose groups. Twenty participants who are randomly assigned to each dose group will provide more than 90% power to detect a difference between dose groups in a 1-way analysis of variance model. PASS and NCSS (NCSS, Kaysville, Utah) software was used to calculate the sample size.

Analyses included all randomly assigned participants. Data from participants who withdrew or were removed from the study were included if available, as were data from 3 ineligible persons who were randomly assigned. Additional analyses, called adherent analyses, were conducted with participants who were eligible for the study and adherent to the protocol, which was defined as having a mean adherence of 80% or higher during the course of the study.

Characteristics of the participants at baseline were descriptively compared among the dose groups, with data presented as means and SDs and counts and frequencies. Mixed-effects models were used to estimate dose–response curves for serum 25-(OH)D and PTH. Dose (as continuous) and time (as categorical, baseline, 6, and 12 months) were included as fixed effects, and participant was included as a random effect.

Quadratic terms and log transformations were explored for dose. Interactions between dose and time were also explored; a significance level of 0.10 was chosen to evaluate the interaction terms. Dose was divided by 1000 to prevent numerical overflow in the model estimation (doses used in the models were 0, corresponding to dosages of 0 IU/d, 0.4 for 400 IU/d, 0.8 for 800 IU/d, 1.6 for 1600 IU/d, 2.4 for 2400 IU/d, 3.2 for 3200 IU/d, 4.0 for 4000 IU/d, and 4.8 for 4800 IU/d). Covariance structures

Table 3—**Continued**

Study Group					
Vitamin D, 1600 IU/d (*n* = 20)	Vitamin D, 2400 IU/d (*n* = 21)	Vitamin D, 3200 IU/d (*n* = 20)	Vitamin D, 4000 IU/d (*n* = 20)	Vitamin D, 4800 IU/d (*n* = 20)	Placebo (*n* = 21)
66 (7.4)	66 (6.3)	69 (7.7)	66 (7.1)	65 (6.1)	66 (6.5)
77 (15)	79 (12)	79 (16)	77 (17)	84 (18)	82 (17)
30.0 (5.4)	30.4 (5.4)	30.2 (5.7)	29.7 (6.4)	32.1 (6.2)	31.1 (5.3)
4 (20)	1 (5)	3 (15)	0 (0)	2 (10)	4 (19)
8 (40)	8 (38)	4 (20)	10 (50)	9 (45)	7 (33)
8 (40)	12 (57)	13 (65)	10 (50)	9 (45)	10 (48)
10 (50)	10 (48)	6 (30)	6 (30)	4 (20)	14 (67)
10 (50)	11 (52)	14 (70)	14 (70)	16 (80)	7 (33)
2.40 (0.075)	2.37 (0.075)	2.35 (0.1)	2.35 (0.1)	2.37 (0.075)	2.37 (0.1)
9.6 (0.3)	9.5 (0.3)	9.4 (0.4)	9.4 (0.4)	9.5 (0.3)	9.4 (0.4)
3.50 (1.8)	3.47 (1.6)	4.42 (2.6)	3.55 (1.8)	3.27 (2.1)	3.6 (1.9)
140 (74)	139 (64)	177 (104)	142 (75)	131 (85)	144 (77)
71 (9)	71 (9)	71 (9)	71 (9)	71 (9)	71 (18)
0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.2)
1.4 (0.3)	1.4 (0.3)	1.2 (0.2)	1.3 (0.4)	1.3 (0.4)	1.4 (0.3)
5.4 (0.5)	5.7 (1.3)	5.6 (1.0)	5.6 (0.9)	5.9 (1.4)	5.5 (0.5)
98.0 (9.3)	103.0 (23.1)	101.0 (18.2)	101.0 (16.7)	106.0 (25.2)	99.0 (9.3)
18.8 (3.3)	18.5 (3.1)	19.5 (4.0)	18.4 (3.9)	20.6 (5.1)	19.5 (6.1)
18.8 (7.9)	17.5 (6.9)	19.9 (7.4)	19.0 (8.0)	20.4 (9.3)	19.8 (8.6)
37.4 (10.2)	38.2 (10.1)	39.8 (8.2)	37.2 (9.2)	38.6 (9.1)	37.7 (9.1)
37.6 (14.1)	35.7 (9.9)	31.9 (10.9)	37.7 (20.1)	35.2 (13.6)	39.6 (14.3)
754 (244)	621 (190)	725 (263)	673 (324)	768 (348)	593 (182)
125 (71)	98 (55)	109 (62)	106 (83)	137 (86)	105 (61)
6 (30)	4 (19)	5 (25)	4 (20)	7 (35)	7 (33)
2 (10)	0 (0)	1 (5)	1 (5)	0 (0)	2 (10)

were compared by using the Akaike information criterion; the autoregressive structure and the compound symmetry structure had similar Akaike information criterion values, so the compound symmetry structure was chosen.

Model fit was examined by looking at various residual plots. A sensitivity analysis was performed by using multiple imputation to determine whether the missing data affected the serum 25-(OH)D and serum PTH models. An additional sensitivity analysis of serum 25-(OH)D was performed by examining total vitamin D intake, measured as supplemental dose plus dietary vitamin D intake at baseline by using the methods described previously.

A goal of the study aside from estimating the dose–response curves was to determine the dosage of vitamin D_3 that meets the RDA and EAR for various 25-(OH)D levels. This dose could be interpreted as that at which 97.5% of new persons will have a 25-(OH)D level greater than 75 nmol/L or 50 nmol/L corresponding to the IOM guidelines (3, 4). One thousand bootstrapped samples were used to determine the 95% prediction limits for the 6- and 12-month 25-(OH)D levels from the predicted values of the random effects, or best linear unbiased predictors, of the mixed-effects model. Repeated sampling for the bootstrap procedure was done at the individual level within each dose group, keeping repeated within-participant measures intact. The dose at which participants reach the RDA is the dose at which the 95% prediction lower limit is greater than 75 nmol/L of 25-(OH)D. The EAR, or the dose at which the 25-(OH)D level is greater than 75 nmol/L in 50% of the participants, was found with a similar method.

Multivariate mixed-effects models were examined by using the same dose and time terms found in the previous analysis for serum 25-(OH)D and PTH. The models were adjusted for known covariates on the basis of clinical experience. Covariates were age, BMI category (normal, $<$25.0 kg/m^2; overweight, 25.0 to 29.9 kg/m^2; or obese, $\geq$30.0 kg/m^2), calcium intake, smoking status, alcohol use, average caffeine intake, and serum creatinine level. Correlations between the covariates were examined before model entry to determine whether multicollinearity existed.

Post hoc comparisons were made by examining BMI categories as predictors of serum 25-(OH)D levels. A mixed-effects model for 25-(OH)D that was similar to the initial model was fit, with dose and time and their interactions, as well as BMI category and the interaction between BMI and time. Interactions among BMI category and dose terms were also explored. At the 12-month time point, the fitted dose–response curves for 25-(OH)D by BMI category were developed. SAS/STAT software, version 9.2 (SAS Institute), was used for the statistical analysis. The statistical computing language R, version 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria), was used to create graphical displays. *P* values less than 0.05 were considered statistically significant.

Reports were sent to the data safety and monitoring board approximately every 6 months to monitor accrual and safety data, including adverse events and serum and urinary calcium data. No interim monitoring of the primary outcome was conducted.

Role of the Funding Source

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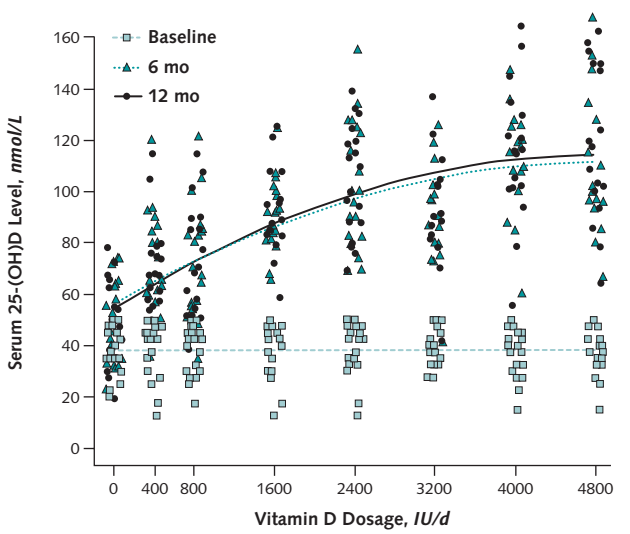
RESULTS

Figure 1 shows the participants who completed the study. However, 1 participant in the 800-IU/d vitamin D_3 group and 1 participant in the placebo group who discontinued the study came in for the study visits, including the final visit. These 2 participants were included in the analysis, as were 3 women who were 56 years of age and thus did not fulfill the age requirement; however, they completed the study. This brings the total number of persons analyzed to 147 (**Figure 1**). The median follow-up of all 163 randomly assigned participants was 12 months (range, 0.9 to 14.0 months); for the 16 participants who withdrew from the study, the median follow-up was 4.4 months (range, 0.9 to 11.4 months). **Table 3** shows the baseline characteristics for the groups.

Figure 2. **Vitamin D dose–response curve.**



Baseline, 6-mo, and final serum 25-(OH)D levels are presented according to dosage of vitamin D or placebo. A quadratic curve was the best fit for data. Levels of 25-(OH)D at 6 and 12 mo were significantly lower in the placebo group compared with all vitamin D dose groups individually ($P < 0.05$). 25-(OH)D = 25-hydroxyvitamin D.

Mean dietary intake of vitamin D_3 and calcium at baseline was 114 IU/d and 685 mg/d, respectively, and was similar among treatment groups. Adherence was measured at 3, 6, 9, and 12 months as a percentage: (number of pills supplied − number of pills returned)/number of pills supplied × 100%. The average of the adherence at 3, 6, 9, and 12 months was calculated to give an overall adherence value for 12 months. Mean adherence averaged over 12 months was 94% for vitamin D_3 and 91% for calcium.

25-(OH)D and PTH Levels at 6 and 12 Months

One hundred sixty-three participants had serum 25-(OH)D levels at baseline, 149 at 6 months, and 147 at 12 months. A quadratic dose–response curve was determined to fit the 25-(OH)D data well. The interaction between dose^2 and time was significant for 25-(OH)D, indicating a quadratic dose–response curve that differs between at least 2 of the time points ($P < 0.001$) (**Figure 2**). The curve started to plateau at approximately 112 nmol/L of 25-(OH)D. The dose–response curves for 6- and 12-month 25-(OH)D levels were almost identical (**Figure 2**).

In **Appendix Table 1** (available at www.annals.org), the coefficients for time and interactions between time and dose and time and dose^2 at 6 months are not significantly different from those at 12 months on the basis of the CIs (**Appendix Table 1**) and the pairwise *P* values between 6 months and 12 months (6-month vs. 12-month time difference [$P = 0.58$], difference between 6-month vs. 12-month time and dose interaction [$P = 0.73$], difference between 6-month vs. 12-month time and dose^2 interaction [$P = 0.54$]). The estimated dose–response curve at 12 months for 25-(OH)D in nmol/L is $54.5 + 24.6 \times \text{dose}/1000 - 2.5 \times \text{dose}^2/1000^2$.

The lower 95% prediction limits reach greater than 75 nmol/L at a dose of 1600 IU/d, indicating that 97.5% of persons will obtain a serum 25-(OH)D level of 75 nmol/L at a vitamin D_3 dosage of 1600 IU/d; therefore, the estimated RDA to achieve a 25-(OH)D level of 75 nmol/L is 1600 IU of vitamin D_3 daily. Analyzing vitamin D_3 intake as total intake by including the dietary intake as well as the dose made no significant difference to the results, probably because dietary intake was low (results not shown). For 97.5% of persons to achieve a 25-(OH)D level greater than

50 nmol/L (as recommended by the IOM), we would require a dosage of vitamin D_3 between 400 and 800 IU/d. If we assume the variability is similar in the 400- and 800-IU/d dose groups, we can extrapolate the lower prediction limits and get a lower prediction limit of 51.3 nmol/L for a dosage of 600 IU/d, which meets the IOM recommendation of 50 nmol/L of vitamin D_3.

Appendix Figure 1 (available at www.annals.org) shows the 12-month 25-(OH)D data with the fitted line from the mixed-effects model with the 95% bootstrapped prediction limits, used to estimate the RDA dose. To estimate the dose of 25-(OH)D that achieves the EAR, 50% of new persons need to achieve a 25-(OH)D level greater than 75 nmol/L. At a dosage of vitamin D_3 between 800 and 1600 IU/d, the median predicted value would be greater than 75 nmol/L for a new person. At a vitamin D_3 dosage of 800 IU/d, the median predicted value is 70 nmol/L at 6 and 12 months; therefore, this model would predict that a dosage of vitamin D_3 slightly higher than 800 IU/d would result in a 25-(OH)D level greater than 75 nmol/L in 50% of persons. To obtain an EAR of 25-(OH)D of 50 nmol/L, a dosage of 400 IU of vitamin D_3 daily is required.

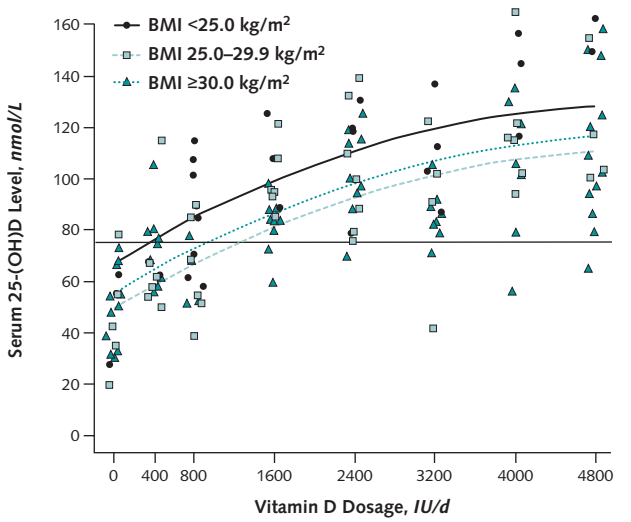
Multivariate Results

The mixed-effects models of dose response described previously were adjusted for clinically important covariates. Average caffeine intake was highly correlated (Pearson correlation coefficient, >0.4) with age and total calcium and creatinine levels and was also associated with smoking status; therefore, it was not considered in the model to help avoid multicollinearity. Final covariates included in the model were age, BMI category, calcium intake, smoking status, alcohol use, and serum creatinine.

Interactions between dose and covariates were explored. All interactions between covariates and doses in the 25-(OH)D model were not significant ($P > 0.20$); therefore, they were removed. **Appendix Table 2** (available at www.annals.org) shows the estimated model for 25-(OH)D. The coefficients of the multivariate mixed-effects model for 25-(OH)D are similar to those in the unadjusted model, with the exception of the intercept term.

Body mass index was the only covariate with a significant effect on 25-(OH)D levels in the model. Because BMI was significantly predictive of 25-(OH)D in the multivariate model, we performed post hoc analyses that more closely examined the relationship between BMI category and 25-(OH)D levels. Mixed-effects models were examined by looking at the effect of BMI, dose of vitamin D_3, and time and their interactions on 25-(OH)D levels. Significant interactions were found between dose and time, dose^2 and time, and BMI and time. None of the other interactions was significant ($P > 0.20$ for all). **Appendix Table 3** (available at www.annals.org) shows the estimated model, and **Figure 3** shows the effect of BMI and dose on 25-(OH)D levels at 12 months.

Figure 3. **Effect of BMI and vitamin D dose on levels of serum 25-(OH)D at 12 months.**



BMI <25 kg/m^2, $n = 31$; BMI 25.0–29.9 kg/m^2, $n = 56$; and BMI ≥30.0 kg/m^2, $n = 76$. 25-(OH)D = hydroxyvitamin D; BMI = body mass index.

Because there was no significant interaction between BMI and dose of vitamin D_3, the 3 BMI curves at 12 months are parallel. Thirty-one participants had a normal BMI, 56 were overweight, and 76 were obese. At 12 months, serum 25-(OH)D levels were higher in normal-weight women than in the overweight women (mean difference, 12.2 nmol/L [95% CI, 4.2 to 20.2 nmol/L]; $P = 0.003$). The mean difference between normal-weight women and obese women was 17.7 nmol/L (CI, 10.2 to 25.2 nmol/L; $P < 0.001$). At 12 months, 25-(OH)D levels were not significantly different between overweight and obese women (mean difference, 5.5 nmol/L [CI, −0. 7 to 12.0 nmol/L]; $P = 0.089$).

Serum PTH levels were available for 163 participants at baseline, 148 at 6 months, and 147 at 12 months. The interaction between vitamin D_3 dose and time was significant, indicating a linear relationship between vitamin D_3 dose and PTH that differed for each time point. The quadratic dose term and interaction between dose^2 and time were not significant in the PTH model ($P > 0.10$ for both).

As **Appendix Figure 2** and **Appendix Table 1** (available at www.annals.org) show that the coefficients for time and the interaction between dose of vitamin D_3 and time at 6 months were not significantly different from those at 12 months on the basis of the CIs and the pairwise P values between 6 and 12 months ($P = 0.11$ and 0.76, respectively). The estimated dose–response curve at 12 months for PTH was $34.2 - 1.6 \times$ dose/1000, with the slope showing a significant decrease in PTH levels as the dose of vitamin D_3 increases ($\beta = -1.6$ [CI, −2.8 to

Table 4. **Adverse Events***

Variable	Study Group							
	Placebo (n = 21)	Vitamin D, 400 IU/d (n = 20)	Vitamin D, 800 IU/d (n = 21)	Vitamin D, 1600 IU/d (n = 20)	Vitamin D, 2400 IU/d (n = 21)	Vitamin D, 3200 IU/d (n = 20)	Vitamin D, 4000 IU/d (n = 20)	Vitamin D, 4800 IU/d (n = 20)
Deaths, *n*	0	0	0	0	0	0	0	0
Withdrawals due to AEs, *n*	0	0	1†	0	0	2	0	0
Patients with any AE, *n*	18	15	17	19	18	17	18	17
Patients with any serious AEs, *n*	2	0	1	2	3	2	0	1
Reported serious AEs	Syncope; total hip replacement	–	Diverticulitis	Cerebrovascular accident; knee replacement	Partial thyroidectomy; tibia–fibula fracture; cholecystectomy	CHF; angina and stent	–	COPD exacerbation

AE = adverse event; CHF = congestive heart failure; COPD = chronic obstructive pulmonary disease.
* Numbers represent frequency.
† Due to persistent hypercalciuria.

−0.4]; $P = 0.031$) (**Appendix Figure 2**). **Appendix Table 1** shows the estimated models for 25-(OH)D and PTH. The results for 25-(OH)D and PTH were similar in the adherent analysis (data not shown).

A multivariate model for PTH was fit similarly to the 25-(OH)D data. All interactions between covariates and dose were not significant and therefore were removed from the model ($P > 0.20$ for all). **Appendix Table 2** shows the estimated model for PTH. The coefficients in the multivariate mixed-effects model for PTH are similar to those in the unadjusted model, although the interaction term between dose and time is no longer as significant ($P = 0.195$). Of the covariates, BMI is marginally significant, with the underweight to normal-weight and overweight groups tending to have lower PTH levels than the obese group ($P = 0.065$); **Appendix Table 2** (available at www.annals.org) shows the coefficients. The results of the sensitivity analysis using multiple imputation models (data not shown) were similar to the missing-completely-at-random models presented here.

Safety and Adverse Events

A total of 11 serious adverse events occurred in 11 patients (**Table 4**). Three of these events were rated as severe, including diverticulitis, congestive heart failure, and a tibia–fibula fracture. Seven of the events were described as moderate, including angina, syncope, hip surgery, radial fracture, an exacerbation of chronic obstructive pulmonary disease, stroke, and partial thyroidectomy for a nodule. No events were attributed to vitamin D_3 use.

Table 5 shows the number of participants with hypercalcemia and hypercalciuria events. All episodes of hypercalcemia were normalized at repeated testing, and 24-hour urinary calcium levels greater than 10 mmol/d (>400 mg/d) were normalized at repeated testing in all but 3 participants. Therapy with calcium supplements was discontinued in 2 of these participants, and both calcium and vitamin D_3 were discontinued permanently in the third.

There were no reports of renal stones. Among the groups, there were no significant changes in levels of serum creatinine, blood urea nitrogen, liver enzymes (aspartate and alanine aminotransferase), glucose, or electrolytes (serum sodium, potassium, chloride, and calcium and 24-hour urinary calcium).

Discussion

To our knowledge, this is the first randomized, controlled, dose–response study of vitamin D in older white women. Its primary finding was that increased levels of serum 25-(OH)D were not linear but followed a quadratic curve suggestive of a rate-limiting mechanism for 25-(OH)D. Further supporting evidence for a control mechanism was the lack of a seasonal increase in serum 25-(OH)D in participants receiving vitamin D_3 at the end of summer and autumn, which is usually approximately 25 nmol/L in our area (12). This could be attributed to 24-hydroxylation and formation of the inactive metabolite 24,24-dihydroxyvitamin D instead of the active metabolite 1,25-$(OH)_2D_3$ (15), a safety mechanism to avoid excessive formation of 1,25-$(OH)_2D_3$.

Control of serum 25-(OH)D levels is also genetically determined. Four genes that help metabolize vitamin D have recently been found; 2 are involved in the metabolism of vitamin D, 1 encodes the *CYP2R1* enzyme that converts vitamin D to 25-(OH)D in the liver, and 1 encodes the *CYP24A1* enzyme that converts 25-(OH)D to 24,24-dihydroxyvitamin D (16). Another gene, *GC*, encodes a protein that transports vitamin D and its metabolites in the blood, and a gene that encodes the enzyme 7-dehydrocholesterol helps to form vitamin D_3 in skin. These results indicate that regulation and maintenance of serum 25-(OH)D is complex.

Body mass index had a significant effect on serum 25-(OH)D levels, as well as on the dose of vitamin D_3. Overweight women had 25-(OH)D levels approximately

12.5 nmol/L lower and obese women had levels approximately 17.5 nmol/L lower than those of women with a normal BMI. Whether the higher BMI causes substantial physiologic changes, such as higher PTH levels and higher bone resorption, is unknown; however, overweight women with lower 25-(OH)D levels do not have lower bone mineral density (17). The parallelism in the 3 dose–response curves in relation to the BMI categories (**Figure 3**) suggests that the difference in 25-(OH)D levels is caused by 1 factor, such as extracellular pool size; had the difference been caused by fat, we would have expected more variability in 25-(OH)D levels.

At 12 months, there was a significant decrease in serum PTH levels associated with increasing vitamin D_3 doses. This was expected, because there is an inverse relation between serum PTH and serum 25-(OH)D levels (18). Also, vitamin D increases 25-(OH)D and thus would be expected to decrease PTH levels. However, this change may be clinically important only in persons with very low 25-(OH)D levels and elevated PTH levels, because a recent review of 70 studies (18) showed that decreases in serum PTH levels reached a plateau (that is, did not decrease further) at serum 25-(OH)D levels that varied greatly between 25 and 125 nmol/L.

Moreover, bone resorption markers that increase with higher PTH levels (19) decrease as 25-(OH)D levels increase and reach a plateau at a low 25-(OH)D level of 42 to 43 nmol/L (18). Many studies show that increased rates of hip fracture and bone loss are only associated with 25-(OH)D levels less than 50 nmol/L; therefore, higher levels are not essential for skeletal health (11, 20–25).

A new finding in our study was the potency of 400 IU/d of vitamin D_3 in increasing serum 25-(OH)D levels by an average of 32.5 nmol/L. Recent data suggest that 400 IU/d of vitamin D_3 would increase 25-(OH)D levels only by 6.7 to 15.0 nmol/L (26). This discrepancy can be explained by the fact that, in our study, baseline serum 25-(OH)D levels were lower than in other studies and treatment started on the steep part of the dose–response curve.

Other evidence also shows that a lower vitamin D_3 dose of 400 IU/d plus calcium is clinically effective. In the Women's Health Initiative (WHI) study of 36 282 women, the adherent group had a 30% reduction in hip fractures (27).

Defining an RDA for vitamin D depends ideally on 3 end points: serum 25-(OH)D level associated with a clinical outcome, the dose of vitamin D that achieves that level, and the dose of vitamin D that prevents or treats the outcome (for example, fractures). By using a 25-(OH)D level less than 50 nmol/L as the end point for the RDA, the 800-IU/d dose group experienced an increase in 25-(OH)D levels greater than 50 nmol/L in 97.5% of women; however, a dosage of 600 IU/d, although not tested, was extrapolated from our model to do the same. The EAR was 400 IU/d. If a serum 25-(OH)D level of 75 nmol/L was used as an optimal value, then the RDA could be defined as the vitamin D_3 dosage greater than 1600 IU/d and the EAR would be between 800 and 1600 IU/d.

In the recent report from the IOM, the investigators also chose a serum 25-(OH)D level of 50 nmol/L on the basis of a clinical outcome of hip fracture incidence that was significantly decreased only in the groups with a 25-(OH)D level less than 50 nmol/L (4, 9). An analysis of literature studies performed in northern latitudes estimated that an EAR of 400 IU/d and an RDA of 600 to 800 IU/d would be adequate to meet that level (4, 9).

The effects of vitamin D in other diseases, such as cancer, immune diseases, diabetes, the metabolic syndrome, and cardiovascular disease, have not been established. These nonclassical actions of vitamin D require the peripheral conversion of 25-(OH)D to 1,25-$(OH)_2$D in local tissues, and the importance of this conversion relative to systemic production in the kidneys and low 25-(OH)D levels is not readily understood in humans at this time. Therefore, the IOM did not find sufficient evidence to recommend an RDA for vitamin D for these conditions.

Regarding the safety of high doses of vitamin D and calcium, our results show that, depending on the cutoff levels of

Table 5. **Occurrence of Hypercalcemia and Hypercalciuria***

Study Group	Serum Calcium Level		Urinary Calcium Level	
	≥2.5 mmol/L (≥10.3 mg/dL)	≥2.7 mmol/L (≥10.6 mg/dL)	>7.5 mmol/d (>300 mg/d)	>10 mmol/d (>400 mg/d)
Placebo	1	0	4	3
Vitamin D, 400 IU/d	1	1	9	2
Vitamin D, 800 IU/d	1	1	7	3
Vitamin D, 1600 IU/d	5	0	5	2
Vitamin D, 2400 IU/d	4	2	6	4
Vitamin D, 3200 IU/d	1	0	6	1
Vitamin D, 4000 IU/d	1	0	6	2
Vitamin D, 4800 IU/d	2	1	5	2
Total	16	5	48	19

* The number of participants who had ≥1 event during the study.

serum and 24-hour urinary calcium for defining hypercalcemia and hypercalciuria, respectively, approximately 2.8% to 9% of participants had an episode of hypercalcemia and 12% to 33% had an episode of hypercalciuria. These numbers may represent a safety risk, as the risk for renal stones increases by almost 2.5 times if the 24-hour urinary calcium level is greater than 7.5 mmol/d (>300 mg/d) (28, 29). It is noteworthy that a 17% increase in kidney stones occurred after 7 years of treatment with vitamin D, 400 IU/d, and a calcium intake of 2000 mg/d in the WHI study (27), and a recent analysis from the same study showed an increase in cardiovascular disease in the subset of women not receiving calcium supplements at random assignment (30).

Vitamin D and calcium intake and their effects on serum and urinary calcium levels are seldom routinely monitored in research studies. Many people take vitamin D and calcium supplements; given the safety concerns in the WHI study and our results, caution is warranted. The hypercalcemia and hypercalciuria events were not related to the vitamin D dose, and whether vitamin D supplements, calcium supplements, or both cause these events is unclear.

Strengths of our study include its design and that it had adequate power to detect differences in 25-(OH)D levels across a broad range of dose groups. Most vitamin D studies have been single-dose studies and did not use a dose–response design. To our knowledge, our study is the first long-term, multiple-dose–response, randomized, double-blind, placebo-controlled trial done in any population.

Our study also has limitations. The sample sizes were relatively small for each dose group. Furthermore, it was conducted in healthy postmenopausal white women, and the findings may not be generalizable to other groups. The highest vitamin D dosage used in our study was 4800 IU/d; thus, it is not possible to accurately predict the dose–response curve beyond this dosage.

In summary, serum 25-(OH)D increased with higher dosages of vitamin D_3 and tended to plateau at approximately 112 nmol/L with vitamin D_3 dosages of 3200 to 4800 IU/d. Vitamin D_3, 800 IU/d, increased 25-(OH)D levels greater than 50 nmol/L in 97.5% of women. This level is associated with significant reductions in hip fractures (21–25). The results from this dose–response study are in good agreement with the IOM's recent recommendation that the RDA for vitamin D should be 600 IU/d in women less than 70 years and 800 IU/d in women older than 70 years (4, 9). For nonskeletal outcomes, optimal 25-(OH)D levels and, therefore, the RDA and EAR will have to be determined in future clinical trials. Our results may be helpful in designing such trials.

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Reproducible Research Statement: *Study protocol*: Available from Dr. Gallagher (e-mail, jcg@creighton.edu). *Statistical code and data set*: Not available.

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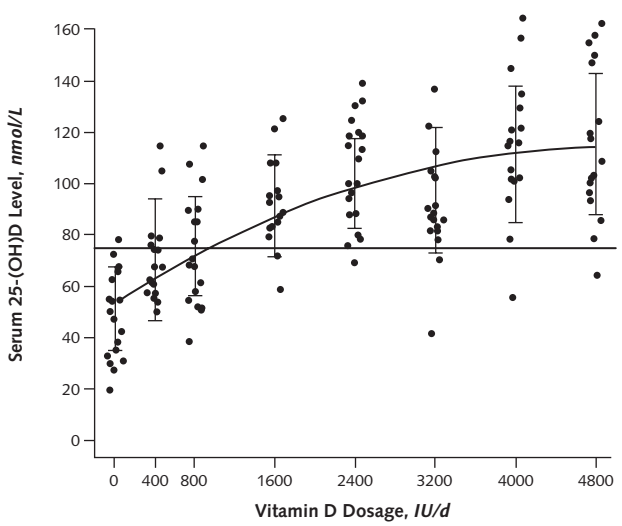
Appendix Figure 1. **Serum 25-(OH)D levels according to vitamin D dosage.**



Levels are shown with a fitted line by using the mixed-effects model, with 95% bootstrapped limits at 12 mo. 25-(OH)D = 25-hydroxyvitamin D.

Appendix Table 1. Dose–Response Mixed-Effects Model, by Estimating the Dose Response of Serum 25-(OH)D and PTH Levels at Each Time Point*

Effect	Serum 25-(OH)D Level, *nmol/L*				Serum PTH Level, *ng/L*			
	β Estimate	SE	95% CI	Overall *P* Value	β Estimate	SE	95% CI	Overall *P* Value
Intercept	54.536	3.161	48.294 to 60.778		34.241	1.675	30.934 to 37.548	
Time								
Baseline	−16.741	3.218	−23.075 to –10.407	<0.001	3.038	1.310	0.459 to 5.617	<0.001
6 mo	1.608	3.247	−4.782 to 7.998		−2.134	1.319	−4.729 to 0.462	
12 mo	0	–	–		0	–	–	
Dose (1000-IU increase)	24.614	3.431	17.861 to 31.367	<0.001	−1.586	0.626	−2.817 to −0.355	0.031
Dose × time								
Baseline	−24.175	3.494	−31.052 to −17.297	<0.001	1.037	0.490	0.072 to 2.002	0.074
6 mo	−1.970	3.525	−8.907 to 4.967		0.148	0.494	−0.824 to 1.121	
12 mo	0	–	–		0	–	–	
Dose^2	−2.535	0.708	−3.928 to −1.142	0.003	–	–	–	
Dose^2 × time								
Baseline	2.462	0.721	1.043 to 3.881	<0.001	–	–	–	
6 mo	0.218	0.728	−1.214 to 1.650		–	–	–	
12 mo	0	–	–		–	–	–	

25-(OH)D = 25-hydroxyvitamin D; PTH = parathyroid hormone.
* Dose was divided by 1000 to fit the models. To estimate the outcome variable, use dose 0 in the models above to correspond to placebo; 0.4 for vitamin D, 400 IU/d; 0.8 for vitamin D, 800 IU/d; 1.6 for vitamin D, 1600 IU/d; 2.4 for vitamin D, 2400 IU/d; 3.2 for vitamin D, 3200 IU/d; 4.0 for vitamin D, 4000 IU/d; and 4.8 for vitamin D, 4800 IU/d.

Appendix Table 2. Multivariate Mixed-Effects Models of Serum 25-(OH)D and PTH Levels*

Effect	Serum 25-(OH)D Level, *nmol/L*				Serum PTH Level, *ng/L*			
	β Estimate	SE	95% CI	Overall *P* Value	β Estimate	SE	95% CI	Overall *P* Value
Intercept	36.398	18.125	0.518 to 72.278		26.300	13.352	−0.126 to 52.725	
Age (1-y increase)	0.126	0.178	−0.224 to 0.477	0.46	−0.077	0.133	−0.338 to 0.185	0.56
BMI								
<25.0 kg/m^2	11.371	3.462	4.554 to 18.189	0.003	−4.716	2.571	−9.779 to 0.347	0.065
25.0–29.9 kg/m^2	3.504	2.871	−2.150 to 9.158		−4.352	2.135	−8.556 to −0.147	
≥30.0 kg/m^2	0	–	–		0	–	–	
Smoking status								
Current	−2.665	4.272	−11.078 to 5.748	0.60	0.309	3.187	−5.966 to 6.583	0.94
Former	1.550	2.732	−3.829 to 6.930		0.741	2.038	−3.272 to 4.754	
Never	0	–	–		0	–	–	
Alcohol use (no vs. yes)	−2.711	2.708	−8.042 to 2.621	0.36	2.417	2.019	−1.560 to 6.393	0.23
Total calcium intake (1-mg increase)	0.002	0.009	−0.016 to 0.019	0.81	0.007	0.007	−0.006 to 0.020	0.28
Serum creatinine (76-μmol [1.0-mg/dL] increase)	7.051	11.600	−15.792 to 29.893	0.53	7.285	8.653	−9.754 to 24.324	0.40
Time								
Baseline	−15.010	3.458	−21.819 to −8.201	<0.001	3.379	1.366	0.688 to 6.069	<0.001
6 mo	2.565	3.458	−4.244 to 9.374		−2.014	1.366	−4.704 to 0.677	
12 mo	0	–	–		0	–	–	
Dose	24.264	3.547	17.280 to 31.248	<0.001	−1.818	0.673	−3.144 to −0.492	0.019
Dose × time								
Baseline	−26.501	3.731	−33.847 to −19.155	<0.001	0.914	0.519	−0.108 to 1.936	0.195
6 mo	−2.368	3.731	−9.714 to 4.978		0.260	0.519	−0.762 to 1.281	
12 mo	0	–	–		0	–	–	
Dose^2	−2.405	0.732	−3.845 to −0.964	0.018	–	–	–	
Dose^2 × time								
Baseline	2.860	0.768	1.348 to 4.373	<0.001	–	–	–	
6 mo	0.243	0.768	−1.269 to 1.756		–	–	–	
12 mo	0	–	–		–	–	–	

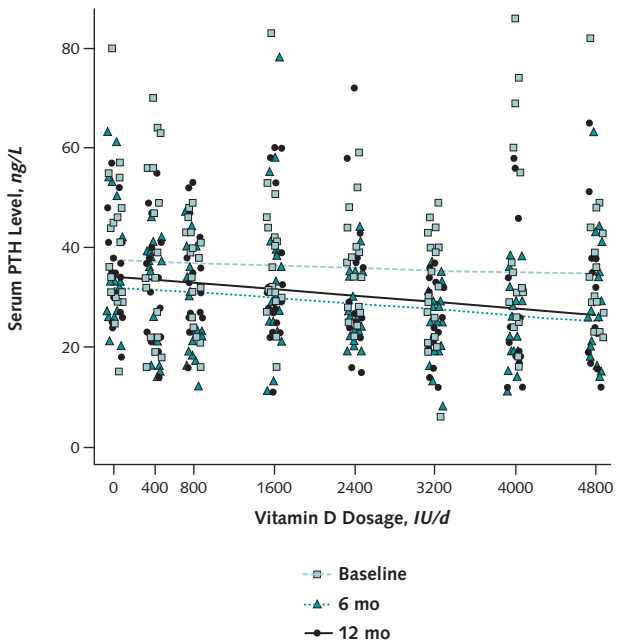
25-(OH)D = 25-hydroxyvitamin D; BMI = body mass index; PTH = parathyroid hormone.
* Dose was divided by 1000 to fit the models. To estimate the outcome variable, use dose 0 in the models above to correspond to placebo; 0.4 for vitamin D, 400 IU/d; 0.8 for vitamin D, 800 IU/d; 1.6 for vitamin D, 1600 IU/d; 2.4 for vitamin D, 2400 IU/d; 3.2 for vitamin D, 3200 IU/d; 4.0 for vitamin D, 4000 IU/d; and 4.8 for vitamin D, 4800 IU/d.

Appendix Table 3. **Mixed-Effects Model of BMI, Dose, and Time on Serum 25-(OH)D***

Effect	β Estimate	SE	95% CI	Overall *P* Value
Intercept	67.502	4.198	59.210 to 75.793	
BMI				
≥30.0 kg/m²	−17.886	3.837	−25.438 to −10.333	<0.001
25.0–29.9 kg/m²	−12.343	4.062	−20.337 to −4.348	
<25.0 kg/m²	0	–	–	
Time				
Baseline	−30.615	4.283	−39.045 to −22.184	<0.001
6 mo	2.524	4.305	−5.950 to 10.998	
12 mo	0	–	–	
Dose	23.176	3.264	16.751 to 29.600	<0.001
Dose × time				
Baseline	−22.792	3.328	−29.343 to −16.241	<0.001
6 mo	−2.319	3.357	−8.927 to 4.289	
12 mo	0	–	–	
Dose²	−2.179	0.675	−3.508 to −0.849	0.011
Dose² × time				
Baseline	2.119	0.689	0.763 to 3.474	0.004
6 mo	0.300	0.695	−1.068 to 1.668	
12 mo	0	–	–	
Time × BMI				
Baseline				
≥30.0 kg/m²	18.609	3.915	10.904 to 26.315	<0.001
25.0–29.9 kg/m²	14.066	4.141	5.915 to 22.217	
<25.0 kg/m²	0	–	–	
6 mo				
≥30.0 kg/m²	−1.964	3.936	−9.712 to 5.785	
25.0–29.9 kg/m²	0.618	4.182	−7.614 to 8.849	
<25.0 kg/m²	0			
12 mo				
≥30.0 kg/m²	0	–	–	
25.0–29.9 kg/m²	0	–	–	
<25.0 kg/m²	0	–	–	

25-(OH)D = 25-hydroxyvitamin D; BMI = body mass index.
* Serum 25-(OH)D levels are measured in nmol/L. Dose was divided by 1000 to fit the models. To estimate the outcome variable, use dose 0 in the models above to correspond to placebo; 0.4 for vitamin D, 400 IU/d; 0.8 for vitamin D, 800 IU/d; 1.6 for vitamin D, 1600 IU/d; 2.4 for vitamin D, 2400 IU/d; 3.2 for vitamin D, 3200 IU/d; 4.0 for vitamin D, 4000 IU/d; and 4.8 for vitamin D, 4800 IU/d.

Appendix Figure 2. **Serum PTH levels, according to vitamin D dosage at 12 months.**



PTH = parathyroid hormone.