Impact of Vitamin D3 Dietary Supplement Matrix on Clinical Response

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Context: As a result of research suggesting increased health risk with low serum 25-hydroxycholecalciferol (25(OH)D), health care providers are measuring it frequently. Providers and patients are faced with treatment choices when low status is identified.

Objective: To compare the safety and efficacy of three vitamin D3 dietary supplements with different delivery matrices.

Setting and Design: A 12-week, parallel group, single-masked, clinical trial was conducted in Seattle, Washington and Kailua Kona, Hawaii. Sixty-six healthy adults with 25(OH)D <33 ng/mL were randomly assigned to take one of three D3 supplements, ie, a chewable tablet (TAB), an oil-emulsified drop (DROP), or an encapsulated powder (CAP) at a label-claimed dose of 10,000 IU/day. Actual D3 content was assessed by a third party and the results adjusted based on the actual D3 content administered. Mean change in 25(OH)D/mcg D3 administered; difference in the proportion of D3 insufficient participants (ie, 25(OH)D <30 ng/mL) reaching sufficiency (ie, 25(OH)D ≥30 ng/mL); and mean change in serum 1, 25-dihydroxycholecalciferol were measured.

Results: In two of the three products tested, the measured vitamin D3 content varied considerably from the label-claimed dose. Differences in 25(OH)D/mcg D3 administered were significantly different between groups (P = .04; n = 55). Pairwise comparisons demonstrated DROP resulted in a greater increase than TAB (P < .05) but not than CAP. TAB was not different from CAP. The proportions reaching sufficiency were: 100% (TAB and CAP) and 80% (DROP) (P = .03 between groups; n = 55). 1, 25-Dihydroxycholecalciferol did not change significantly in any group.

Conclusions: Oil-emulsified vitamin D3 supplements resulted in a greater mean change in serum 25(OH)D concentration, but fewer patients reaching vitamin D sufficiency, than chewable or encapsulated supplements. (J Clin Endocrinol Metab 99: 2720–2728, 2014)
strategies with structured analysis of adverse events (12–
14). Recent evidence suggests high doses of D₃ are safe and may be necessary to achieve cholecalciferol “sufficiency” (15–17). Vitamin D₃ dosages of up to 10,000 IU/d have been determined to be safe, whereas dosages greater than 40,000 IU/d have been associated with vitamin D toxicity (18–21). Clinical trials evaluating the effectiveness of dietary supplementation to correct D₃ insufficiency have demonstrated relative safety without hypercalcemia or other manifestations of toxicity (21–25). However, the effectiveness of different repletion strategies to reach “optimal” 25(OH)D concentration was highly variable and ranged from 33–86% in participants receiving oral D₂ (23, 24) and 5–89% in the participants receiving oral D₃ (22, 25).

It is important to consider sources of potential variability when interpreting the results of these and other clinical trials of vitamin D, especially when dietary supplements are being used. One potential source of variability in the clinical effectiveness of dietary supplements, which may also influence patient preference and adherence, is the delivery matrix used in the supplement; numerous matrices exist in the marketplace; however, the most common matrices include dry powder in capsules, chewable tablets, and oil-emulsified drops. Thus, if absorption and overall effectiveness differs by delivery matrix, it is important to establish dosing equivalents of these products to ensure patient safety and to assist providers.

Another source of potential variability in results from vitamin D clinical research is dosing disparities from the label-claimed dose and the actual vitamin D content. Industry guidance for the manufacturer of vitamin D supplements comes from the US Pharmacopoeial Convention National Formulary (USP-NF) and the US Food and Drug Administration (FDA) (26–28). The USP-NF guidance to dietary supplement manufacturers allows D₃ dietary supplements to contain no less than 90% and no more than 165% of the product label claim for cholecalciferol (D₂). The FDA (21CFR101.9) provides the following regulatory guidance on D₃ supplements: 1) D₃ content in the composite that is at least equal to the D₃ content of the label claim; 2) reasonable excesses are acceptable within current good manufacturing practices; and 3) D₃ content does not exceed 120% of the label claim or falls within the variability generally recognized for the analytical method for analysis (28). Therefore, label-claimed doses are not always accurate for vitamin D supplements, and third-party measurement and quantification of vitamin D content of dietary supplements used in clinical trials should be considered a “best practice.”

To better assess the degree of inconsistency between product label claims and actual vitamin D content, and to directly compare different delivery matrices on clinical response, we performed a randomized, pragmatic clinical trial of three common supplement matrices and adjusted our results for the actual dose administered based on third-party analysis of each product.

**Materials and Methods**

The clinical trial protocol was approved by the Bastyr University Institutional Review Board (no. 09A-1241) and was registered at clinicaltrials.gov as NCT01524874.

**Settings and participants**

The trial protocol was standardized and implemented in three clinical settings: Bastyr Center for Natural Health (Seattle, Washington); Bastyr University Clinical Research Center (Kenmore, Washington); and Lokahi Health Center (Kailua Kona, Hawaii). Participants were recruited from active patients at each clinical site, as well as through flyers posted throughout the greater Seattle, Washington metropolitan area. Candidate participants were informed about the study by their physicians and/or a study coordinator and invited for screening at the discretion of their physician.

Candidate participants provided informed consent and completed a baseline health assessment and laboratory screening including measurement of serum 25(OH)D and 1,25(OH)₂D, a comprehensive metabolic panel and complete blood count. We then enrolled participants who were generally healthy adults, ages 18–65 y, with serum 25(OH)D <33 ng/mL (82.5 nmol/mL), aspartate transaminase (AST) >60 U/L; alanine transaminase >65 U/L, alkaline phosphatase >120 U/L, total bilirubin >1.5 mg/dL, serum creatinine >1.4 mg/dL, blood urea nitrogen >25 mg/dL, pregnancy or unwillingness to avoid pregnancy by using contraceptives, osteoporosis, parathyroid disorder, difficulty swallowing pills, psychological conditions or substance abuse that may challenge adherence to the protocol, cardiac arrhythmia, other severe illness limiting activities of daily living, and/or currently taking medications that interfere with the metabolism of vitamin D (eg, anticonvulsants, anticoagulants, oral corticosteroids, or barbiturates) (29, 30).

**Randomization and interventions**

Randomization was conducted centrally in blocks of three. Upon confirmation of participation criteria each participant was randomized to one of three D₃ treatments for 12 weeks. Candidates were excluded if they met any of the following criteria: unwilling to provide consent, extradietary vitamin D intake >1,000 IU/d, unwilling to use sunscreen, allergy to sunscreen or sesame oil, serum 25(OH)D ≥33 ng/mL (82.5 nmol/mL), aspartate transaminase (AST) >60 U/L; alanine transaminase >65 U/L, alkaline phosphatase >120 U/L, total bilirubin >1.5 mg/dL, serum creatinine >1.4 mg/dL, blood urea nitrogen >25 mg/dL, pregnancy or unwillingness to avoid pregnancy by using contraceptives, osteoporosis, parathyroid disorder, difficulty swallowing pills, psychological conditions or substance abuse that may challenge adherence to the protocol, cardiac arrhythmia, other severe illness limiting activities of daily living, and/or currently taking medications that interfere with the metabolism of vitamin D (eg, anticonvulsants, anticoagulants, oral corticosteroids, or barbiturates) (29, 30).

Randomization was conducted centrally in blocks of three. Upon confirmation of participation criteria each participant was randomized to one of three D₃ treatments donated by three different private manufacturers: an oil-emulsified drop (DROP); a dry, encapsulated powder (CAP); or a chewable tablet (TAB). Participants were each instructed to take five dosage units per day equaling a label-claimed daily dose of 10,000 IU. The TAB and CAP were from a single manufacturing batch; the DROP was from two manufacturing batches due to the short shelf life of this product. The supplements were provided free of charge in adequate quantity for the entire 12-week study period. Study participants were not masked to their product assignment due to the inherent challenges in masking the administration of the chew-
able tablets and the oil-emulsified drops; the trial was intended as pragmatic.

**Assessment of D₃ content of each supplement**

Each of the supplements was sent to an independent analytical lab (Flora Research Laboratories) to measure the D₃ content of each product before the trial began, and at the end of the trial to evaluate for product degradation. A sample from each product was prepared in triplicate with an additional sample for spike recovery, and the samples were analyzed using ion-trap liquid chromatography tandem mass spectrometry (LC-MS/MS).

**Assessment and control of potential residual confounders**

Participants completed a standardized food frequency questionnaire to quantify their dietary intake of vitamin D (31–33) and a standardized sun exposure questionnaire to quantify their use of sun protection (eg, long-sleeved clothing), and exposure (ie, hours per day) (34). To further reduce potential sources of variability in our results, participants were provided with an ample supply of SPF 30 sunscreen (SolRx or MyChelle Dermaceuticals, LLC) and instructed to apply it daily to sun-exposed skin. Special attention was paid to the measurement of human PTH using the HBN1B-51K Milliplex Map Kit (Millipore Corp); PTH kits were read on a Luminex 100/200 analyzer (Luminex Corporation). PTH concentrations were analyzed from banked samples (stored at −70°C) for participants from the Seattle, Washington site (N = 40; missing = 1). PTH was not analyzed in samples from the Hawaii site due to potential sample degradation during overnight transit. Additional clinical parameters were collected at baseline and again after 12 weeks: height, weight, heart rate, blood pressure (BP), body mass index (BMI), hemoglobin Alc (HbA1c), total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, calcium, fasting insulin and glucose, and a comprehensive metabolic panel.

**Assessment of safety, dose-reduction protocol, and trial-stopping criteria**

Safety was assessed by inquiry at clinical research visits, safety monitoring telephone interviews at 6 weeks (the trial midpoint), and self-report throughout the trial via telephone or e-mail. Adverse event (AE) data were collected using a standardized adverse event reporting form, based on the Washington State Department of Social and Health Services form 10–334, which quantifies severity on a 0–4 scale where 0 = no symptom and 4 = FDA serious AE. If participants reported a symptom associated with hypercalcemia or vitamin D toxicity at any time during the trial, serum calcium and 25(OH)D were measured immediately. To ensure participant safety, a two-tiered dose-reduction protocol was implemented for participants who reported symptoms associated with hypercalcemia for three days or longer despite an absence of laboratory-measured hypercalcemia. Dosage was first reduced to 6000 IU/d and subsequently to 2000 IU/d if symptoms did not resolve within 3 days. Trial-stopping criteria included hypercalcemia occurring in ≥10% of participants, greater than three deaths from any cause, or one death attributable to a dietary supplement.

**Outcomes**

The primary, secondary, and tertiary outcome comparisons between groups were change in mean serum 25(OH)D/mcg vitamin D₃ administered, difference in proportion of participants with D₃ “insufficiency” (ie, 25(OH)D < 30 ng/dL) reaching “sufficiency” (ie, 25(OH)D ≥ 30 ng/dL), and mean change in serum 1,25(OH)₂D₃.
University of Washington (36). One of the principal investigators (PI) of this trial (R.B.) did not have access to interim trial results and was separated from all data entry. The other PI of this trial (M.T.) did have access to individual participants’ results for the purposes of ongoing clinical care at the Kona, Hawaii site; M.T. did not have access to composite trial results and was not involved in data analysis. The data analysis plan was finalized by R.B. and J.F. before any data were analyzed. The unmasking of both PIs to the allocation groups occurred only after the results were considered final. STATA 11.0 (StataCorp) was used to conduct statistical analyses.

Role of the funding source

This project was supported by the National Institutes of Health National Center of Complementary and Alternative Medicine (NCCAM) Grant T32AT00815 and National Center for Research Resources (NCRR) Grant 1KL2RR025015-01. REDCap was supported by Award Number ULTR000114 from the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH). Additional support was provided by the Diabetes Action Research and Education Foundation and Bastyr University. The funding sources and manufacturers had no influence on any aspects of the trial, including data collection, manuscript preparation, or decisions regarding where to submit the manuscript for publication; however, the product manufacturers were allowed up to 30 days to review the manuscripts prior to submission and offer their comments.

Results

A total of 302 individuals were screened for inclusion based on their interest in the trial (Figure 1). One hundred and ninety-nine participants were excluded for reasons summarized in Figure 1. Of the 103 participants screened for eligibility based on 25(OH)D status, 37 were sufficient in serum 25(OH)D. Sixty-six participants met participation criteria and were randomly assigned. Loss to follow-up was evenly distributed among treatment groups and totaled 11 (16.7% of randomized sample) participants, leaving a remaining sample of 55 (83% of randomized sample) participants in which to assess outcomes. Baseline characteristics (Table 1)

![Figure 1. CONSORT flow diagram of three-arm comparative effectiveness trial.](image)

### Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Group Characteristic</th>
<th>All (n = 55) Mean (SD) or Frequency, %</th>
<th>TAB (n = 18) Mean (SD) or Frequency, %</th>
<th>DROP (n = 20) Mean (SD) or Frequency, %</th>
<th>CAP (n = 17) Mean (SD) or Frequency, %</th>
<th>Group Comparison P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Site, % from WA</td>
<td>75 (13.7)</td>
<td>72 (13.5)</td>
<td>75 (13.6)</td>
<td>76 (14.7)</td>
<td>.43</td>
</tr>
<tr>
<td>Age, y</td>
<td>39.9 (13.7)</td>
<td>40.3 (13.5)</td>
<td>39.5 (13.6)</td>
<td>39.9 (14.7)</td>
<td>.98</td>
</tr>
<tr>
<td>Female, %</td>
<td>85 (13.7)</td>
<td>83 (13.5)</td>
<td>85 (13.6)</td>
<td>85 (14.7)</td>
<td>.92</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>111.3 (12.7)</td>
<td>112.4 (11.2)</td>
<td>107.7 (9.8)</td>
<td>114.4 (16.4)</td>
<td>.25</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>73.1 (9.5)</td>
<td>75.9 (9.4)</td>
<td>69.8 (9.9)</td>
<td>73.8 (8.5)</td>
<td>.13</td>
</tr>
<tr>
<td>Height, in</td>
<td>65.8 (3.1)</td>
<td>65.5 (3.0)</td>
<td>65.5 (3.1)</td>
<td>66.4 (3.4)</td>
<td>.67</td>
</tr>
<tr>
<td>Weight, lbs</td>
<td>161.5 (39.6)</td>
<td>168.9 (46.9)</td>
<td>154.8 (27.6)</td>
<td>161.6 (44.1)</td>
<td>.56</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 (5.3)</td>
<td>27.4 (6.5)</td>
<td>25.2 (4.0)</td>
<td>25.6 (5.2)</td>
<td>.39</td>
</tr>
<tr>
<td>Dietary Vitamin D intake, IU/wk</td>
<td>1525 (1826)</td>
<td>1545 (1933)</td>
<td>1502 (1869)</td>
<td>1530 (1770)</td>
<td>.10</td>
</tr>
<tr>
<td>Sun exposure, h/wk</td>
<td>9 (10)</td>
<td>10 (13)</td>
<td>10 (10)</td>
<td>8 (5)</td>
<td>.73</td>
</tr>
<tr>
<td>Sun protection, %</td>
<td>76 (13.7)</td>
<td>83 (13.5)</td>
<td>80 (13.6)</td>
<td>88 (14.7)</td>
<td>.09</td>
</tr>
<tr>
<td>PTHa, pg/mL</td>
<td>36.6 (51.9)</td>
<td>21.2 (19.3)</td>
<td>53.2 (78.3)</td>
<td>31.7 (25.7)</td>
<td>.26</td>
</tr>
<tr>
<td>Baseline 25(OH)D, ng/mL</td>
<td>22.6 (6.7)</td>
<td>21.9 (7.8)</td>
<td>24.2 (5.1)</td>
<td>21.5 (7.2)</td>
<td>.41</td>
</tr>
</tbody>
</table>

a PTH measured in Seattle site samples only: total, n = 40; n_TAB = 12; n_DROP = 15; n_CAP = 13.
did not differ significantly between groups (all $P > .05$ by ANOVA), including important covariates that correlate with vitamin D status, (eg, baseline vitamin D status, age, sex, BMI, dietary intake, sun protection, sun exposure, state and enrollment season).

**Dose of vitamin D**

Third-party testing using LC-MS/MS determined each mean dose as follows: TAB = 3943 IU/dosage unit or 98.6 mcg/dosage unit (ie, 197% label claim); DROP = 2208 IU/dosage unit or 55.2 mcg/dosage unit (ie, 110% label claim); and CAP = 4131 IU/dosage unit or 103.3 mcg/dosage unit (ie, 207% label claim).

**Change in Serum 25(OH)D**

Within group analysis unadjusted for vitamin D content (Table 2) demonstrated all treatments increased 25(OH)D significantly between baseline and 12-week follow-up ($P < .0001$ for all groups).

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline Mean (SD), ng/mL</th>
<th>Week 12 Mean (SD), ng/mL</th>
<th>Mean (SD) Change, ng/mL</th>
<th>95% Confidence Interval</th>
<th>$P$ Value for Change$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAB (n = 18)</td>
<td>21.9 (7.8)</td>
<td>55.1 (15.2)</td>
<td>+33.3 (5.7)</td>
<td>21.9–44.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>DROP (n = 20)</td>
<td>24.2 (5.1)</td>
<td>58.6 (27.4)</td>
<td>+34.4 (5.4)</td>
<td>23.6–45.1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>CAP (n = 17)</td>
<td>21.5 (7.2)</td>
<td>75.1 (22.8)</td>
<td>+53.6 (5.8)</td>
<td>41.9–65.2</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Results are reported by randomization group, and are not standardized by actual dose administered.

$^a$ $P$ value corresponds to within group changes by 2-sided, paired $t$ test.

Changes in 25(OH)D per mcg D$_3$ administered, based on the results of third-party analysis, were TAB = 0.068 ± 0.016 ng/mL 25(OH)D/mcg D$_3$; DROP = 0.125 ± 0.015 ng/mL 25(OH)D/mcg D$_3$; and CAP = 0.106 ± 0.017 ng/mL 25(OH)D/mcg D$_3$, as shown in Figure 2. Between-group ANOVA demonstrated significant between-group differences ($P = .04$) and subsequent Tukey’s test revealed significant differences between DROP vs TAB ($P < .05$), but not between TAB vs CAP or between DROP vs CAP ($P > .05$ for both). The primary result did not change in post hoc ANCOVA analyses. Between-group ANCOVA demonstrated significant between-group differences ($P = .03$) and subsequent Tukey’s test mirrored the unadjusted analyses with differences between DROP and TAB showing significance ($P < .05$), but not TAB vs CAP or DROP vs CAP ($P > .05$ for both).

**Proportion attaining 25(OH)D sufficiency status**

We report the proportion of participants attaining sufficiency per the reference range of our clinical reference

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**Figure 2.** A) Unadjusted between group changes in serum 25-hydroxycholecalciferol concentration ng/mL/mcg vitamin D$_3$ administered (ng/mL 25(OH)D/mcg D$_3$). B) Adjusted between group changes in serum 25-hydroxycholecalciferol concentration ng/mL/mcg vitamin D$_3$ administered (ng/mL 25(OH)D/mcg D$_3$).

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laboratory, Pacific Physician’s Laboratories (PPL) and per guidelines set by the Institute of Medicine. Per the PPL-defined reference range, the proportion of participants with D3 “insufficiency” (ie, 25(OH)D < 33 ng/dL; n = 55) reaching “sufficiency” (ie, 25(OH)D ≥33 ng/dL) between treatment arms was 100% for TAB and CAP, and 80% for DROP (P = .03). Per the Institute of Medicine clinical definitions, the proportion of participants with D3 “insufficiency” (ie, 25(OH)D < 30 ng/dL; n = 46) reaching “sufficiency” (ie, 25(OH)D ≥30 ng/dL) between treatment arms was 100% for TAB and CAP, and 82% for DROP (P = .07).

Change in serum 1,25(OH)2D
There were no clinically or statistically significant changes in 1,25(OH)2D within or between allocation groups; P > .05 for all within-group comparisons and between-group ANOVA (Table 3).

Changes in other clinical parameters
Changes in serum calcium and plasma PTH levels were not significant within or between groups (all P > .05).

Adverse events
Three mild AEs occurred including tachycardia, anxiety, itching, muscle cramping, and nausea; all occurred without hypervitaminosis D or hypercalcemia (verified by laboratory tests per protocol). Two dose reductions to 6000 IU D3/d were required per protocol, and all AEs resolved.

Discussion
Our study demonstrates several novel and important clinical results. First, we identified considerable variability between label-claimed content and independently measured content of two of the three supplements evaluated in this trial, with one product containing double the label-claimed dose. We further compared the relative effectiveness of three different delivery matrices for vitamin D3 in dietary supplements and measured statistically significant differences in clinical response, with the oil-emulsified product being the most effective of the products evaluated. We also measured an overall effectiveness of 93% for the repletion strategy tested here, compared with other repletion strategies reported in the literature (ie, 5–89%). Finally, all three vitamin D3 dietary supplements were safe to administer for 12 weeks at 10,000 IU/d (based on label claim), without hypercalcemia or other clinically significant adverse events.

Strengths
We employed a rigorous, comparative effectiveness trial design in order to compare commonly available vitamin D3 dietary supplements representing three different delivery matrices. The trial was appropriately powered to measure a clinically significant 25% relative difference between groups for changes in mean for 25(OH)D, a difference which was exceeded by one product tested. In addition, we assessed for allocation imbalance between potential confounders (ie, BMI, dietary intake of vitamin D, sun exposure, and sunscreen use), which did not differ among randomization groups. To ensure even minor residual confounding did not affect our interpretation, we also adjusted our results for covariates that may predict response to vitamin D supplements (eg, baseline status, enrollment season, dietary intake, and BMI) (37, 38). We also employed standardized AE reporting, clinical laboratory monitoring, and dose-reduction protocols to ensure safety of study participants and detect any cases of hypercalcemia. The generalizability of our results is greatly improved by the conduct of the trial in two very different geographical regions (ie, Seattle, Washington and Kona, Hawaii). We also conducted our trial over an entire calendar year, recruiting participants during all seasons (ie, participants were not enrolled only during the summer or the winter).

The greatest strength of this trial is that we contracted independent measurement of the vitamin D3 content of each of three dietary supplements evaluated in the trial, before and after the intervention period, in order to ac-

Table 3. Mean Changes in Serum 1,25-Dihydroxycholecalciferol (1,25(OH)2D)a

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline Mean (SD), ng/mL</th>
<th>Week 12 Mean (SD), ng/mL</th>
<th>Difference Mean (SD), ng/mL</th>
<th>95% Confidence Interval</th>
<th>P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAB (n = 16)</td>
<td>41.4 (2.5)</td>
<td>44.9 (4.3)</td>
<td>+3.5 (3.9)</td>
<td>−11.9, 4.9</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>DROP (n = 19)</td>
<td>43.4 (3.2)</td>
<td>43.2 (2.9)</td>
<td>−0.2 (4.0)</td>
<td>−8.2, 8.5</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>CAP (n = 17)</td>
<td>42.8 (3.7)</td>
<td>39.0 (2.5)</td>
<td>−3.8 (4.3)</td>
<td>−5.4, 12.9</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

a Results are reported by randomization group and not standardized by actual dose administered.
b P value corresponds to within group 2-sided paired t tests. Between-group ANOVA P > .05.

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count for dosing variability and potential product degradation. In the absence of independent product testing, our results would be based solely on the product’s label claimed dose, which would have led to erroneous results (data not shown). Because this source of error is likely common (38) and often not quantified, we recommend manufacturer-independent testing of vitamin D₃ dietary supplements in all future clinical trials.

Limitations

We did not employ an inactive or placebo control group in this study because all participants had clinically low vitamin D status warranting active treatment, and because the primary intention was to compare the efficacy of these active treatments. Similarly, participants were not masked to their allocation group due to difficulties in masking the emulsified drops. There is little reason to suspect group crossover or other sources of bias due to their group assignment because all supplements contained active D₃, all were provided free of charge, and each was dispensed in adequate supply for the entire duration of the trial. Although all other baseline characteristics were balanced during randomization, we did not assess participant ethnicity and thus were unable to measure any impact differences in skin pigmentation may have had on our results. Similarly, because we were primarily interested in determining whether the supplements tested would produce clinically significant increases in 25(OH)D generalizable to most patients, we did not measure the contribution of highly individualized, genetically-determined covariates (eg, DNA methylation levels of CYP2R1 and CYP24A1), or vitamin D receptor, or vitamin D binding protein (39–41).

There remains considerable debate about the “gold standard” for the determination of serum 25(OH)D. We used the DiaSorin method used by most clinical laboratories. However, measuring serum 25(OH)D using recent methodology such as LC-MS/MS may result in improved sensitivity and specificity in future clinical trials when absolute concentrations, rather than relative changes, are critical to interpretation (43).

Although we attempted to control for sun exposure and season by dispensing sunscreen to all participants, and advising them to apply it daily to all sun-exposed surfaces, we were unable to determine participants’ adherence to the use of sunscreen. However, because baseline sun protection behaviors (eg, use of sun hats, long-sleeved shirts, and sunscreen), were equivalent between randomization groups, we would expect the use of sunscreen we provided to be equivalent between groups as well. Current research has not definitively demonstrated that the level of skin pigmentation affects changes in serum 25(OH)D induced by supplementation with exogenous sources of vitamin D; therefore the affect of this potential confounder was not assessed (15).

The interpretation of our results regarding the apparent clinical effectiveness of 10,000 IU of D₃/d as an appropriate repletion strategy must be qualified with our finding that only the emulsified drop had a D₃ concentration relatively consistent with its label claim of 2000 IU per dosage unit, whereas the tablet and the capsule contained higher concentrations of vitamin D₃ than were claimed on the label. This finding exemplifies the need for improved standardization in quantification of the potency of D₃ dietary supplements for both clinical practice and research. In fact, a recent study of the quality of over-the-counter D₃ dietary supplements showed similar variability (44).

Sources of dosing variability to consider in dietary supplements include the USP-NF and FDA (21CFR101.9) criteria allowing overages in D₃ dietary supplements to account for current good-manufacturing practices, degradation of raw material, and/or to ensure the label accuracy upon the expiration date, and the variability inherent in the analytical methodologies used to measure D₃ (26, 28). The fact that the oil-emulsified drop resulted in the greatest mean change in 25(OH)D despite being the lowest-dosed product in the trial is surprising and further supports the relative quality of this preparation.

Although we had only three self-reported adverse events, no cases of overt hypercalcemia, and no significant changes in either 1,25(OH)₂D or PTH, we did not assess 24-h urinary calcium excretion, which is considered a more sensitive indicator of vitamin D toxicity. Thus, conclusions regarding the safety of the repletion strategy in this trial must be interpreted accordingly.

Finally, our finding that only 82% of participants who received the oil-emulsified drop achieved D₃ “sufficiency” is surprising considering the high dose administered, the excellent overall effectiveness of this product, and that participants were instructed on proper mixing of the liquid product as instructed on the label prior to dosing. We are unable to explain this finding; however, speculative explanations for this observation may include limited adherence, poor mixing of the product, intestinal malabsorption, and/or genetic polymorphisms in vitamin D receptor, or vitamin D binding protein (39–41).

Significance

The Endocrine Society Clinical Practice Guidelines recommends a prescribed dosage of 50,000 IU Vitamin D₂ per wk for 8 wks as a safe, effective, and well-tolerated method to correct vitamin D deficiency (45). We conclude that the strategy used in this pragmatic clinical trial, dosing
commercially available vitamin D₃ dietary supplements at 10,000 IU/d for 12 wks, is another safe, effective, and well-tolerated strategy for clinical vitamin D₃ repletion in people with suboptimal 25(OH)D. There remains inherent variability in the manufacture and acceptable potency in D₃ dietary supplements based on current regulatory frameworks (26–28); therefore, periodic clinical monitoring is recommended when providers and/or their patients use D₃ dietary supplements in high-dose strategies to increase serum 25(OH)D concentration.

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