A Systematic Review: Influence of Vitamin D Supplementation on Serum 25-Hydroxyvitamin D Concentration

Philippe Autier, Sara Gandini, and Patrick Mullie
International Prevention Research Institute (P.A., P.M.), 69006 Lyon, France; and European Institute of Oncology (S.G.), 20139 Milano, Italy

Context: Few studies in subjects over 50 yr of age have evaluated the influence of variable doses of vitamin D supplementation on serum 25-hydroxyvitamin D levels.

Objective: We performed a meta-analysis of changes in circulating 25-hydroxyvitamin D level associated with vitamin D supplementation in Caucasian subjects over 50 yr old.

Data Sources: We conducted a systematic search in literature databases and in references of past reviews.

Study Selection: Randomized placebo or open-label trials that evaluated the influence of vitamin D supplementation on clinical outcomes were included in the study.

Data Extraction: We reviewed trial characteristics and serum 25-hydroxyvitamin D concentrations at baseline and during the trial.

Data Synthesis: Seventy-six trials published from 1984 to March 2011 included 6207 subjects allocated to 101 intervention groups that tested supplement doses ranging from 5 to 250 μg/d (median, 20 μg/d). For similar doses, trials could obtain increases in 25-hydroxyvitamin D three to four times lower than other trials. A meta-regression showed that in the absence of concomitant use of calcium supplements, the average increase in serum 25-hydroxyvitamin D concentrations was 0.78 ng/ml (1.95 nmol/liter) per microgram of vitamin D3 supplement per day. Compared to the vitamin D3, the vitamin D2 was associated with significantly lower increases (P = 0.03). Concomitant use of calcium supplementation and high 25-hydroxyvitamin D concentration at baseline was nonsignificantly associated with lower increases in 25-hydroxyvitamin D concentrations.

Conclusions: Dietary recommendations and randomized trials on vitamin D supplementation should evaluate whether increases in circulating 25-hydroxyvitamin D levels match expectations—for instance, the average increases obtained by trials on vitamin D3 without concomitant calcium supplements. (J Clin Endocrinol Metab 97: 2606–2613, 2012)

The serum level of 25-hydroxyvitamin D is a result of skin exposure to sunlight and of total dietary and supplemental vitamin D intake. Other factors such as age, skin pigmentation, smoking, and adiposity are well-known determinants of serum 25-hydroxyvitamin D level (1). This level is often considered as reflecting long-term individual vitamin D status. The use of vitamin D supplements increases serum 25-hydroxyvitamin D levels, and their use is mainly prevalent in subjects over 50 yr of age for improving bone health and preventing osteoporosis and falls (1, 2). Some observational studies suggest extraskeletal benefits of increasing the vitamin D status (1, 3), but their results may reflect the influence of confounding factors and not true cause-effect relationships (4, 5).
Although many trials have evaluated the influence of vitamin D supplementation on clinical outcomes and that use of vitamin D supplements is more widespread at older ages, few attempts have been made for evaluating the influence of variable doses of vitamin D supplementation on serum 25-hydroxyvitamin D levels in these older age groups (6, 7). This question is, however, crucial for setting dietary recommendations as well as for determining up to which point randomized trials testing the ability of vitamin D to prevent chronic diseases occurring for the most part after 50 yr of age actually improved the vitamin D status of subjects allocated to intervention groups. In particular, the Women’s Health Initiative (WHI) trial was criticized for the vitamin D3 dose and for the low adherence of women to supplementation (8, 9). The WHI trial was a 7-yr randomized trial in which 18,176 mainly white postmenopausal women 50–79 yr of age were assigned to daily oral intake of 10 μg of vitamin D3 and 1 g of elemental calcium, whereas placebos were given to a control group of 18,106 women of the same age. The trial showed that low doses of vitamin D and calcium supplementation had no influence on the risk of fracture, colorectal and breast cancer, cardiovascular events, coronary artery calcification, circulating lipids, and blood pressure (10–16). Because of its experimental design, the results of the WHI trial are not likely to be due to confounding, and they point to an absence of cause-effect relationship or simply to a totally insignificant dose of vitamin D. Women enrolled in the WHI trial had a blood draw at randomization, and the blood was stored for nested case-control studies built within the trial. The published report on colorectal cancer stated that “two years after randomization, a comparison of serum 25-hydroxyvitamin D levels in 227 women in the group given calcium with vitamin D and 221 women in the placebo group revealed that the levels were 28% higher in the supplement group” (12).

For addressing the dose-response issue and for ascertaining the clinical significance of changes in 25-hydroxyvitamin D level obtained in the WHI trial, we made a systematic review of changes in circulating 25-hydroxyvitamin D level reported by randomized trials that tested supplementation with vitamin D in mainly Caucasian subjects over 50 yr old.

Materials and Methods

A systematic literature search and quantitative analysis were conducted based on a protocol developed for this study following the QUOROM statement (17).

Data search and selection criteria

We performed a systematic search of published randomized trials on vitamin D supplementation in subjects over 50 yr old, whatever the endpoint of interest. The intervention was oral or i.m. supplements of vitamin D2 (ergocalciferol) or D3 (cholecalciferol), with or without concomitant calcium supplement.

We searched the published literature from 1980 to March 2011 in several databases including the ISI Web of Science, Science Citation Index Expanded (SCI Expanded), and PubMed. We first made searches without language restriction using various combinations of keywords: “vitamin D,” “cholecalciferol,” “ergocalciferol,” and limitation criteria “age 45+” and “clinical trials.”

We made a second search in the reference lists of five recent systematic reviews of randomized trials with oral intake or i.m. injection of vitamin D supplements in Caucasian 50-yr-old subjects. These five systematic reviews were: 1) the International Agency for Research on Cancer report on vitamin D and cancer (4) that searched randomized trials on vitamin D supplements for a multiplicity of outcomes, including nonskeletal or postural endpoints; 2) the Cochrane review on prevention of postmenopausal fractures (18); 3) a review on the prevention of fracture and falls commissioned by the U.S. Institute of Medicine (19); 4) a meta-analysis on vitamin D supplementation for fall prevention (20); and 5) a review of vitamin D and calcium supplements on health outcomes commissioned by the U.S. Institute of Medicine (21).

We managed to have copies of original publications and we selected trials on the basis of predefined criteria:

- Trials had to have a control group, i.e., a group of subjects receiving a placebo instead of the vitamin D supplement, or subjects not receiving vitamin D supplement (i.e., open-label trials).
- To evaluate the effect of intervention on serum 25-hydroxyvitamin D levels, trials had to report serum levels at some point during the trial for the control and the intervention group(s) separately.
- Trials had to be independent; when the results of the same trial were published in more than one article, we used the reporting results on the largest sample of individuals, or the most recently published, or the more detailed results.

We excluded:

- Trials involving mainly non-Caucasian subjects;
- Trials not based on individual randomization (i.e., cluster randomization);
- Trials in hospitalized and nonhospitalized patients with chronic conditions such as end-stage renal disease or chronic health failure;
- Trials in which vitamin D was administered as fortified food;
- Trials testing compounds other than cholecalciferol and ergocalciferol;
- Trials in subjects mainly under 50 yr old.

Retrieval of articles

The articles found via keywords or search in reference lists were first screened for the method used (randomized trial vs. other design) and the compounds tested (oral vitamin D2 or D3 vs. other vitamin D analog). In a second step, full text of the first step survivors were retrieved and screened for inclusion and exclusion criteria. In a third step, the relevant data
from the eligible articles were extracted in a structured database. Data extraction was made by two authors (P.A. and P.M.), and discrepancies were solved by consensus between the three authors.

Many trials included only one gender or did not report results by sex. We could delineate two broad age categories, from 50 to 79 and 80 or more, although some trials categorized as “80 or more” could include a minority of younger subjects, and some trials categorized as “50 to 79” could include a minority of older subjects. We classified subjects as community-dwelling or institutionalized (in nursing home) according to authors.

Vitamin D supplements were sometimes administered as weekly or monthly doses, principally in trials that used vitamin D2 in subjects over 79 yr old. One trial suggested that daily intake may be more efficient at raising serum levels than monthly intakes (22), whereas another suggested that dose efficiency did not correlate with dose frequency (23). We chose to compute daily doses for all trials, and when administration was not daily, we took as the standard way for calculation of daily dose the transformation we used in a previous study (24) for the Trivedi et al. trial (25) in which 2500 μg oral vitamin D was taken every 4 months, representing a daily dose of 20.75 μg.

Few trials did not report the type of vitamin D used (26–28), and we assumed it was vitamin D3 (cholecalciferol). An intervention group of a trial (29) used both vitamin D2 and D3 at the same time, and we arbitrarily considered that this intervention group tested the vitamin D2.

A number of trials also had oral calcium supplements as part of the intervention, and we abstracted this information.

For 25-hydroxyvitamin D assessment, most trials had archived blood samples of subjects and performed measurement of 25-hydroxyvitamin D levels at baseline and during the trial on random samples of the control and intervention groups. Measurement of serum 25-hydroxyvitamin D levels could take place at several occasions during the trial, and we always took the 25-hydroxyvitamin D level measured at baseline and at the latest blood draw after randomization.

Some trials did not report baseline 25-hydroxyvitamin D levels (25, 30), and we took the in-trial level in the control group as surrogate for baseline levels in both control and intervention groups. In the WHI trial, the baseline median level reported in the control group of the three nested case-control studies was 16.9 ng/ml (10–12). This baseline level in the control group was taken as a surrogate for the baseline level of the intervention and for the in-trial level of the control group.

Statistical analysis

For both intervention and control groups, we evaluated the absolute changes in serum 25-hydroxyvitamin D levels between intervention and control groups at last measurement and the average values between intervention and control groups at baseline.

We first performed graphical exploration of data and then an investigation of variability of changes in 25-hydroxyvitamin D levels due to doses, type of vitamin D, calcium supplementation, 25-hydroxyvitamin D baseline values, gender, mean age, type of population, length of follow-up, country, and publication year through meta-analytic methods.

Multivariate meta-regression analysis was carried out to investigate heterogeneity, and normal distribution of residuals was graphically checked. Vitamin D dose was included with Nep-erian logarithmic transformation. Trials reported variability in 25-hydroxyvitamin D levels between intervention and control groups in various ways—as confidence intervals (CI), or variance of mean levels in each group, or interquartile ranges, or SE. We used these parameters for estimating the variance specific for each category of dose within each trial. Some trials did not report any indication on the variance of measured levels. To circumvent gaps in information on variances, we used methods proposed by Wiebe et al. (31) for handling missing variance data.

Summary estimates were estimated by pooling the study-specific estimates with the mixed effects models. PROC MIXED in SAS (SAS Windows version 8.02, 1999; SAS Institute Inc., Cary, NC) with maximum likelihood estimates was used to take into account two sources of variations: between-study and within-study variabilities.

We fitted random effects meta-regression models for prediction of linear change in 25-hydroxyvitamin D concentration. Summary estimates according to supplementation doses were estimated. We tested the influence of various covariates such as mean age, institutionalized or community-dwelling populations, and baseline 25-hydroxyvitamin D level on heterogeneity and summary results through multivariate meta-regression (32). Selection of the model best describing the data was based on clinical and statistical reasons. On the clinical side, a predictive model had to include the 25-hydroxyvitamin D concentration at baseline because changes in concentrations are usually smaller in subjects with high baseline concentration (4). Because of digestive tolerance problems, concomitant calcium supplementation may affect compliance to vitamin D supplementation and thus needs to be accounted for in statistical models. Statistical selection was based on changes in residual between-study variance (32), with the lowest Akaike information criterion indicating greatest explanation of the total variability in data.

Sensitivity analyses were carried out to investigate the influence of different variance imputations. To investigate whether publication bias might affect the validity of the estimates, we constructed funnel plots of the regression of changes in serum levels on the sample size, weighted by the inverse of the variances.

Results

The Medline searches returned 2560 papers for the various combinations of keywords and limits (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://jem.endojournals.org). The search in references of major reviews retrieved eight additional articles. A first screening of titles and abstracts looked for keywords “randomized” or “randomised.” After application of inclusion and exclusion criteria on the title and abstracts, 159 articles were selected. Full copies of these 159 articles were obtained and read. Eighty-three articles were excluded due to the absence of reporting of serum 25-hydroxyvitamin D levels during the trial (42 articles), absence of a control group receiving a placebo or no vitamin D (i.e. open-label trials; six articles), use of compounds other than chole- and ergocalciferol (28 articles), and trials done in chronically ill subjects (e.g. patients with
TABLE 1. Number of intervention groups and of subjects in intervention groups in 76 randomized trials on vitamin D supplements

<table>
<thead>
<tr>
<th>In all 76 trials</th>
<th>In trials done in community-dwelling subjects</th>
<th>In trials done in institutionalized subjects</th>
<th>In all 76 trials</th>
<th>In trials done in community-dwelling subjects</th>
<th>In trials done in institutionalized subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>101</td>
<td>73</td>
<td>28</td>
<td>6207</td>
<td>4933</td>
</tr>
<tr>
<td>Age 50–79 yr</td>
<td>67</td>
<td>67</td>
<td>1</td>
<td>4606</td>
<td>4778</td>
</tr>
<tr>
<td>Age 80 + yr</td>
<td>33</td>
<td>6</td>
<td>27</td>
<td>1601</td>
<td>355</td>
</tr>
<tr>
<td>Women</td>
<td>36</td>
<td>30</td>
<td>6</td>
<td>2531</td>
<td>2126</td>
</tr>
<tr>
<td>Men</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td>1138</td>
<td>1003</td>
</tr>
<tr>
<td>Men and women</td>
<td>51</td>
<td>30</td>
<td>21</td>
<td>2538</td>
<td>1804</td>
</tr>
<tr>
<td>Vitamin D2</td>
<td>14</td>
<td>11</td>
<td>3</td>
<td>673</td>
<td>584</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>87</td>
<td>62</td>
<td>25</td>
<td>5534</td>
<td>4349</td>
</tr>
<tr>
<td>Calcium supplements part of the intervention</td>
<td>No</td>
<td>41</td>
<td>26</td>
<td>2147</td>
<td>1505</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>60</td>
<td>47</td>
<td>4060</td>
<td>3428</td>
</tr>
<tr>
<td>Trial placebo-controlled</td>
<td>No</td>
<td>13</td>
<td>9</td>
<td>999</td>
<td>860</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>88</td>
<td>64</td>
<td>5208</td>
<td>4073</td>
</tr>
</tbody>
</table>

The best multivariate model, displayed in Table 3, includes the baseline serum 25-hydroxyvitamin D level (mean between trial arms) and the type of vitamin D supplementation. Summary estimates from this model confirmed a significant association between the supplement dose and the change in 25-hydroxyvitamin D level. Compared with vitamin D2, vitamin D3 led to increases of 4.29 ng/ml higher on average (P = 0.03). Inverse associations were found between increases in 25-hydroxyvitamin D levels and the baseline serum 25-hydroxyvitamin D levels or the concomitant intake of calcium supplements, but these associations were not statistically significant. So, for
a daily supplementation of 20 \mu g of vitamin D3 combined with calcium in subjects having a mean concentration of 10 ng/ml, the expected average change in serum 25-hydroxyvitamin D level would be: 

\[
[2.28 - 0.12 \times 10 + (6.78 \times \ln (20)) - 2.72] = 14.1 \text{ ng/ml}.
\]

Age was strongly correlated with the type of population and could thus not contribute to improving the fit of the full model. Further analysis showed no association between change in 25-hydroxyvitamin D level and use of a placebo or length of follow-up. The influence of gender could not be explored owing to the numerous trials that did not report details on this variable. No indication of publication bias was found (\(P = 0.67\)).

In a sensitivity analysis, we restricted the analysis to trials that published the estimates of precision of changes (i.e. 95% CI, SE, variance, sd), and results did not change (data not shown).

On average, the baseline serum 25-hydroxyvitamin D level of community-dwelling subjects included in trials was 19.9 ng/ml, and these subjects took 20 \mu g of vitamin D supplement per day (Table 2). According to the model in Table 3, if supplementation is done with vitamin D3 and if calcium supplements are not given simultaneously, the serum 25-hydroxyvitamin D concentration of these subjects should increase by 15.6 ng/ml; that is an increase of 0.78 ng/ml (1.95 nmol/liter) per microgram of vitamin D3 supplement per day.

In the WHI trial, the baseline 25-hydroxyvitamin D level was 16.9 ng/ml (12). After 2 months of trial, the 25-hydroxyvitamin D levels were on average 28% higher in the supplement group of the WHI trial (12). Hence, the observed increase in this was \((0.28 \times 16.9) = 4.7 \text{ ng/ml}\). According to the model in Table 3, a supplementation with a daily dose of 10 \mu g of vitamin D3 plus calcium should lead to an average increase of 8.6 ng/ml.

**Discussion**

Our systematic review shows that changes in serum 25-hydroxyvitamin D level obtained with vitamin D supple-
mentation in Caucasian subjects age 50 and older depend on the dose taken and on the type of vitamin (D2 or D3). The lower potency of vitamin D2 (ergocalciferol) is in line with results of most randomized trials that tested the specific ability of the two vitamin D isoforms to raise and maintain circulating 25-hydroxyvitamin D concentrations (35). Variables such as being institutionalized or being old did not influence the dose-response relationship. Other factors known to be associated with low vitamin D status (1) cannot explain our results because, by the virtue of randomization, they were evenly distributed among intervention and control group subjects.

Trials that used similar supplement doses could obtain substantially different changes in 25-hydroxyvitamin D concentrations. Adherence to the supplementation regimen was surely a strong source of between-trial variability, but few trials reported data on adherence to supplementation, which was often difficult to compare. Digestive tolerance problems associated with calcium supplements are well documented (36). Results of our meta-analysis are compatible with the hypothesis that concomitant calcium supplementation can reduce the compliance to vitamin D supplementation. The fear of lower compliance due to calcium-induced digestive problems probably underpinned the decision made in some trials to test vitamin D supplementation only (25).

A second source of variability was the type of vitamin D used, bearing in mind that the vitamin D2 was usually used for elderly people and administered in large doses deemed to cover needs for one or several months.

A third source of variability could have been the proportions of overweight and obese subjects included in trials because overweight and obesity markedly decrease response to vitamin D supplementation (37, 38). However, the mean body mass index in clinical trials included in our review ranged from 24 to 28 kg/m², and such a range is not likely to explain most of the range in response to oral administration.

A fourth source of variability was probably the different laboratory tests used for measuring serum 25-hydroxyvitamin D concentrations (39). The dosage methods were quite variable across trials and were sometimes not reported. Therefore, we could not explore the influence of this factor on meta-analysis results.

Our review has several limitations inherent to the type of data reported in publications. For instance, two thirds of the trials did not report results by gender. We also had to deal with different age distributions. Few trials used supplement doses above 50 μg/d, and therefore one should be cautious with estimations of changes in vitamin D status to be expected with a higher dose of supplement. We did not address effects of supplementation in non-Caucasian subjects or in patients with chronic conditions, and specific meta-analyses should be done in these groups.

Two recent dose-escalation, placebo-controlled, randomized trials in community-dwelling women age 64 and older using 0, 5, 10, and 15 or 20 μg of vitamin D3 per day suggested a 0.64 to 0.79 ng/ml (1.6 to 1.97 nmol/liter) increase in 25-hydroxyvitamin D concentrations per microgram of daily intake (6, 7). These two trials were included in the present meta-analysis. The meta-regression model in Table 3 that we derived from our meta-analysis of 74 randomized trials that used doses ranging from 5 to 50 μg/d yields predicted changes in serum 25-hydroxyvitamin D similar to those obtained by the two dose-escalation trials. However, increases per microgram of supplement are likely to be lower if adherence to supplementation is not optimal (especially when calcium supplements are taken concomitantly) and if vitamin D2 is used instead of vitamin D3.

Prospective cohort studies and their meta-analysis generally found no association between circulating 25-hy-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>–2.28</td>
<td>–7.79</td>
<td>3.22</td>
</tr>
<tr>
<td>Ln dose (μg/d)</td>
<td>6.78</td>
<td>5.38</td>
<td>8.18</td>
</tr>
<tr>
<td>Type of supplement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3 Ref.</td>
<td>–4.18</td>
<td>–7.92</td>
<td>–0.44</td>
</tr>
<tr>
<td>D2 Ref.</td>
<td>–2.72</td>
<td>–5.97</td>
<td>0.54</td>
</tr>
</tbody>
</table>

The three trials that tested doses greater than 100 μg/d were not included in the model.
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choxyvitamin D concentrations and cancer risk excepting for colorectal cancer (3, 40). A meta-analysis of eight prospective cohort studies showed that a 10 ng/ml increase in serum 25-hydroxyvitamin D concentration was associated with a 15% (95% CI, 0.08–0.21) decrease in the risk of colorectal cancer (3). In contrast, the WHI trial showed a slight nonsignificant increase of 8% (95% CI, −0.14 to 0.34) in the risk of colorectal cancer associated with vitamin D and calcium supplementation (10). The results of observational studies could be the consequence of confounding factors, whereas the WHI trial findings would indicate a genuine absence of effect of vitamin D status on colorectal cancer risk. However, according to the model derived from the meta-regression, the increase obtained by the WHI trial was about half the expected average increase based on randomized trials that reported the influence of vitamin D3 supplements on serum 25-hydroxyvitamin D concentration. Concomitant use of calcium supplements in the WHI trial may have contributed to reduced adherence to vitamin D supplementation. The aforementioned meta-analysis of observational studies (3) indicates that with the 4.7 ng/ml increase in 25-hydroxyvitamin D level obtained in the WHI trial, a 7% decrease in the risk of colorectal cancer could have been expected. The WHI trial size was far too small for assessing a 7% risk reduction. Hence, the possibility cannot be dismissed that, because of dosage and compliance problems, changes in vitamin D status in the WHI trial might not have been sufficient for observing a substantial influence on the risk of colorectal cancer.

In conclusion, dietary recommendations and randomized trials on vitamin D supplementation should evaluate whether increases in circulating 25-hydroxyvitamin levels match expectations like, for instance, the average increases obtained by trials using vitamin D3 without concomitant calcium supplements.

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Address all correspondence and requests for reprints to: Philippe Autier, M.D., International Prevention Research Institute (iPRI), 95 Cours Lafayette, 69006 Lyon, France. E-mail: philippe.autier@i-pri.org.

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