Response of Vitamin D Concentration to Vitamin D_3 Administration in Older Adults without Sun Exposure: A Randomized Double-Blind Trial

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OBJECTIVES: To determine the dose-response relationship between 25-hydroxyvitamin D (25(OH)D) and supplemental vitamin D_3 in elderly nursing home residents.

DESIGN: Randomized double-blind investigation.

SETTING: Nursing home.

PARTICIPANTS: Of 81 women (n = 51) and men (n = 30) (mean age 87.4 ± 8) enrolled, 72 completed the study.

INTERVENTION: Sixteen weeks of oral vitamin D_3 at 800, 2,000, or 4,000 IU/d or 50,000 IU/wk.

MEASUREMENTS: The main outcome was 25(OH)D concentrations (tandem mass spectrometry) after 16 weeks. Free 25(OH)D and intact parathyroid hormone (iPTH) were also analyzed. Safety monitoring of calcium and estimated glomerular filtration rate was performed, and adherence and clinical status were measured.

RESULTS: 25(OH)D concentrations increased with dose ($P < .001$) and were higher with 50,000 IU/wk ($P < .001$) than other doses and with 4,000 IU/d than 800 or 2,000 IU/d, but 800 IU and 2,000 IU/d did not differ. One subject receiving 800 IU/d had concentrations less than 20 ng/mL. All subjects receiving more than 2000 IU/d had concentrations of 20 ng/mL and greater. Free 25(OH)D concentrations rose with total 25(OH) vitamin D. Total and free 25(OH)D were related to calcium concentrations; only free 25(OH)D was related to iPTH.

CONCLUSION: 25(OH)D increased linearly with 800 to 4,000 IU/d and 50,000 IU/wk of vitamin D_3, without a ceiling effect. Data suggest that some elderly adults will require more than 800 IU/d of vitamin D_3 to ensure adequate vitamin D levels. Changes in 25(OH)D with vitamin D_3 were related to starting concentrations (greatest with the lowest concentrations and unchanged with 800 and 2,000 IU/d if 20–40 ng/mL). Relationships between serum calcium and iPTH and free 25(OH)D suggest the potential for free 25(OH)D in defining optimal 25(OH)D concentrations. **J Am Geriatr Soc 64:65–72, 2016.**

Key words: vitamin D; elderly; 25(OH)D; iPTH; vitamin D dosing

Vitamin D has a role in health and disease, with inadequate vitamin D levels negatively affecting health.[1–24] Institute of Medicine recommendations for daily vitamin D intake of 800 IU are the same for all adults aged 70 and older but acknowledge the paucity of data from very old people, in whom physiological, disease-related, and environmental exposure differences from younger people could affect vitamin D pharmacokinetics and pharmacodynamics.[15,25–27]

The goal of the current study was to perform a double-blind randomized trial in very elderly adults to determine steady-state circulating concentrations of total 25-hydroxyvitamin D (25(OH)D) in response to vitamin D_3 doses of 800, 2,000, 4,000 IU/d (tolerable upper intake level), and 50,000 IU/wk used for treatment of vitamin D deficiency. Steady-state relationships between total and free 25(OH)D and calcium and intact parathyroid hormone (iPTH) concentrations were also determined as biomarkers of vitamin D effects.

METHODS

Overall Design

This was a 16-week double-blind study of long-term stay nursing home residents aged 65 and older randomized to vitamin D_3 doses of 800, 2,000, or 4,000 IU/d, or 50,000 IU/wk.

Participants

Participants were elderly (≥65) clinically stable long-term stay nursing home residents (Jewish Home, San Francisco).

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There were no changes in medications or diagnoses within the month before enrollment or hospitalizations within 6 months of enrollment. Subjects had no hypercalcemia, history of hypercalcemia, uncontrolled thyroid or parathyroid disorders, severe renal failure (estimated glomerular filtration rate (eGFR)[28] <30 mL/min per 1.73 m^2), active malignancies (except nonmelanoma skin cancer), intestinal bypass surgery or small bowel resection, granulomatous diseases, contraindications or allergy to vitamin D, osteoporosis or a history of fractures and receiving more than 800 IU/d of vitamin D, or treatment for severe vitamin D deficiency or an investigational agent in the prior 6 months. They received no vitamin D supplements (vitamin D naïve) or had stable vitamin D doses for longer than 2 months before entry. All were able to provide informed consent or had an agent able to provide consent to the project approved by the University of California at San Francisco Committee on Human Research.

Intervention

Supplements of 800, 2,000, of 4,000 IU/d or 50,000 IU/wk of vitamin D_3 were given orally for 16 weeks. Randomization was in blocks of four stratified according to sex. Nurses administered one capsule daily (identical-appearing vitamin D or placebo in the 50,000 IU/wk group) with the meal estimated to have the highest fat intake (breakfast in 90%). Status was monitored every 2 weeks in interviews and medical and nursing record reviews. Chemistry panels were analyzed at baseline, midstudy, and study end; 25(OH)D and iPTH were measured at baseline and study end.

Vitamin D Formulations

Capsules containing 800, 2,000, and 4,000 IU vitamin D_3 were custom produced (Bio-Tech Pharmacal, Inc., Fayetteville, AR, www.Bio-Tech-Pharm.com) to be identical in appearance to their commercially produced capsules containing 4,000 and 50,000 IU vitamin D_3. Analyses of capsule content were performed before the study and at 6-month intervals (Tai C. Chen, PhD, Boston University, Boston, MA).

Vitamin D Measurements

Total serum 25(OH)D3 plus 25(OH)D2 (including C3 epimer) concentrations were determined using liquid chromatography tandem mass spectrometry (Mayo Clinical Laboratories, Rochester, MN, a participant in the National Institute of Standards and Technology quality assurance program for analysis of vitamin D metabolites in human serum, funded by the National Institutes of Health Office of Dietary Supplements). The assay has approximately 10% coefficient of variation at concentrations of 10 ng/mL or greater.

Free 25(OH)D was directly measured using immunoassay (Future Diagnostics B.V., Wijchen, the Netherlands, http://www.future-diagnostics.nl/), as previously described.[29,30] The limit of detection of the assay was 1.9 pg/mL. In the range of concentrations measured, the coefficient of variation was 7% or less.

iPTH was measured (San Francisco General Hospital Clinical Laboratories, San Francisco, CA) using a two-site sandwich immunoassay using direct chemiluminometric technology (ADVIA Centaur, Siemens, Malvern, PA).

Adherence was calculated from capsules remaining in blister packs retrieved at 2-week intervals.

Statistical Design and Data Analysis

Demographic and clinical characteristics and baseline laboratory test results of groups were compared using analysis of variance (ANOVA). Comparisons of concentrations in vitamin D_3 dosing groups at study end were made using ANOVA. Adherence of 80% was prespecified for inclusion in analyses. Results presented were Bonferroni-corrected for a midpoint safety analysis. Distribution of dropout and side effects was tested using chi-square analysis. Relationships between total and free 25(OH)D, albumin-adjusted calcium concentrations, and iPTH were tested using linear regression. In residents without a history of supplemental vitamin D, time and dose effects were tested for using repeated-measures ANOVA. Based on conservative estimates of variability and dose responses, a sample size of 24 per group was the prestudy target to detect a dose response in the form of any difference between two dose groups with an omnibus one-way ANOVA, with an α of 0.05 and power of 0.8. Midstudy estimates based on trial data and corrected for multiple comparisons estimated that 18 per group would provide power of 0.88 to 0.94 to detect a dose effect and to detect differences between groups except for between 800 and 2,000 IU/d.

The trial was registered with Clinical Trials.gov as NCT01554241. A Data Safety and Monitoring Board appointed by the National Institute on Aging monitored the study before, during, and at the termination of the study.

RESULTS

Participants

Of 363 long-stay residents screened, 277 met exclusion criteria or declined to participate. Informed consent was obtained in 86, with four ineligible based on baseline laboratory test results and one with consent withdrawn. The study was initiated in 81 residents. Subject characteristics at baseline are presented in Table 1. Mean Charlson Comorbidity Index was 7 ± 3, and mean activity of daily living score was 2 ± 2 (requiring assistance in 4 of 6 activities of daily living on average); 25 were prefrail, 38 were frail, and one was not frail according to the Fried criteria,[31] with frailty assessment unable to be performed in 17. Resident activity was tracked. Ninety percent of participants did not leave the indoor units of the facility; the few who left the indoor units were fully dressed for the San Francisco climate, without sun exposure to the skin. At baseline, 16 residents had not previously received vitamin D supplements. The mean dose in the 65 residents receiving prior supplementation was $1,391 \pm 904$ IU/d (range 200–4,400 IU/d).

Seventy-two participants (89%) completed the study. Five died during the study (pneumonia, n = 2; stroke,

Table 1. Participant Characteristics at Study Entry

Characteristic	Enrolled, n = 81	Completed, n = 72	Oral Vitamin D_3 Dose			
			800 IU/d, n = 20	2,000 IU/d, n = 19	4,000 IU/d, n = 20	50,000 IU/wk, n = 13
Age, mean ± SD (range)	87.4 ± 7.9 (65–105)	87.4 ± 8.0 (65–105)	84.9 ± 8.7 (66–98)	85.9 ± 8.5 (66–101)	89.5 ± 6.6 (75–103)	90.1 ± 6.6 (79–105)
Sex, n						
Male	30	23	6	7	7	3
Female	51	49	14	12	13	10
Race, n						
White	80	72	19	19	20	13
Asian	1	0	1	0	0	0
Weight, kg, mean ± SD	69.7 ± 16.2	70.2 ± 16.2	71.1 ± 18.3	71.1 ± 13.2	69.6 ± 17.3	69.6 ± 16.4
Height, cm, mean ± SD	159.6 ± 9.3	159.4 ± 9.4	160.7 ± 10.9	160.4 ± 7.5	159.0 ± 9.8	156.5 ± 9.7
BMI mean ± SD	27.4 ± 5.7	27.5 ± 5.7	27.5 ± 6.8	27.6 ± 5.3	27.0 ± 5.4	28.2 ± 5.7
Creatinine, mg/dL, mean ± SD	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	0.8 ± 0.3	1.0 ± 0.4	1.1 ± 0.3
Estimated glomerular filtration rate, mL/min per 1.73 m^2, mean ± SD[a]	69.5 ± 25.0	68.3 ± 24.5	67.9 ± 27.9	81.3 ± 23.3	65.9 ± 22.2	54.5 ± 16.5
Calcium, mg/dL[b]	9.4 ± 0.3	9.4 ± 0.3	9.5 ± 0.4	9.3 ± 0.3	9.3 ± 0.4	9.4 ± 0.4
Phosphorus, mg/dL, mean ± SD	3.7 ± 0.6	3.7 ± 0.5	3.8 ± 0.6	3.7 ± 0.4	3.7 ± 0.5	3.5 ± 0.6
Albumin, g/dL, mean ± SD	3.6 ± 0.4	3.6 ± 0.4	3.6 ± 0.4	3.7 ± 0.4	3.6 ± 0.4	3.7 ± 0.4
25-hydroxyvitamin D, mean ± SD						
Total, ng/mL	29.1 ± 9.5	29.7 ± 9.5	29.4 ± 10.4	28.9 ± 10.4	29.0 ± 9.9	32.3 ± 6.0
Free, pg/mL	7.9 ± 2.1	8.0 ± 2.2	7.8 ± 2.6	8.1 ± 2.1	8.1 ± 2.3	7.7 ± 1.5

SD = standard deviation; BMI = body mass index calculated as weight in kg divided by the square of height in meters.

[a] Calculated using the Modified Diet in Renal Disease equation.

[b] Adjusted for the albumin concentration.

n = 2; sepsis, n = 1), two were withdrawn during hospitalization, and two met predetermined stopping criteria of change in renal status or a renal stone. No effect of vitamin D dose assignment on dropout rates was detected (dropouts were 3/20 assigned to 800 IU/d, 1/20 assigned to 2,000 IU/d, 4/20 assigned to 4,000/d, 1/14 assigned to 50,000 IU/wk), nor were differences detected in clinical characteristics between those who completed the study and those who did not. An interim safety analysis of subjects who completed the study included 13 randomized to 50,000 IU/wk and found 25(OH)D concentrations greater than 50 ng/mL in eight and greater than 80 ng/mL in one.

Samples were obtained at a mean of 71 ± 56 hours after dosing and were unrelated to time after dose. Hypercalcemia did not occur, nor were corrected calcium concentrations changed by more than 5% (maximum changes were 0.5 and 0.6 mg/dL in two subjects).

The Data Safety Monitoring Board recommended no further randomization to this dose, resulting in fewer subjects in the 50,000 IU/wk group.

Study Capsule Content

No significant changes in capsule content were detected during the study (April 2013 to September 2014). Mean content of capsules was 858 ± 29 IU and 861 ± 45 for 800-IU capsules, 2,467 ± 69 and 2,482 ± 73 IU for 2,000-IU capsules, 4,839 ± 202 and 4,807 ± 108 IU for 4,000-IU capsules, and 68,354 ± 2,296 IU and 57,542 ± 356 IU for the commercially available 50,000-IU capsules at study initiation and end, respectively.

Adherence

Mean adherence was 96 ± 7% and did not differ according to dose assignment. Only one subject (2,000-IU/d group) had adherence of less than 80%.

25(OH)D Concentrations

Total 25(OH)D concentrations at study end were related to dose ($P < .001$). Mean data are presented in Table 2 and Figure 1 and individual responses in Figure 2. Concentrations were higher with 50,000 IU/wk than with all other doses, and 4,000 IU/d produced concentrations greater than 800 or 2,000 IU/d. No difference was detected in results between 800 and 2,000 IU/d ($P = .50$), although the only person failing to reach a 25(OH)D of 20 ng/mL was assigned 800 IU/d. 25(OH)D concentrations at start influenced responses ($P < .001$). Greatest changes

Table 2. Total and Directly Measured Free 25-Hydroxyvitamin D (25(OH)D) Concentrations According to Vitamin D_3 Dosing Assignment

Vitamin D_3 Dosing Assignment (n/n, Completed/Randomized)	Total 25(OH)D, ng/mL, Mean ± SD (Range)	Between-Group Differences	Free 25(OH)D, pg/mL, Mean ± SD (Range)	Between-Group Differences
800 IU/d (20/23)	33 ± 6 (19–42)	vs 2,000/d, $P = .56$ vs 4,000/d, $P < .001$ vs 50,000/wk, $P < .001$	8.7 ± 2.1 (5.7–13.6)	vs 2,000/d, $P = .48$ vs 4,000/d, $P < .002$ vs 50,000/wk, $P < .001$
2,000 IU/d (19/20)	34 ± 6 (24–46)	vs 4,000/d, $P < .003$ vs 50,000/wk, $P < .001$	9.5 ± 1.7 (7.3–13.2)	vs 4,000/d, $P < .02$ vs 50,000/wk, $P < .001$
4,000 IU/d (20/24)	43 ± 10 (26–59)	vs 50,000/wk, $P < .001$	12.2 ± 4.3 (7.3–23)	vs 50,000/wk, $P < .001$
50,000 IU/wk (13/14)	61 ± 14 (46–83)		16.8 ± 4.3 (11.6–25.4)	
Dose effect	$P < .001$		$P < .001$	

SD = standard deviation.
Statistical results are for effect of assignment (analysis of variance for dose effect with Bonferroni correction for midpoint testing).

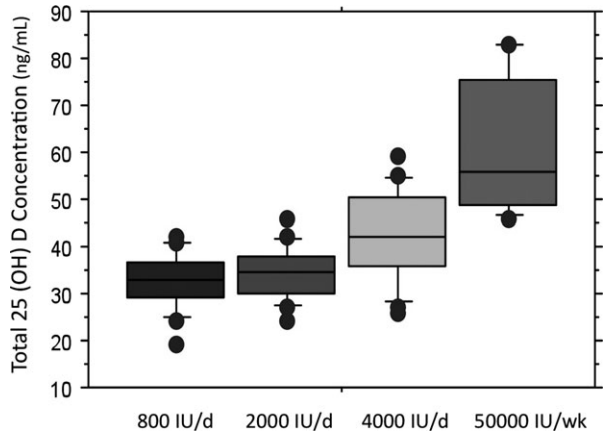

Figure 1. 25-hydroxyvitamin D (25(OH)D) concentrations according to dosing assignment (800, 2,000 IU/d, 4,000 IU/d, 50,000 IU/wk of oral vitamin D_3). The box plot shows the 10th, 25th, median, 75th, and 90th percentile values. Circles represent individual values above the 90th and below the 10th percentile.

in 25(OH)D were seen in participants with the lowest concentrations at study start; those with concentrations of 20 to 40 ng/mL at study start did not have changes in 25(OH)D with doses of 800 or 2,000 IU/d (Figures 2 and 3). Decreases in 25(OH)D concentrations were seen in participants with concentrations greater than 40 ng/mL at baseline randomized to 800 or 2,000 IU/d, which can be explained on the basis of the higher vitamin D dosage that they had received before the study (mean 2,267 IU/d, range 1,600–2,800 IU/d).

Sixteen participants received crushed vitamin D with food. Post hoc analysis found vitamin D concentrations in these subjects similar to concentrations of those receiving intact capsules (800 IU/d (n = 5), 30 ± 4 ng/mL vs 34 ± 7; 2,000 IU/d (n = 3), 31 ± 4 vs 35 ± 6, 4,000 IU/d (n = 6), 43 ± 12 vs 43 ± 9; 50,000 IU/wk (n = 2), 63 ± 10 vs 61 ± 16), although power was insufficient to eliminate a type II error ($P = .64$; power = 0.1). 25(OH)D2 concentrations were detected in 10 participants at baseline (15–50% of total 25(OH)D) and in four at study end (10%–25% of total). C3-epi-25(OH)D3 was detected in one participant at baseline (8% of total of 47 ng/mL) and in two other participants at study end (10% of 44 ng/mL total 25(OH)D, 15% of 59 ng/mL total 25(OH)D).

Free 25(OH)D Concentrations

Concentrations of free 25(OH)D correlated with total 25(OH)D (coefficient of determination (r^2) = 0.62, $y = 1.08 + 0.25 \times X$, $P < .001$) and were related to dose (Table 2). No differences were found between 800 and 2,000 IU/d, but the lowest free 25(OH)D concentrations were seen with 800 IU/d. The participant with total 25(OH)D of less than 20 ng/mL had a free 25(OH)D concentration of 6.3 pg/mL, and two other participants assigned to 800 IU/d had the only lower concentrations (5.5 and 5.7 pg/mL).

Relationships Between Calcium and iPTH and 25(OH)D

Calcium concentrations at study end did not differ between groups (9.4 ± 0.3 mg/dL for 800 IU/d, 9.3 ± 0.3 for 2,000 IU/d, 9.4 ± 0.4 for 4,000 IU/d, 9.5 ± 0.3 for 50,000 IU/wk; $P = .42$). Total and free 25(OH)D were positively correlated with calcium concentrations but explained a small amount of the variation ($r^2 = 0.08$, $y = 9.109 + 0.007 \times X$, $P = .02$; r^2=0.07, $y = 9.154 + 0.022 \times X$, $P = .02$, respectively). Study-end iPTH concentrations did not differ between dosing groups (56 ± 29 pg/mL for 800 IU/d, 43 ± 22 for 2,000 IU/d, 45 ± 32 for 4,000 IU/d, 48 ± 23 for 50,000 IU/wk; $P = .52$). Free, but not total, 25(OH)D was inversely related to iPTH ($r^2 = 0.08$; $y = 67.9–1.75 \times X$, $P = .02$; r^2= 0.03; $y = 61.8–0.34 \times X$; $P = .15$, respectively).

Adverse Effects

Hypercalcemia occurred in one subject assigned to 4,000 IU/d that resolved with discontinuation of supplemental calcium. One participant assigned to 50,000 IU/wk was withdrawn because of a renal stone on study Day 4; unblinding showed only placebo on days 1 to 4 (i.e., subject had not yet received the weekly dose of 50,000 IU). One participant assigned to 800 IU/d was withdrawn

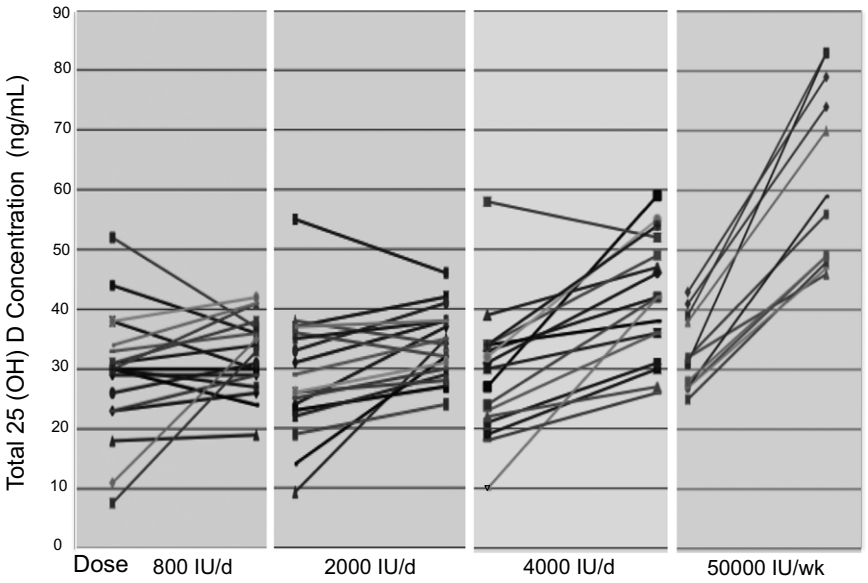

Figure 2. Individual 25-hydroxyvitamin D (25(OH)D) concentration data according to dosing group assignment at study entry and end. The main study outcome was concentration at study end, but individual dose-response data demonstrate greater changes for individuals with baseline 25(OH)D concentrations of less than 20 ng/mL, minimal changes in concentrations for those with baseline concentrations between 20 and 40 ng/mL assigned to 800 or 2,000 IU/d of vitamin D_3, and decreases in those with concentrations greater than 40 ng/mL at baseline and assigned to 800–4,000 IU/d. ($P < .001$ for effect of baseline concentration).

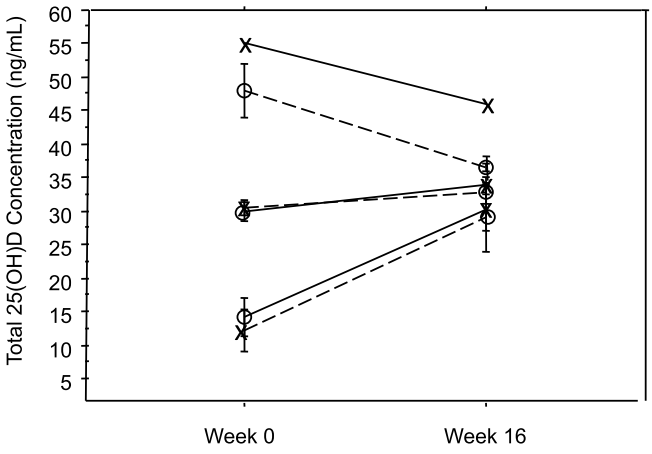

Figure 3. Responses of 25-hydroxyvitamin D (25(OH)D) to oral vitamin D_3 supplementation with 800 and 2,000 IU/d grouped according to study entry 25(OH)D concentration (<20, 20–39, ≥40 ng/mL); open circles and dashed lines represent data with 800 IU/d, and x and solid lines represent data with 2,000 IU/d. Data are mean ± standard error (except n = 1 for 2,000 IU/d with baseline 25(OH)D > 40 ng/mL). The greatest changes are seen in the group with baseline concentrations less than 20 ng/mL, with no change in those with baseline concentrations of 20 to 39 ng/mL ($P < .001$). Concentrations decreased with dosing of 800 or 2,000 IU/d in those with baseline concentrations of 40 ng/mL or greater.

because of a decrease in eGFR to the prespecified exclusion criteria of severe renal disease (<30 mL/min per 1.73 m^2), which was a minimal change from 30 to 28 mL/min per 1.73 m^2 at week 8. eGFR returned to baseline with study discontinuation and did not change with clinical rechallenge of 800 IU/d.

DISCUSSION

There are limited data on concentration responses to vitamin D in very elderly adults, yet there is consensus that they are highly likely to require vitamin D supplementation because of absent or limited ultraviolet B (UVB) exposure, poor conversion of precursor in the skin with UVB exposure, and low dietary intake of vitamin D.[27,32,33] Daily intake of 800 IU is the current recommendation of the Institute of Medicine (IOM) for people aged 70 and older based on estimates that this dose will produce or exceed a threshold of 25(OH)D of 20 ng/mL in 97.5% of people.[15] In this randomized, double-blind study of elderly nursing home residents, mean circulating 25(OH)D concentration after 16 weeks of 800 IU/d was 33 ng/mL. The data support the IOM premise that most older people will achieve concentrations greater than 20 ng/mL with 800 IU/d of a formulation with documented content based on studies of somewhat younger populations.[15] Lack of detection of differences between the group receiving 800 and 2,000 IU/d also supports the IOM recommendations, although one of 20 residents did not reach a threshold of 20 ng/mL with 800 IU/d, suggesting that some nursing home residents need higher doses to ensure 25(OH)D concentrations of 20 ng/mL or greater. With 2,000 IU/d, all participants had total 25(OH)D concentrations of 20 ng/mL or greater, and none had concentrations of 50 ng/mL or greater, a concentration at which hypercalciuria may occur. These results are similar to the those of the only prior study of U.S. nursing home residents receiving vita-

min D_3 (n = 15), which reported a mean concentration of 32 ng/mL after 6 weeks of 2,000 IU/d.[34] With 4,000 IU/d of vitamin D_3, mean 25(OH)D concentrations were 43 ng/mL or 20% higher than with 800 or 2,000 IU/d. Slightly more than half reached concentrations of 40 ng/mL or greater, and one-quarter had 25(OH)D concentrations of 50 ng/mL or greater. These 25(OH)D data with doses from 800 to 4,000 IU/d are similar to those reported in double-blind studies of postmenopausal white and African-American women randomized to 800 to 4,800 IU/d of vitamin D_3 for 1 year.[35,36] The data also demonstrate the complex relationship between baseline 25(OH)D status and responses to supplemental vitamin D. With doses of 800, 2,000, and 4,000 IU/d, increases in 25(OH)D were greatest in those with the lowest baseline concentrations. Conversely, less of a change to no change was seen in those with adequate or higher baseline concentrations. This supports the viewpoint that supplementation will have the greatest effect in people with the lowest vitamin D concentrations and that vitamin D concentration, and not just dosing, is an important consideration when evaluating responses clinically and for research.[37]

Concentrations with 50,000 IU/wk of vitamin D_3, or approximately 7,143 IU/d, were 42% higher than with 4,000 IU/d, whereas mean concentrations increased only 21% on average between the 2,000 and 4,000 IU/d doses. No plateau in concentrations of 25 (OH)D in response to increasing vitamin D_3 dose was seen. This finding differs from quadratic equation model–based predictions of a plateau in 25(OH)D at approximately 46 ng/mL with doses of 3,200 IU/d of vitamin D_3 or greater, although doses of 4,800 IU/d or greater were not part of the data for the model.[35] The lack of a plateau in 25(OH)D concentration in response to vitamin D_3 doses from 1,000 to 10,000 IU/d has been demonstrated in young men who achieved 25(OH)D concentrations of approximately 30, 60, and 84 ng/mL after oral doses of 1,000, 5,000, and 10,000 IU of vitamin D_3 per day for 20 weeks.[38] These data demonstrate similar concentration responses to supplemental oral vitamin D_3 across the adult age span and between races.

The results with dosing of 50,000 IU/wk were somewhat unexpected. Retrospective studies of responses to 50,000 IU/wk of vitamin D_2 for clinical vitamin D deficiency report mean 25(OH)D concentrations of approximately 33 ng/mL after at least 60 days[39] or 3 to 12 months.[40] In the current study, mean 25(OH)D concentrations were 61 ng/mL with concentrations of 40 ng/mL or greater in all receiving 50,000 IU/wk vitamin D_3 and 50 ng/mL or more in more than half. Potential explanations for the differences include greater adherence in this prospective study than in retrospective studies and that vitamin D_3 and vitamin D_2 differ in pharmacokinetics and pharmacodynamics.[41–45] Most, but not all, of the literature suggests that lower total 25(OH)D concentrations are reached with vitamin D_2 compared to vitamin D_3,[41,42,46,47] including a single-blind direct comparison with 50,000 IU/wk in healthy middle-aged people.[48] This may be clinically important because 50,000 IU vitamin D_3 formulations are commercially available, and results with 50,000 IU/wk of vitamin D_3 may differ from dosing with the prescription dose of 50,000 IU/wk of vitamin D_2.

A novel aspect of this study is the direct measurement of free 25(OH)D. The "free hormone" hypothesis postulates that protein-bound ligands cannot freely cross the cell membrane to interact with cytoplasmic or nuclear-binding proteins, whereas unbound "free" small lipophilic ligands can cross cell membranes and access cytoplasmic or nuclear bound proteins to exert effects. Circulating concentrations of 25(OH)D are 3 log scales higher than those of the active hormone: $1,25(OH)_2$ vitamin D. A number of tissues express CYP27B1 and so are able to convert the circulating 25(OH)D to the active $1,25(OH)_2$D within the target cell. Thus, circulating free 25(OH)D could represent the driving "free" hormone of the vitamin D system. Free 25(OH)D concentrations were positively correlated with vitamin D_3 dose and serum calcium and inversely correlated with iPTH. No normal range for free 25(OH)D has been established, but free 25(OH)D concentrations ranging from 1 to 8 pg/mL have been reported in healthy people and 3.5 to 15 pg/mL in individuals with cirrhosis.[30] In this study, the lowest free 25(OH)D concentrations were seen in the 800 IU/d group, giving further support to a potential need for doses greater than 800 IU/d in some elderly adults, although all free 25(OH)D concentrations were within the range seen in healthy people. Conversely, free 25(OH)D concentrations in some participants receiving 4,000 IU/d and in all receiving 50,000 IU/wk of vitamin D_3 exceeded concentrations seen in healthy subjects, stable individuals, and even individuals with cirrhosis. The implications are uncertain but warrant further investigation.

Potential Limitations

The 16-week duration was based on prior investigations showing a 3- to 4-month duration of vitamin D_3 dosing to reach steady-state in younger and middle-aged adults.[38,41,47,49] Small increases in 25(OH)D from 8 to 16 weeks were found with 4,000 IU/d and 50,000/wk in the 12 participants who were vitamin D supplement naïve at study entry ($P = .03$ with Bonferroni Dunn criteria for significance of $P = .02$; n = 5). There may have been a small underestimation of peak steady-state concentrations for these doses in vitamin D–naïve people. Sample sizes were small (~20 per group) but the same as or larger than in other vitamin D dose-ranging studies.[35,36,41,46,47] The current results were obtained using vitamin D_3 capsules that met U.S. Pharmacopeial Convention standards of having at least 100% of labeled content and within 100% to 140% of labeled content. Commercially available and compounded formulations can vary greatly in content, and results may not be the same with formulations that do not have the same content. Nursing home residents were studied, but because of lack of financial assistance for assisted living, board and care, or retirement facilities in California, many participants were of similar age and health status to older people with limited sunshine exposure living in the community in other states. Finally, concentration responses to vitamin D_3 doses have been reported to be the same in blacks as whites,[36] but only whites were studied, and potential racial differences cannot be addressed.

In summary, this study provides the first data from randomized, double-blind investigations of 25(OH)D responses to 800 to 4,000 IU/d and 50,000 IU/wk of vita-

min D_3 in very elderly adults without sunshine exposure. Important clinical implications of this study include the potential need for doses of greater than 800 IU/d of oral vitamin D_3 to ensure adequate vitamin D levels in some elderly adults and that more than 8 weeks of dosing is required to achieve steady-state conditions. The lack of changes in 25(OH)D with vitamin D_3 supplementation with 800 and 2,000 IU/d in people with normal basal concentrations calls into question the rationale for supplementation with these doses in such individuals; 25(OH)D concentrations with vitamin D_3 dosing from 800 IU/d to 50,000 IU/wk did not plateau, and 25(OH)D concentrations with 4,000 IU/d and 50,000 IU/wk were higher than expected and possibly in the hypercalciuric range. The 25(OH)D concentrations achieved with 50,000 IU/wk of vitamin D_3, especially, do not support the routine use of this dose of vitamin D_3 for supplementation and suggest that responses should be monitored to avoid potential adverse effects during its use. The data also suggest the potential of free 25(OH)D in further defining optimal 25(OH)D concentrations.

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Author Contributions: Schwartz: study concept and design, data collection and analysis, manuscript preparation. Kane: data collection, entry, and preliminary analysis; manuscript preparation. Bikle: study concept and design, data analysis, manuscript preparation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Study recruitment and completion schematic.

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