Vitamin D Supplement Doses and Serum 25-Hydroxyvitamin D in the Range Associated with Cancer Prevention

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Abstract. *Background: Studies indicate that intake of vitamin D in the range from 1,100 to 4,000 IU/d and a serum 25-hydroxyvitamin D concentration [25(OH)D] from 60-80 ng/ml may be needed to reduce cancer risk. Few community-based studies allow estimation of the dose–response relationship between oral intake of vitamin D and corresponding serum 25(OH)D in the range above 1,000 IU/d. Materials and Methods: A descriptive study of serum 25(OH)D concentration and self-reported vitamin D intake in a community-based cohort (n=3,667, mean age 51.3±13.4 y). Results: Serum 25(OH)D rose as a function of self-reported vitamin D supplement ingestion in a curvilinear fashion, with no intakes of 10,000 IU/d or lower producing 25(OH)D values above the lower-bound of the zone of potential toxicity (200 ng/ml). Unsupplemented all-source input was estimated at 3,300 IU/d. The supplemental dose ensuring that 97.5% of this population achieved a serum 25(OH)D of at least 40 ng/ml was 9,600 IU/d. Conclusion: Universal intake of up to 40,000 IU vitamin D per day is unlikely to result in vitamin D toxicity.*

The recent increase in interest in vitamin D by the general public has fueled a better than 200% increase in sales of over-the-counter vitamin D preparations from 2008 to 2009, and a more than 6-fold increase since 2001 (1). Additionally, products with progressively increasing content of vitamin D have been introduced with similar rapidity. There seems to have been little precedent for a change of this magnitude and duration for other nutrients (*e.g.*, vitamins C and E) that have enjoyed brief periods of popularity among the general public. There is essentially no information on how the public uses these products or on their impact on the vitamin D status of consumers.

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GrassrootsHealth (GRH), a non-profit community service organization dedicated to promoting public awareness about vitamin D, has assembled a database that includes information on supplemental vitamin D intake by a self-selected population cohort, and links these intakes to measured values for serum 25(OH)D, various demographic variables, and a variety of health status measures. GRH data include values from many individuals with daily supplemental intakes in and above the ranges often used today for cancer prevention and co-therapy (2, 3).

This study used the GRH database to describe the relationship of measured vitamin D status to vitamin D supplementation, both as practiced by health conscious individuals and as related to cancer prevention.

Materials and Methods

Participants. The initial participants in the study were individuals who responded to an invitation issued to all attendees at a Vitamin D Seminar hosted by GRH in December, 2008, supplemented by extensive recruitment from internet invitations since then. There were no exclusion criteria, and participants included both genders and a wide range of ages, nationalities and levels of health status. Participation included receiving a test of serum 25(OH)D concentration and an on-line health questionnaire to be completed each six months for a suggested period of five years. The purpose of the latter was to enable determination of what health outcomes are associated with various serum 25(OH)D concentrations. GRH provided the participants with a blood spot 25(OH)D test kit manufactured by ZRT Laboratory (Beaverton, OR, USA). After each test, the participants received an email message from GRH indicating that their test scores were available. If desired, they then logged into their account to view the results. Included in the test results were the normal reference ranges, information about potential toxicity levels, and suggested serum 25(OH)D concentrations (40-60 ng/ml). Participants chose for themselves what actions to take. The project costs were funded entirely by participant fees. This project, analyzing anonymized GRH data, was reviewed by the Creighton University Institutional Review Board and declared 'exempt'.

Analytical methods. Serum 25(OH)D concentrations were determined by the ZRT blood spot test kit. The analytical method used was high-performance liquid chromatography followed by

Table I. *Demographic variables†.*

	All	Non-Hispanic White	Black	Other
n	3667	3403	33	230
Age (yr)	51.3 (13.4)	51.7 (13.3)	45.8 (11.8)	46.6 (13.9)
Wt (kg)	73.8 (17.1)	74.0 (17.0)	79.4 (18.8)	69.9 (18.2)
Ht (m)	1.70 (0.10)	1.71 (0.10)	1.73 (0.09)	1.68 (0.11)
Latitude of residence (°)	40.2 (6.7)	40.2 (6.7)	40.6 (5.1)	39.3 (7.6)
Gender (M/F)	1436/2231	1312/2091	16/17	107/123

†Except for gender, values given as mean (standard deviation).

mass spectroscopy and has been validated against the DiaSorin RIA method with an r^2 value of 0.91 and with a slope not different from 1.0 (4). Participants obtained their own blood spots, dried them, and returned them to GRH in supplied mailers. The dried blood spots have been shown to be stable at room temperature with regard to serum 25(OH)D concentration for at least four months.

Statistical analysis. The accumulated data were stored in a MySQL database (Ver. 5.0.77 Oracle USA, Redwood City, CA, USA), operating behind a firewall, and password protected. Data extracts were exported to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Analysis was by the various statistical routines of Excel and SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA). The relationship of oral vitamin D supplement intake to serum 25(OH)D concentration was fitted to the following equation, using the curve-fitting routine of SigmaPlot.

$$Y = Y_0 + a(1 - e^{-bX}) + cX , \qquad \text{Eq. 1}$$

where Y=serum 25(OH)D, X=vitamin D dose (in 1,000s IU/d). As the equation shows, it contains three terms: (i) the zero dose value of 25(OH)D (Y_0); (ii) an expression describing the saturable exponential component relating to hepatic 25-hydroxylation; and (iii) a linear term relating to zero-order kinetics for 25-hydroxylase (5). Specifically: a=the 25(OH)D increment at maximum saturation of the hepatic 25-hydroxylase, b=the rate constant of the process, and c=the coefficient of the linear rise in serum 25(OH)D. In addition to other statistics, the curve-fitting routine provides the standard error of the estimate (SEE) around the fitted mean. The 95% probability range for the 25(OH)D concentration values is thus ±1.96 SEE.

Results

Table I sets forth the pertinent demographic information with respect to the participant cohort, and Figure 1 presents a frequency distribution of self-reported daily vitamin D intakes. A large majority of the participants were non-Hispanic whites (N-H Whites), ingesting 5,000 IU/d or lower. Approximately one-fourth of the cohort reported no supplemental vitamin D intake; another 47% reported intakes up to 2,000 IU/d; and 1.8% reported intakes above 10,000 IU/d (n=60). There is an evident skewing of the intake distribution to the right. The relationship between reported vitamin D intake and measured serum 25(OH)D concentration is plotted in Figure 2, which

includes also the best fit line for the data using Equation 1. Figure 2 demonstrates several points: (i) the tendency for serum 25(OH)D to rise with increasing dosage is much more gradual than might have been anticipated from extrapolation of the relationship at more usual, lower intakes; (ii) there is a very large spread of values around the regression line, consistent with what most other studies have found (*e.g.*, 6); and (iii) despite there being in some individuals clearly supraphysiological inputs, very few individuals had serum 25(OH)D values above the 200 ng/ml lower boundary for potential toxicity described by Hathcock *et al.* (7) and Vieth (8).

The value of the Y_0 parameter (32.9 ng/ml ±0.483 SEM, Figure 2) is the zero supplement value for this cohort, reflecting vitamin D inputs solely from cutaneous solar UVB photosynthesis and food. In brief, the X-axis zero value does not reflect actual zero input, just zero *supplemental* input. Using Equation 1 and extrapolating the curve to the left produces a true zero 25(OH)D value at approximately –3,300 IU/d. In other words 3,300 IU/d is the approximate magnitude of the rightward translation exhibited by the X-axis and, correspondingly, that value approximates the mean non-supplemental vitamin D input for this participant cohort.

The fitting routine was applied, not only to the whole data set, as in Figure 2, but to various subsets, based on gender and ethnicity. Men and women exhibited nearly identical fits at intakes below 10,000 IU/d, but the rise at intakes above 10,000 was nearly flat for men. However, there were relatively few instances of such intakes in the 1,436 men in this sample; hence this issue remains uncertain. There were too few data for those in the 'Black' category (n=33) to permit curve-fitting, but by direct calculation, their zero supplement serum 25(OH)D concentration was 18.0 (±9.5) ng/ml, significantly lower than for N-H Whites, for whom Y_0=33.4 (±26.4) ng/ml ($p<0.001$). The 'Other' ethnicity category (largely Eastern Asiatics; n=230) had sufficient data to permit fitting to Equation 1. Its parameters did not differ appreciably from those of the N-H White group, except for the Y_0 estimate, which was 26.6 (±23.7) ng/ml, also significantly lower than the Y_0 parameter estimate for N-H Whites ($p<0.001$). These differences are consistent with expectations based on skin pigmentation.
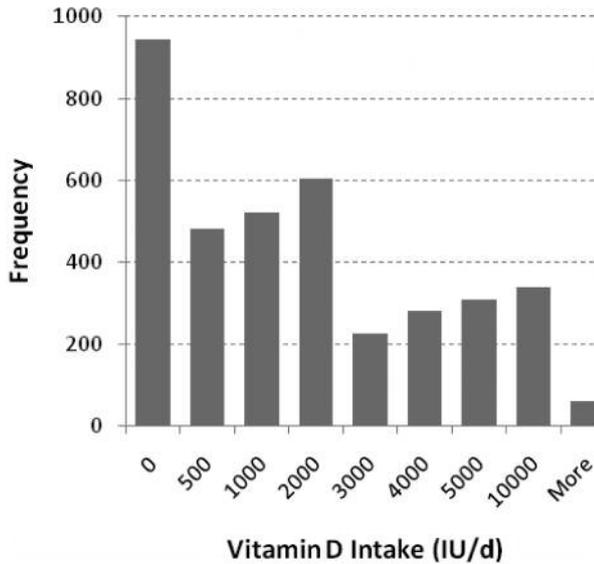
Figure 1. *Frequency distribution of individual vitamin D daily doses among 3,667 participants. Each bar shows the number of participants ingesting a dose up to the specified magnitude and greater than the next lower daily input. (Copyright Robert P. Heaney, 2011. Used with permission)*
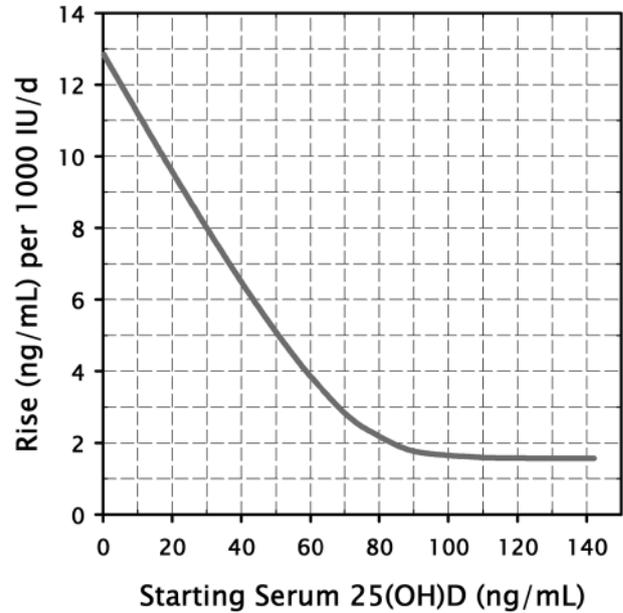

Figure 3. *Plot of the expected rise in serum 25(OH)D for every 1,000 IU vitamin D3 supplemented daily, as a function of basal 25(OH)D value. (Copyright Robert P. Heaney, 2011. Used with permission.)*

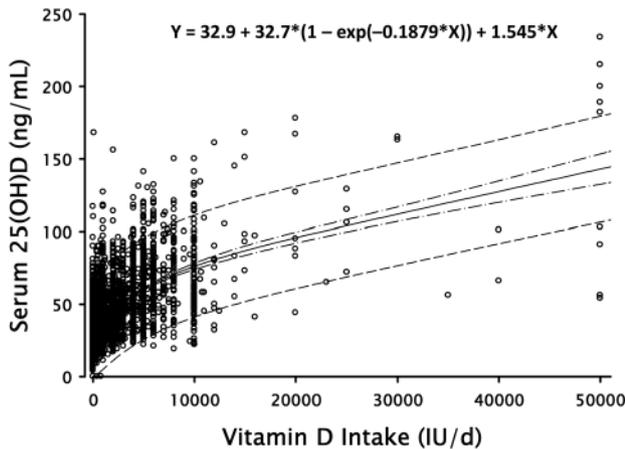$$Y = 32.9 + 32.7*(1 - exp(-0.1879*X)) + 1.545*X$$

Figure 2. *Serum 25(OH)D as a function of daily vitamin D intake, together with the best fit line to Eq. 1, its confidence limits, and the 95% probability band (outer dashed lines) for the entire cohort. For convenience in reading, the exponential constant and the coefficient of the linear term in the equation are in units of 1,000 IU/d. (Copyright Robert P. Heaney, 2011. Used with permission)*
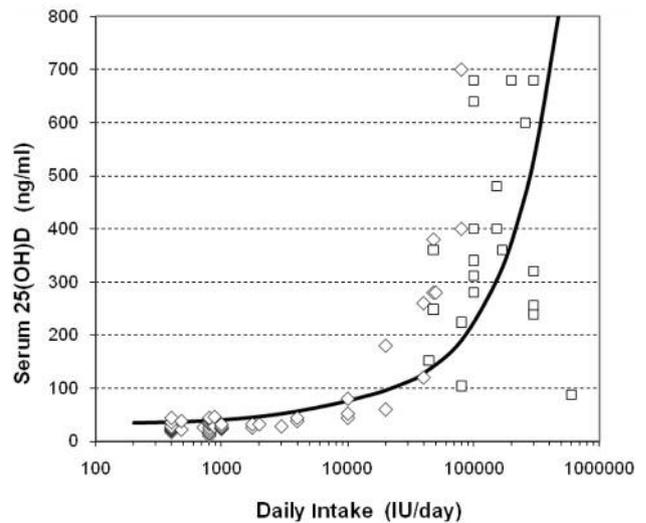

Figure 4. *Plot of the regression line from Figure 2 on previously published high-dose data (8). Note that the horizontal axis is logarithmic. The diamond-shaped symbols are the means of controlled dosing studies (n=48) and the square symbols, individual values from reported cases (n=21) of vitamin D intoxication. (Copyright Robert P. Heaney, 2011. Used with permission)*

Because obesity is recognized to be associated with low vitamin D status, the residuals from the above curve fit were regressed against body weight and demonstrated the expected inverse relationship (r^2=0.03). While statistically significant ($p<0.01$), this relation failed to account for most of the between-participant variance.

A question frequently asked by clinicians is how much of an increase in serum 25(OH)D should be expected for a given additional oral dose of vitamin D. Figure 3 provides an answer by plotting the first derivative of the equation in

Figure 2 at various starting values. For example, at a starting value of 10 ng/ml, the mean increment that would be expected to be produced by an additional 1,000 IU/d is 11 ng/ml, whereas at 30 ng/ml it is 8 ng/ml, and at 50 ng/ml, only 5 ng/ml. Above a starting value of 90 ng/ml, the response is nearly flat at about 1.6 ng/ml/1,000 IU/d.

Because no serum 25(OH)D method has been specifically calibrated against standards above 100 ng/ml, the accuracy of the 25(OH)D values was assessed by superimposing the regression line from Figure 2 on previously published data (8) relating high-dose vitamin D intake to serum 25(OH)D. The results are shown as Figure 4. It is immediately apparent that the regression line from the blood spot method used in the present study superimposes on the data points previously published by Vieth (8), at least out to 100,000 IU/d, which is as far as the GRH data extend.

Discussion

To the Authors' knowledge this is the first analysis of the relation of vitamin D status to voluntary vitamin D supplementation as practiced in the community. The community base, the size of the sample, and the completeness of the pertinent data are strengths. Weaknesses include the fact that no single vitamin D product was used, the products themselves were not evaluated for exact vitamin D content and the doses are self-reported. Additionally, the data are cross-sectional and cannot give a true picture of individual responses to dose changes. Nevertheless, several features of the current findings indicate that these limitations do not preclude drawing useful conclusions from these data.

As noted above, the fit derived from the total data set superimposes on the high-dose data assembled by Vieth (8). Additionally, the general shape of the curve (exponential at low intakes and linear at high) is precisely mirrored in an earlier publication by Heaney *et al.* (5), relating the serum concentrations of 25(OH)D and cholecalciferol, in which the inflection point between the linear and exponential components occurred at a serum 25(OH)D concentration of ~35 ng/ml, corresponding to a serum vitamin D concentration of ~4 ng/ml. These values are very similar to the pertinent parameters of the equation used here to describe the GRH data set. Both of these agreements among studies support the overall validity of the data in the present report.

The 95% probability bands in Figure 2 provide useful information on the dosages required to ensure that a specified fraction of the population would have 25(OH)D concentrations above any given level. Such inferences are not affected by the cross-sectional character of the data. The points where that lower band crosses 30, 40, and 50 ng/ml (75, 100, and 125 nmol/l) are the supplemental intake values that ensure that 97.5% of the population would be above the

specified serum 25(OH)D concentrations concerned. These are, respectively, 6,100, 9,600, and 14,100 IU/d for this population. Observed mean (SD) 25(OH)D concentrations at these intakes are, respectively 64.6 (±18), 75.1 (±18), and 85.2 (±18) ng/ml. Given that the average, non-supplemental intake in this cohort was estimated to be ~3,300 IU/d, the total intake from a vitamin D-deprived basal state which would be required to ensure that all but 2.5% of the population would reach the specified serum 25(OH)D levels would be 9,400, 12,900, and 17,400 IU/d. Although an order of magnitude higher than currently recommended oral intakes (9), these calculated daily intakes are of the same magnitude as produced by a single, minimal erythemal dose of UV-B radiation, such as would be obtained during a few minutes of solar UVB exposure near noon in midsummer, assuming nearly complete skin exposure (10).

Although this data set provides no information with respect to serum or urine calcium values in these individuals, at the same time it is clear that there were no clinical evidences of toxicity. Indeed, since virtually all of the values, at whatever dose, were associated with 25(OH)D values below 200 ng/ml [and no doses below 50,000 IU/d produced serum 25(OH) D values above 200 ng/ml], the absence of apparent toxicity is not surprising. The very slow rise in serum 25(OH)D concentration for each 1,000 IU increment at serum values above 80-100 ng/ml (Figure 3) is firm expression of the general safety of even relatively high doses.

A prominent feature of this cohort is that it is self-selected for health consciousness. Hence, in terms of dosing choices, this cohort cannot be considered representative of the general population. Accordingly, given this cohort's likely high degree of adherence to supplementation, the achieved vitamin D status values are almost certainly higher than would be expected in less-motivated members of the general public.

Finally, and as an incidental observation, these data suggest a possible insight into the pathogenesis of toxicity. It is suggested that such an outcome requires two conditions: (i) high dose and (ii) high individual responsiveness to any given dose. As Ilahi *et al.* (6) reported previously, values for C_{max} following a single dose of 100,000 IU spanned a six-fold range from 4.9 ng/ml to 30.8 ng/ml. Had that dosing been continued (as in the high-dose members of the GRH cohort), a person with a 30.8 ng/ml increase would likely have exceeded a serum 25(OH)D concentration of 200 ng/ml, whereas a person at the low end of the range for C_{max}, would not have.

Acknowledgements

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