Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration[1–3]

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ABSTRACT

Background: Indirect evidence suggests that optimal vitamin D status is achieved with a serum 25-hydroxyvitamin D [25(OH)D] concentration >75 nmol/L.

Objective: We aimed to determine the intake of vitamin D_3 needed to raise serum 25(OH)D to >75 nmol/L.

Design: The design was a 6-mo, prospective, randomized, double-blinded, double-dummy, placebo-controlled study of vitamin D_3 supplementation. Serum 25(OH)D was measured by radioimmunoassay. Vitamin D_3 intake was adjusted every 2 mo by use of an algorithm based on serum 25(OH)D concentration.

Results: A total of 138 subjects entered the study. After 2 dose adjustments, almost all active subjects attained concentrations of 25(OH)D >75 nmol/L, and no subjects exceeded 220 nmol/L. The mean (±SD) slope at 9 wk [defined as 25(OH)D change/baseline dose] was 0.66 ± 0.35 (nmol/L)/(μg/d) and did not differ statistically between blacks and whites. The mean daily dose was 86 μg (3440 IU). The use of computer simulations to obtain the most participants within the range of 75–220 nmol/L predicted an optimal daily dose of 115 μg/d (4600 IU). No hypercalcemia or hypercalciuria was observed.

Conclusions: Determination of the intake required to attain serum 25(OH)D concentrations >75 nmol/L must consider the wide variability in the dose-response curve and basal 25(OH)D concentrations. Projection of the dose-response curves observed in this convenience sample onto the population of the third National Health and Nutrition Examination Survey suggests a dose of 95 μg/d (3800 IU) for those above a 25(OH)D threshold of 55 nmol/L and a dose of 125 μg/d (5000 IU) for those below that threshold. *Am J Clin Nutr* 2008;87:1952–8.

INTRODUCTION

Nutritional recommendations for vitamin D initially aimed to prevent overt deficiency states such as rickets and osteomalacia. In recent years, it has been appreciated that vitamin D insufficiency may lead to osteoporotic fractures, and the concept of optimal intake in the prevention of chronic disease was developed. More recently, it has been appreciated that vitamin D may have important extraskeletal roles in the prevention of cancer, autoimmune disease, diabetes, and other disorders (1).

The serum 25(OH)D concentration is accepted as the nutritional biomarker of vitamin D sufficiency. Optimal 25(OH)D concentrations for skeletal health are determined by relating serum concentrations to functional outcomes, such as the incidence of falls and fractures, and the loss of bone density. An optimal serum 25(OH)D concentration must be determined before making population recommendations for vitamin D intake or setting goals for serum 25(OH)D in individuals. Controversy exists, however, over what cutoff should be recommended, with European experts favoring 50 nmol/L and US experts favoring 75 nmol/L (2, 3). An evidence-based report by the Office of Dietary Supplements of the National Institutes of Health was carried out by the Agency for Healthcare Research and Quality (4). The report concluded that there is currently insufficient evidence to recommend a specific cutoff of serum 25(OH)D that indicates vitamin D sufficiency.

In addition to the lack of consensus as to the optimal level, there is no unanimity as to the dose that will bring an individual patient to a given level The literature characterizing the dose-response curve of 25(OH)D contains varied results. The heterogeneity in findings is due to the use of unassayed vitamin D supplements, the methodologic variability in serum 25(OH)D assay methods, and the variability in individual laboratory performance. Many studies are sex- or age-specific and are limited to a single group, whereas other studies confound age, sex, and race. Accordingly, there is an inconsistency in public policy statements between recommended serum 25(OH)D thresholds and the vitamin D intake necessary to achieve the recommended levels. For example, although the *Dietary Guidelines for Americans* and the Surgeon General's report on osteoporosis seem to endorse a serum concentration of 25(OH)D in excess of 80 nmol/L as desirable, those reports recommend daily intakes of only 800–1000 IU/d (5, 6). Whatever the cutoff for 25(OH)D selected, the dose-response curve of serum 25(OH)D to vitamin D intake is critical in choosing optimal vitamin D intake for individuals and for the general population.

African Americans are particularly susceptible to vitamin D insufficiency because the darker color of their skin limits the amount of ultraviolet light that penetrates, thereby reducing the cutaneous synthesis of vitamin D. In our recent longitudinal

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study of vitamin D supplementation in 208 African American women, 50 μg/d (2000 IU/d) failed to raise 25(OH)D to >75–80 nmol/L in 40% of the sample (7). Thus, there was concern that the responses to vitamin D intake may differ between races (7). We therefore undertook a dose-finding study in African American and white men and women with the objective of investigating an algorithm for raising 25(OH)D concentrations to between 80 and 140 nmol/L.

SUBJECTS AND METHODS

This study was a 6-mo, prospective, randomized, double-blind, placebo-controlled study of vitamin D_3 supplementation in healthy white and African American men and women aged 18–65 y. Publications subsequent to the initiation of this study suggest an optimal serum 25(OH)D range of 75–220 nmol/L (8–12). Subjects were recruited from areas on Long Island surrounding our institution in Mineola, NY, through flyers and direct mailing during the 3 winters (November through March) of 2004–2006. Before entry, all subjects signed a written informed consent, underwent a complete history and physical exam, and provided blood and urine specimens for testing. The project was approved by the Institutional Review Board of Winthrop-University Hospital. Because the goal of the study when first proposed was to attain 25(OH)D concentrations >80 nmol/L, any subject with a baseline 25(OH)D concentration >80 nmol/L was excluded. Furthermore, subjects with chronic medical conditions, bone disease, or those taking medications known to interfere with vitamin D metabolism were excluded.

Description of the dosing algorithm

The dose of vitamin D supplemented was based on the initial 25(OH)D concentration. The parameters of the algorithm were estimated from our prior longitudinal vitamin D studies. Those with a basal concentration between 50 and 80 nmol/L were started on 50 μg/d, whereas those with a basal concentration <50 nmol/L were started on 100 μg vitamin D_3/d. Subjects were followed at 8-wk intervals, and the dose was adjusted after each visit to achieve and maintain serum 25(OH)D concentrations >80 nmol/L (and <140 nmol/L) throughout the study. Doses were adjusted in 50- or 20-μg (200- or 800-IU) increments or decrements on the basis of assayed serum 25(OH)D. On subsequent visits, dose adjustments were done according to the following algorithm (with the constraint that the total dose not exceed 250 μg/d, or 10 000 IU): if 25(OH)D was <50 nmol/L, increase supplement by 50 μg/d (2000 IU); if between 50 and 80 nmol/L, increase by 50 μg/d (2000 IU); if between 80 and 140 nmol/L, do not change; if >140 nmol/L, decrease by 50 μg/d (2000 IU) [unless current dose was 50 μg/d (2000 IU) or less; in that case, decrease dose to 20 μg/d (800 IU)].

Dietary vitamin D and calcium were monitored by use of 3-d food diaries administered at baseline and at the end of the study. Travel history was recorded at each visit to evaluate whether sunlight exposure might have had a significant effect on serum 25(OH)D. Vitamin D_3 and matching placebo were custom manufactured (Tishcon Corp, Westbury, NY). The capsules contained either 50 μg (2000 IU) or 20 μg (800 IU) of vitamin D_3. The manufacturer's assay was verified by an independent laboratory (Vitamin D, Skin, and Bone Research Laboratory, Department of Medicine, Boston University School of Medicine, Boston, MA).

Randomization to treatment or placebo was done according to a computer-generated pseudo-random code by using the method of random permuted blocks. One-half of the subjects in each race were randomly assigned to active vitamin D_3 and the other one-half to matching placebo. Treatment assignments in labeled sealed envelopes were provided to the research pharmacist by the study statistician. Patients and investigators were blinded. Because doses for the active patients were titrated up or down according to the algorithm above, the blind was maintained by randomly adjusting the placebo dose to match the distribution of changes in the active patients who were at the same point in the study (a double-dummy design).

Serum 25(OH)D was measured by a radio-receptor assay purchased from DiaSorin Inc (Stillwater, MN). The intraassay CV was 4.1%, and the interassay CV was 7.0%. We also sent internal standards and samples to the Mayo Medical Laboratories (Rochester, MN) for measurement by the gold standard method of HPLC-tandem mass spectrometry (HPLC-TMS). The regression r value between the DiaSorin assay and HPLC-TMS was 0.98 in samples <100 nmol/L. The r value decreased to 0.92 when sample values were <200 nmol/L; the results of the DiaSorin assay were greater than that with the HPLC-TMS. Because 81% of our participants' serum 25(OH)D concentrations were <100 nmol/L, the agreement with the Mayo values is adequate in this study. Our laboratory had participated in (and is certified by) the Vitamin D External Quality Assessment Scheme (DEQAS) since November 2004 (13, 14). Total body fat and bone mineral density of the spine and femur were measured with a dual-energy X-ray absorptiometer (model QDR 4500; Hologic Inc version 9.80D, Hologic, Waltham, MA).

Statistical analysis

Multiple linear regression was used to model serum 25(OH)D response as a function of predictor variables including dose, baseline values, season, BMI, and percentage body fat. Slope at a specific time point was defined as the change in serum 25(OH)D from baseline divided by the dose assigned during the preceding visit. Prima facie evidence of noncompliance in active patients was a slope <0.1; those values were treated as missing. Differences between groups of patients were analyzed with the independent t test or Fisher's exact test for continuous or categorical variables, respectively. Changes in slope over time were analyzed with a repeated-measures mixed model analysis of variance. The structure of the covariance matrix assumed in the mixed model analysis was determined empirically by the Akaike's Information Criterion (15–17). Pearson's correlation was used to quantify the linear association between variables.

A two-tailed P value < 0.05 was deemed statistically significant. Results are expressed as means ± SDs and, where appropriate, their 95% CIs. Analyses were done both with all available data (intent-to-treat) and by using subjects with complete data. Data analysis used the statistical package SAS 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Baseline characteristics

There were 262 persons screened during the wintertime of 2004 to 2006. One hundred twenty-four persons were excluded from the study secondary to withdrawal of consent, higher than

TABLE 1
Baseline patient characteristics[1]

Baseline characteristics	Female blacks	Female whites	Male blacks	Male whites	P^2
Number (%)	50	62	12	14	NA
Age (y)	44.8 ± 12.2[3]	47.8 ± 9.4	48.0 ± 12.4	52.2 ± 9.6	NS
Menopausal [*n* (%)]	15 (30)	23 (37)	NA	NA	NS
BMI (kg/m^2)	26.8 ± 3.5	24.8 ± 4.2	27.8 ± 4.0	28.8 ± 3.3	S
Current smoker [*n* (%)]	4 (8)	7 (11)	1 (8)	1 (7)	NS
Dietary vitamin D intake (IU/d)	86.2 ± 117.0	88.4 ± 106.9	52.9 ± 41.2	54.6 ± 62.9	NS
GFR (mL · min^{-1} · 1.73^{-1} m^{-2})	105.7 ± 27.6	97.2 ± 26.8	102.2 ± 14.3	109.1 ± 25.0	NS
Serum calcium (mg/dL)	9.0 ± 0.36	8.9 ± 0.43	9.0 ± 0.41	9.2 ± 0.47	NS
Calcium intake (mg/d)	617 ± 247	755 ± 321	558 ± 290	730 ± 320	R
24-h Urinary calcium (mg/d)	99.3 ± 62.1	117.5 ± 111.4	165.2 ± 92.9	161.9 ± 89.7	NS
25(OH)D (nmol/L)	40.9 ± 14.4	57.3 ± 14.6	34.9 ± 16.4	59.9 ± 12.3	R
PTH (pg/mL)	45.8 ± 18.6	41.6 ± 17.7	49.3 ± 26.6	43.5 ± 8.2	NS
T-score, spine	0.60 ± 1.3	−0.16 ± 1.4	0.64 ± 1.1	−0.35 ± 1.2	R
T-score, total femur	0.48 ± 1.1	−0.29 ± 0.9	0.88 ± 0.9	−0.10 ± 1.0	R
Total body fat (%)	35.4 ± 5.5	32.5 ± 6.8	19.2 ± 4.8	25.7 ± 6.1	I, S

[1] NA, not applicable. GFR, glomerular filtration rate; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.
[2] Two-way ANOVA. S, male vs female, $P < 0.01$; R, black vs white, $P < 0.01$; I, = sex × race interaction, $P < 0.01$.
[3] $\bar{x}$ ± SD (all such values).

optimal 25(OH)D concentrations, morbid obesity, osteoporosis, history of nephrolithiasis or hypercalciuria, and other abnormal laboratory test results. Of the 138 patients enrolled, 62 (45%) were African Americans and 76 were white. Twenty-six (19%) men and 112 women participated. Those with chronic medical conditions, morbid obesity, or disorders of bone metabolism or who were taking medications known to interfere with bone or vitamin D metabolism were excluded. The sample's demographic characteristics are found in **Table 1**.

Thirty-six (58%) African Americans were given placebo and 26 were given the active medication. Of the 76 white participants enrolled in the study, 37 (49%) received placebo and 39 were given the active medication. Although mean parathyroid hormone concentrations were 4.5 pg/mL higher in African Americans than in whites, the difference was not significant. The most salient baseline differences between African Americans and whites were weight, serum 25(OH)D, dietary calcium, and the T-scores for bone mineral density of the spine and of the femur. These differences are detailed in Table 1. One hundred eleven (80%) of the patients completed the 6-mo study.

Compliance

Compliance at the 3 follow-up visits was estimated from a pill count of returned pills. Overall, pill consumption compliance for active patients had a mean value of 65% (20%). Active and placebo patients were almost identical with respect to their compliance. Compliance assessed by pill count in the active group was highly correlated with the slope of dose versus serum 25(OH)D.

Dose

The dosing algorithm began at baseline with an intake of 50 μg/d (2000 IU) for 27 active patients above the 25(OH)D threshold of 50 nmol/L and an intake of 100 μg (4000 IU) for the 38 patients below it. The intake was modified over the next 2 visits and the dose prescribed ranged between 20 μg (800 IU) and 170 μg/d (6800 IU) with a median of 95 μg/d (3800 IU). The distribution of all doses at all time points for both black and whites and the changes in dose over time are found in **Figure 1**.

Safety

The study protocol listed 3 safety criteria: the development of hypercalcemia (defined as serum calcium >10.6 mg/L), hypercalciuria (fasting urine calcium/creatinine ratio of >0.16 mg/mg), and an upper limit of serum 25(OH)D of 200 nmol/L. The distribution of 25(OH)D concentrations across all visits is found in **Figure 2**. On only one occasion did a patient's 25(OH)D concentration exceed 200 nmol/L.

No patient showed a serum calcium value >10.6 mg/dL, but 4 patients had hypercalciuria on several occasions. It is difficult to attribute this result to the vitamin D because there was no difference in the number of active and placebo patients evidencing hypercalciuria. In all cases (except one) when the test was repeated within 1 wk, it proved to be within the reference range. Serum creatinine remained in the reference range in both groups.

Efficacy

The goal of exceeding 75 nmol/L was achieved by virtually all patients in the active group at week 27 (final visit). However,

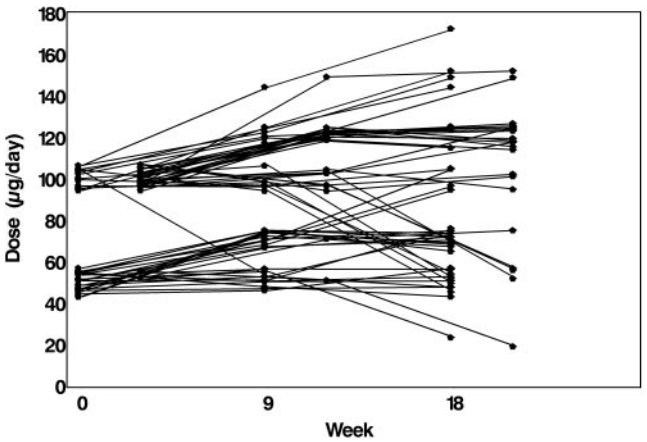

FIGURE 1. Distribution of active doses and changes over time, by race. For legibility, blacks are shifted to the right 3 wk and a small random between −7 and + 7 is added to each data point; $n = 65$ patients taking vitamin D.

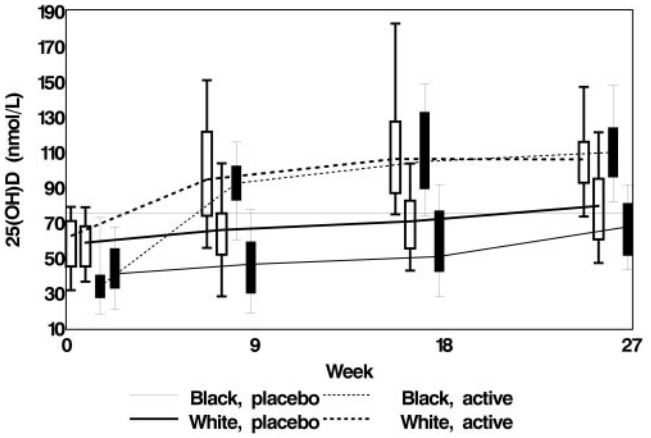

FIGURE 2. Serum 25-hydroxyvitamin D [25(OH)D] over time by race and randomization group ($n = 138$). In addition to the significance of the main effects (group, $P < 0.0001$; race, $P < 0.0001$; time, $P < 0.0001$), after baseline there was a significant group × time interaction ($P < 0.035$) due to the upward trend in vitamin D in the placebo group across the study but the leveling off after week 18 in the active group. The only other significant interaction, group × race ($P = 0.02$), was due to the rise in active blacks and whites to the same level (from different starting points), whereas within the placebo group the blacks and whites maintained their relative distance throughout the study. *P* values were generated by using a 3-way repeated-measures ANOVA model.

increasing sun exposure partly contributed to this result, because seasonal increases were also observed in the placebo group. Seventy-six percent of African Americans and 70% of whites in the active group exceeded 75 nmol/L at week 9; 90% of African Americans and 88% of whites exceeded 75 nmol/L at week 18.

Although both African Americans and whites achieved the goal of 75 nmol/L by week 18, the dose needed to accomplish that goal was 50% higher in the African American patients. Because of their lower baseline 25(OH)D concentrations, African Americans were more often assigned a higher dose than were whites. The mean daily dose over all 3 visits was 97.9 ± 21.0 μg (3916 ± 840 IU) for African Americans and 76.0 ± 28.4 μg (3040 ± 1136 IU) for whites. The mean daily dose for all patients in the active group across the study was 86 μg (3440 IU), and the mean daily dose assigned for the patients in the placebo group was 85 μg (3400 IU), which indicates the success of the double-dummy control.

Potential influences on 25(OH)D concentrations: season, race, sex, age, body composition, baseline 25(OH)D, dose-slope interaction

Placebo (and active) patients entered the study from November through March. They ended the study from May through October, on average 30 wk later. Among the patients in the placebo group, there was a significant change (19.5 ± 16.0 nmol/L) in 25(OH)D at the last visit compared with baseline. This was mainly attributed to the effect of sun exposure on 25(OH)D concentrations. Among the African American placebo patients, the change from baseline after 27 wk (an average of 212 ± 23 d, or ≈7 mo from the midwinter starting point) was 20.4 ± 12.9 nmol/L; among the whites it was 18.7 ± 18.4 nmol/L ($P = 0.69$).

There were no statistically significant sex or race differences in slope. We observed no difference in slopes between the 9 active males and 52 active females in this study at week 9. The female slope was 0.59 ± 0.40, and the male slope was 0.64 ± 0.35 ($P = 0.74$). In multiple regression analyses, age, body mass index, and percentage body fat did not significantly influence the response to vitamin D. The response of serum 25(OH)D to 1 μg of vitamin D_3, ie the slope, was inversely dependent on the basal 25(OH)D concentration. This was seen in the active cohort as a significant negative correlation between baseline and the change at 9 wk ($r = -0.46$, $P = 0.0002$).

Dose-response findings

The primary data for the analysis of slopes [defined as the change in 25(OH)D concentration divided by the dose] consists of the changes from baseline to week 9. Slopes were stable across study visits. Slope did not differ by race or dose. African Americans, most of whom (23/25) were taking 100 μg/d (4000 IU), had a slope of 0.59 ± 0.24. This was similar to that of whites (0.72 ± 0.41), who more often than not (18/31) were taking 50 μg/d (2000 IU). The slope for those whites taking 50 μg (2000 IU) was 0.81 ± 0.41; the slope for whites taking 100 μg (4000 IU) was 0.59 ± 0.39 ($P = 0.13$). There was a wide degree of intersubject variability in the slope measurement. Pooling active data from both sexes and races and baseline doses of 50 μg/d (2000 IU) and 100 μg/d (4000 IU) resulted in an overall slope of 0.66 ± 0.35. The individual slopes ranged from 0.15 to 1.49, virtually a 10-fold increase. Corresponding increases in serum 25(OH)D concentrations ranged from 7.5 to 141.8 nmol/L. During the first 9 wk, African Americans in the active group increased their serum 25(OH)D concentration by 55.7 ± 19.0 nmol/L while taking a mean dose of 96 μg/d (3840 IU). The corresponding serum increase for whites was 40.9 ± 33.7 nmol/L while taking a mean dose of 71 μg/d (2840 IU).

DISCUSSION

The dosing algorithm that we developed on the basis of findings from our previous study of African American women proved safe but suboptimal in raising 25(OH)D concentrations in this study of black and white men and women. A striking finding in this study was the high variability in the slopes for the dose-response curves. Similar variability was found recently by other investigators and appears to also occur in the serum 25(OH)D response to sunlight exposure (8, 18). When this variability is considered along with the wide range of baseline serum 25(OH)D values in the general population, it is clear why a single dose of vitamin D may not be satisfactory for achieving a range of 75–220 nmol/L for serum 25(OH)D in almost everyone.

Our approach to the data was to optimize the projected range of serum 25(OH)D, defined as 75–220 nmol/L. Because this was a convenience sample with over-representation of African Americans [who have lower 25(OH)D concentrations], we projected our observed slopes onto the population sample of the third National Health and Nutrition Examination Survey (NHANES III). The optimal algorithm was not very different from that developed from our sample: the threshold and lower dose remained the same at 55 nmol/L and 95 μg/d (3800 IU). However, the high dose for those below the threshold was 125 μg/d (5000 IU). This proposed algorithm would have only 5% out of the optimal range.

Selection of the upper limit of the reference range for serum 25(OH)D remains somewhat subjective. It has been reasoned that natural exposure to sunlight cannot be harmful (except for the skin), and the maximal value for serum 25(OH)D should be

those values resulting from natural sun exposure (8, 12, 19). This argument may be criticized in that it is based on a perceived design in nature and the assumption that man has reached a steady state and is now "perfected." On the basis of earlier studies with limited populations, Hollis (12) recommended 220 nmol/L as the maximum for the reference range (DiaSorin assay) on the basis of this maximal sun exposure principle. A more recent study of 93 subjects in Hawaii suggested a maximal value of 172 nmol/L (DiaSorin assay) (8). It should be appreciated that the maximum in a sample depends to some extent on the size of the sample, and these sample sizes were small. If the Binkley et al (8) study had observed 18 882 patients (the NHANES III population) instead of 93, "extreme value" mathematics predicts an observed maximum >200 nmol/L. Indeed, in the NHANES III sample, the maximum was 243.6 nmol/L. Thus, an upper limit for 25(OH)D of 220 nmol/L appears to be a reasonable goal for the present. Hypervitaminosis D (hypercalcemia and hyperphosphatemia) has been reported with serum 25(OH)D concentrations ranging from 700 to 1600 nmol/L.

Although dose was shown to be independent of sex, race, and body weight (at least in the nonobese), dose is dependent (in a nonlinear way) on the response to a microgram of vitamin D (ie, the slope). For those studies with a mean dose <35 μg/d, the slope was inversely proportional to the dose. For those studies done at doses >35 μg/d, the slope was constant. Both African Americans and whites in the present study (taking doses >50 μg/d) had slopes that were similar to the women taking 50 μg/d (2000 IU) in our previous study of African American women (7). But both of these slopes differed substantially and significantly from the mean slope in the African American women in that same study while taking 20 μg/d (800 IU). The slope in that previous study, while taking 20 μg/d (800 IU), was 1.1 at 3 mo. The slope for these same women while taking 50 μg/d (2000 IU) was 0.76 (nmol/L)/(μg/d). Similar findings of different slopes for doses above and below 35 μg/d were reported by Vieth et al (20), who found an increment of 1.15 nmol$\cdot L^{-1} \cdot \mu g^{-1}$ D_3 for a 25-μg/d (1000-IU) dose and an increment of 0.56 nmol $\cdot L^{-1} \cdot \mu g^{-1}$ for a 100-μg/d (4000-IU) dose. This relation between dose and slope also appeared in the current study. For doses >50 μg/d (2000 IU), the slope was statistically constant. In addition, the dose-response ratio of the 23 African Americans taking 100 μg (4000 IU) in the current study was comparable with that of the 59 African Americans taking 50 μg (2000 IU) in our previous study and is similar to the slope of 0.70 reported by Heaney et al (21). Our analysis of published dose-response curves (*see* Appendix A) demonstrates that nonlinearity is not a concern in implementing the algorithm when doses >35 μg (1400 IU) are used.

We carried out the study for 6 mo in part to observe the response to input from sunlight in our region. There was a significant seasonal increase in serum 25(OH)D in both black and white patients in the placebo group. The contribution of ultraviolet light exposure must be considered when planning optimal intake; the slopes in the studies discussed were determined in the winter to avoid the confounding effect of sunlight on our intake-response evaluation. Future research will allow us to accommodate a seasonal factor into a model projecting vitamin D concentrations throughout the year.

The variability in assays for serum 25(OH)D has obscured the interpretation of studies of vitamin D outcomes. Membership in DEQAS, an international quality-control program, and the availability of a standard serum from the National Institute of Standards and Technology should help to reduce the variability among laboratories (22, 23).

We found no evidence of toxicity in this 6-mo study, just as we found no evidence of toxicity in our previous 3-y study (7). The Food and Nutrition Board and the European Commission Scientific Committee on Food have selected 50 μg (2000 IU) as a safe tolerable upper intake level. The selection of the upper limit is now considered to have been based on insufficient evidence. A risk assessment, using the safe tolerable upper intake method, was recently published (10). No evidence of toxicity was found in doses up to 1250 μg/d (50000 IU) leading to selection of a NOAEL (no adverse event limit) of 250 μg/d, or 10000 IU (24, 25).

The baseline differences in 25(OH)D between African Americans and whites in this study are similar to findings reported by us and others in the past (26). There was no significant racial difference in response of serum 25(OH)D per 1 μg of vitamin D intake. In addition to sex and ethnic considerations, our study did not include children and obese or elderly individuals, so that further studies in these groups must be conducted.

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The contributions of the authors were as follows—JFA: designed and wrote the manuscript; SAT and MM: were responsible for medical supervision of the study participants; JFA: designed and supervised the study; MP, RD, and ML-N: were research fellows who were responsible for clinical care, data gathering, data presentation, and analysis and review of the manuscript; SP: the study statistician, was responsible for the data and statistical analyses and contributed to the writing of the manuscript; JKY: the laboratory director, was responsible for the biochemical assays. None of the authors had a personal or financial conflict of interest.

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APPENDIX A: OPTIMIZATION OF THE ALGORITHM

A. THE IMPACT OF DOSE ON THE DOSE RESPONSE CURVE

To further our understanding of the impact of dose on slope, we analyzed the dose-response relation found in 42 studies summarized in an article paper by Vieth et al (1) and another 24 found from a literature search post-1999 (2-14).

Analyses of the nonlinear relation between slope and vitamin D dose was estimated by the Joinpoint program, which provided a permutation test for deciding on the number of splines that best fit the data (15). A confirmation of the model fitting the data was obtained by a LOESS graphical analysis of the data. LOESS (16) is a technique for determining the shape of the function that best summarizes the scatter plot between 2 continuous variables. The LOESS procedure makes no assumptions about the parametric form of the regression surface. The smoothing parameter (the fraction of the data used around each point) was optimally chosen on the basis of objective criteria described by Hurvich and Simonoff (17). The Joinpoint estimate of the regression line reveals a plateau beginning at 35 μg (95% CI: 22, 45) (*see* **Figure A1**)

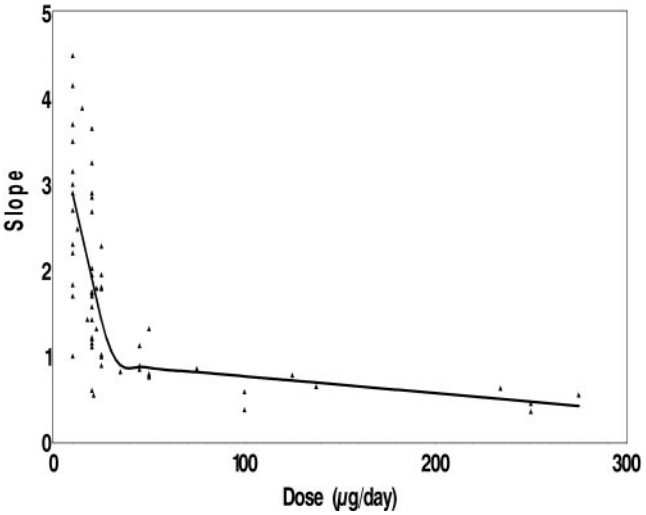

FIGURE A1. Joinpoint (spline) analysis of the vitamin D response (nmol/L)/dose (μg/d) relation as a function of dose. Data were taken from the literature (n = 65 studies); because of data compression, only results for the 62 studies with doses <300 μg/d are displayed. The Joinpoint permutation test determined (P = 0.0002) that the data are best fit by a single joinpoint (or knot in spline terminology) at 35 μg joining 2 lines (95% CI: 22, 45). The rate of decline per μg for doses <35 μg was −0.085 (P = 0.0001); for doses >35 μg, the rate of decline was −0.0003 (not significantly different from 0, P = 0.51). The results were almost identical if the 3 studies with doses >300 were included or excluded. Those 3 studies had doses (slopes) of 500 (0.27), 1000 (0.25), and 1269 (0.51).

(2, 11). Thus, the Joinpoint analysis confirmed the visual impression that the graph of slope regressed on dose is flat after 35 μg. That is, the serum 25(OH)D response to an intake >35 μg (1400 IU) is proportional to the dose. This independence of slope and dose makes the calculation of serum 25(OH)D concentration for various doses (>35 μg, or 1400 IU) feasible. When projecting response to other doses a patient could hypothetically take (from knowledge of an assumed distribution of slopes), there is no need to modify or adjust the distribution of slope as a function of dose.

B. ALGORITHM REFINEMENT AND SINGLE DOSE PROJECTION BASED ON A CONVENIENCE SAMPLE

The slopes and baseline values obtained in this study allow us to test the hypothesis that a single dose can be prescribed to all patients with satisfactory results. Multiplying a supposed dose by the observed slope of an individual study subject and adding that product to the baseline vitamin D for that patient results in a distribution of projected serum 25(OH)D concentrations for that dose when repeated across subjects. By varying the dose from, say, 35 to 200 μg (1400 to 8000 IU), we projected the serum 25(OH)D response to various single doses of vitamin D. After suitable optimization criteria have been defined, a dose can be selected that is optimal for the entire sample. We used here the criterion of maximizing the number of patients projected to be within the 75–220 nmol/L range. This computer-intensive program was carried out and the single dose of 115 μg/d (4600 IU) produced the lowest number (13%) of patients outside the range; in contrast, a dose of 50 μg/d (2000 IU) left 39% outside the range.

An algorithm with 2 doses, 95 μg/d (3800 IU) for those above a threshold of 55 nmol/L and 130 μg/d (5200 IU) for those below the threshold of 55 nmol/L only marginally improved the success

rate, with 10% of the values falling outside the range. In contrast, the suboptimal algorithm for this study using lower doses [a threshold of 50 nmol and lower and higher doses of 50 and 100 μg/d (2000 IU and 4000 IU)] produced 26.2% below 75 nmol/L (and none higher than 220 nmol/L).

Using multiple threshold values resulted in a small decrease (from 10% to 9.2%) in the percentage of patients who fell outside the optimal range. Although the extra expense and inconvenience of using thresholds is not necessarily justified by the small decrease in the number of patients outside the desired range, it has the advantage that the mean dose of vitamin D is minimized. In our projections, the mean dose using the best combination resulted in a mean dose of 107 μg/d (4280 IU), compared with the mean dose of 120 μg/d (4800 IU) when one dose is prescribed for all.

C. ALGORITHM REFINEMENT BASED ON POPULATION PROJECTIONS ESTIMATE

This study was conducted by using a convenience sample. Our sample consisted of more subjects with baseline serum 25(OH)D <75 nmol/L than is expected from the national 25(OH)D distribution obtained from NHANES III. This is to be expected because the study excluded all patients with concentrations >80 nmol/L and we recruited a greater proportion of blacks than their representation in the general population [blacks have baseline concentrations of 25(OH)D below average]. We computed that if the one baseline measurement algorithm is projected onto the NHANES population, the mean dose (including the 44% at zero dose) would be 65 μg/d (2600 IU) and 5% would be out of range. Interestingly, although the optimum threshold and lower dose remains 55 nmol/L and 95 μg/d (3800 IU), respectively, in the NHANES III projection, the optimal high dose for those below the threshold is 125 μg/d (5000 IU). The percentage out of range and the mean dose are almost identical if the upper dose is 125 or 135 μg/d. By contrast, the mean dose over the first 9 wk was 79.2 ± 24.8 μg/d (3168 ± 992 IU). Whereas the study patients actually achieved a 25(OH)D concentration of 95.3 ± 26.5 nmol/L at 9 wk, with the higher doses that we are now suggesting are optimum, the median serum 25(OH)D concentration attained is projected to be 111 nmol/L in the study patients and 105 nmol/L in the NHANES III population.

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