# Impact of Vitamin $D_3$ 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 $D_3$ 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 $D_3$ 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 $D_3$ content was assessed by a third party and the results adjusted based on the actual $D_3$ content administered. Mean change in 25(OH)D/mcg $D_3$ administered; difference in the proportion of $D_3$ insufficient participants (ie, 25(OH)D $\leq$30 ng/mL) reaching sufficiency (ie, 25(OH)D $\geq$30 ng/mL); and mean change in serum 1, 25-dihydroxycholecalciferol were measured.

**Results:** In two of the three products tested, the measured vitamin $D_3$ content varied considerably from the label-claimed dose. Differences in 25(OH)D/mcg $D_3$ 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 $D_3$ 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)**

The importance of cholecalciferol, ie, vitamin $D_3$ ($D_3$), in human health conditions has gained increased research attention in recent years. As a result of observational findings suggesting increased health risk with low serum 25-hydroxycholecalciferol (25(OH)D) (1–11), health care providers of all categories are measuring serum 25(OH)D more frequently, and providers and patients are faced with choices in treatment to achieve repletion when low status is identified, including prescription and over-the-counter dietary supplement forms of $D_3$.

The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine suggested substantial gaps in $D_3$ research requiring attention, including the effectiveness of high-dose supplementation

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Abbreviations: AE, adverse event; ANCOVA, analysis of covariance; BMI, body mass index; BP, blood pressure; CAP, encapsulated powder; DROP, oil-emulsified drop; FDA, US Food and Drug Administration; LC-MS/MS, liquid chromatography tandem mass spectrometry; PPL, Pacific Physicians Laboratory; TAB, chewable tablet; USP-NF, US Pharmacopoeial Convention National Formulary.

strategies with structured analysis of adverse events (12–14). Recent evidence suggests high doses of $D_3$ are safe and may be necessary to achieve cholecalciferol "sufficiency" (15–17). Vitamin $D_3$ 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 $D_3$ 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 $D_2$ (23, 24) and 5–89% in the participants receiving oral $D_3$ (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 $D_3$ dietary supplements to contain no less than 90% and no more than 165% of the product label claim for cholcalciferol ($D_3$). The FDA (21CFR101.9) provides the following regulatory guidance on $D_3$ supplements: 1) $D_3$ content in the composite that is at least equal to the $D_3$ content of the label claim; 2) reasonable excesses are acceptable within current good manufacturing practices; and 3) $D_3$ 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 and willing to be randomized onto one of three $D_3$ 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 $\geq$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 dietary supplements 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 $D_3$ content of each supplement

Each of the supplements was sent to an independent analytical lab (Flora Research Laboratories) to measure the $D_3$ 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 A1c (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 $D_3$ administered, difference in proportion of participants with $D_3$ "insufficiency" (ie, 25(OH)D <30 ng/dL) reaching "sufficiency" (ie, 25(OH)D ≥30 ng/dL), and mean change in serum $1,25(OH)_2D$.

## Measurement of 25(OH)D

Serum 25(OH)D was measured at Pacific Physicians Laboratory, LLC, Lynnwood, Washington (PPL) using a chemi-luminescence immunoassay (LIAISON 25-OH Vitamin D Total Assay code 310600; DiaSorin Inc) (35). Serum $1,25(OH)_2D$ was measured by liquid chromatography tandem mass spectroscopy, using the extraction, chromatography, radioreceptor assay (Quest Diagnostics). Samples from Seattle, Washington were refrigerated and transported on the same day to PPL via courier; samples from Kailua-Kona, Hawaii, were stored on ice (−4°C) and shipped overnight directly to PPL for processing. All samples were stored for less than 24 h.

## Statistical analysis and data management

Our target sample size of 60 total participants allowed for detection of a 25% differential response between groups with 80% power at a significance level of $\alpha = 0.05$. Baseline characteristics were compared between randomization groups by ANOVA to confirm balanced allocation. ANOVA was conducted to determine whether there was a significant difference in 25(OH)D/mcg $D_3$ administered (primary outcome) and/or $1,25(OH)_2D$ (tertiary outcome) between groups, ie, $H_0$ = difference in mean change between groups = 0 ng/mL. If the null hypothesis was not accepted, we compared the final distributions of 25(OH)D and $1,25(OH)_2D$ between group pairs using Tukey's test, ie, $H_0$= difference in mean change between groups = 0 ng/mL. Analysis of covariance (ANCOVA) was performed post hoc to determine whether adjustment for baseline covariates (ie, age, sex, study site, BMI, baseline 25(OH)D, dietary intake of vitamin D, sun protection behavior, sun exposure, and enrollment season) modified the primary results. For both adjusted and unadjusted analyses, we calculated our primary outcome based on the actual (ie, not label-claimed) dose of vitamin D administered to each group by calculating the change in 25(OH)D in ng/mL for each participant and dividing by the actual vitamin D dose in micrograms they received based on the results of third-party measurement of each supplement. Actual vitamin D dose was calculated by averaging the results from pretrial and posttrial triplicate measurements of $D_3$ in each supplement, conducted by a third-party laboratory. Because two participants in the study reported adverse events and required a dose reduction, we calculated the average dose over the course of the entire study for these participants based on the number of days they took the full dose of 10,000 IU daily and the number of days they took the reduced dose of 6,000 IU daily to generate a time-weighted average dose for these two participants. We then calculated the "mean change in 25(OH)D ng/mL per mcg vitamin $D_3$ administered" for the entire group. To determine the proportion of participants with baseline $D_3$ "insufficiency" who reached "sufficiency" at follow-up (secondary outcome), we applied two definitions of "insufficiency, (ie, both as defined by our clinical laboratory [ie, 25(OH)D <33 ng/dL] and as defined by the Institute of Medicine [ie, 25(OH)D <30 ng/dL]). Proportions were compared between groups using Fisher's exact test.

Study data were managed using REDCap v4.0 (Research Electronic Data Capture, Vanderbilt University) hosted at the

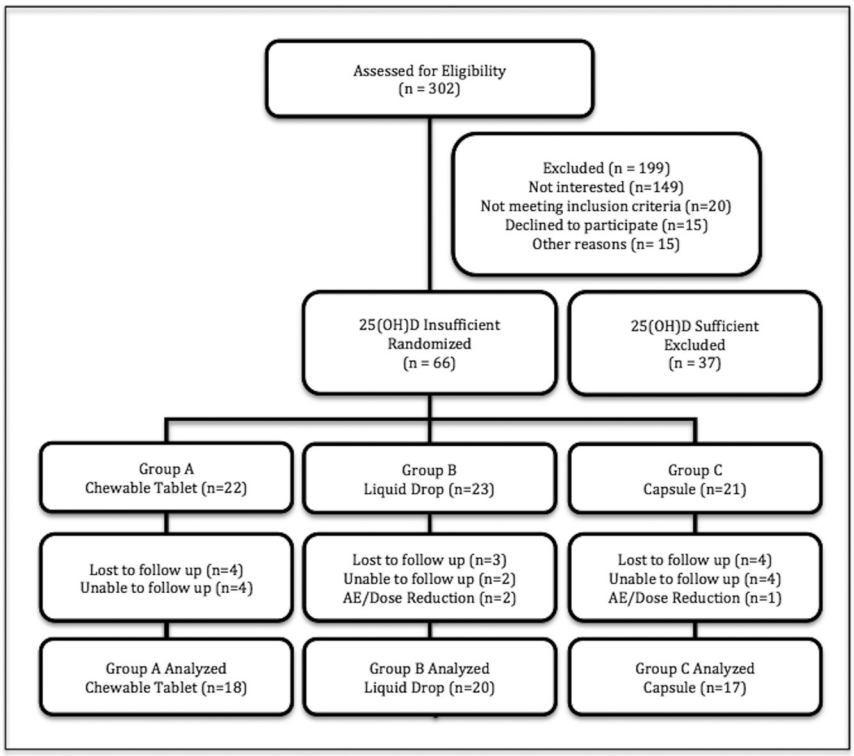


**Figure 1.** CONSORT flow diagram of three-arm comparative effectiveness trial.

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

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## 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)

**Table 1.** Baseline Characteristics

| Group Characteristic | All (n = 55) Mean (SD) or Frequency, % | TAB (n = 18) Mean (SD) or Frequency, % | DROP (n = 20) Mean (SD) or Frequency, % | CAP (n = 17) Mean (SD) or Frequency, % | Group Comparison *P* Value |
|---|---|---|---|---|---|
| Study Site, % from WA | 75 | 72 | 75 | 76 | .43 |
| Age, y | 39.9 (13.7) | 40.3 (13.5) | 39.5 (13.6) | 39.9 (14.7) | .98 |
| Female, % | 85 | 83 | 85 | 88 | .92 |
| Systolic BP, mm Hg | 111.3 (12.7) | 112.4 (11.2) | 107.7 (9.8) | 114.4 (16.4) | .25 |
| Diastolic BP, mm Hg | 73.1 (9.5) | 75.9 (9.4) | 69.8 (9.9) | 73.8 (8.5) | .13 |
| Height, in | 65.8 (3.1) | 65.5 (3.0) | 65.5 (3.1) | 66.4 (3.4) | .67 |
| Weight, lbs | 161.5 (39.6) | 168.9 (46.9) | 154.8 (27.6) | 161.6 (44.1) | .56 |
| BMI, $kg/m^2$ | 26.1 (5.3) | 27.4 (6.5) | 25.2 (4.0) | 25.6 (5.2) | .39 |
| Dietary Vitamin D intake, IU/wk | 1525 (1826) | 1545 (1933) | 1502 (1869) | 1530 (1770) | .10 |
| Sun exposure, h/wk | 9 (10) | 10 (13) | 10 (10) | 8 (5) | .73 |
| Sun protection, % using | 76 | 83 | 60 | 88 | .09 |
| PTH[a], pg/mL | 36.6 (51.9) | 21.2 (19.3) | 53.2 (78.3) | 31.7 (25.7) | .26 |
| Baseline 25(OH)D, ng/mL | 22.6 (6.7) | 21.9 (7.8) | 24.2 (5.1) | 21.5 (7.2) | .41 |

[a] PTH measured in Seattle site samples only: total, n = 40; $n_{TAB}$ = 12; $n_{DROP}$ = 15; $n_{CAP}$ = 13.

**Table 2.** Mean Changes in Total Serum 25-Hydroxycholecalciferol (25(OH)D)

| | 25(OH)D | | | | |
|---|---|---|---|---|---|
| Group | Baseline Mean (SD), ng/mL | Week 12 Mean (SD), ng/mL | Mean (SD) Change, ng/mL | 95% Confidence Interval | *P* Value for Change[a] |
| TAB (n = 18) | 21.9 (7.8) | 55.1 (15.2) | +33.3 (5.7) | 21.9–44.6 | <.0001 |
| DROP (n = 20) | 24.2 (5.1) | 58.6 (27.4) | +34.4 (5.4) | 23.6–45.1 | <.0001 |
| CAP (n = 17) | 21.5 (7.2) | 75.1 (22.8) | +53.6 (5.8) | 41.9–65.2 | <.0001 |

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

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 $D_3$

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).

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

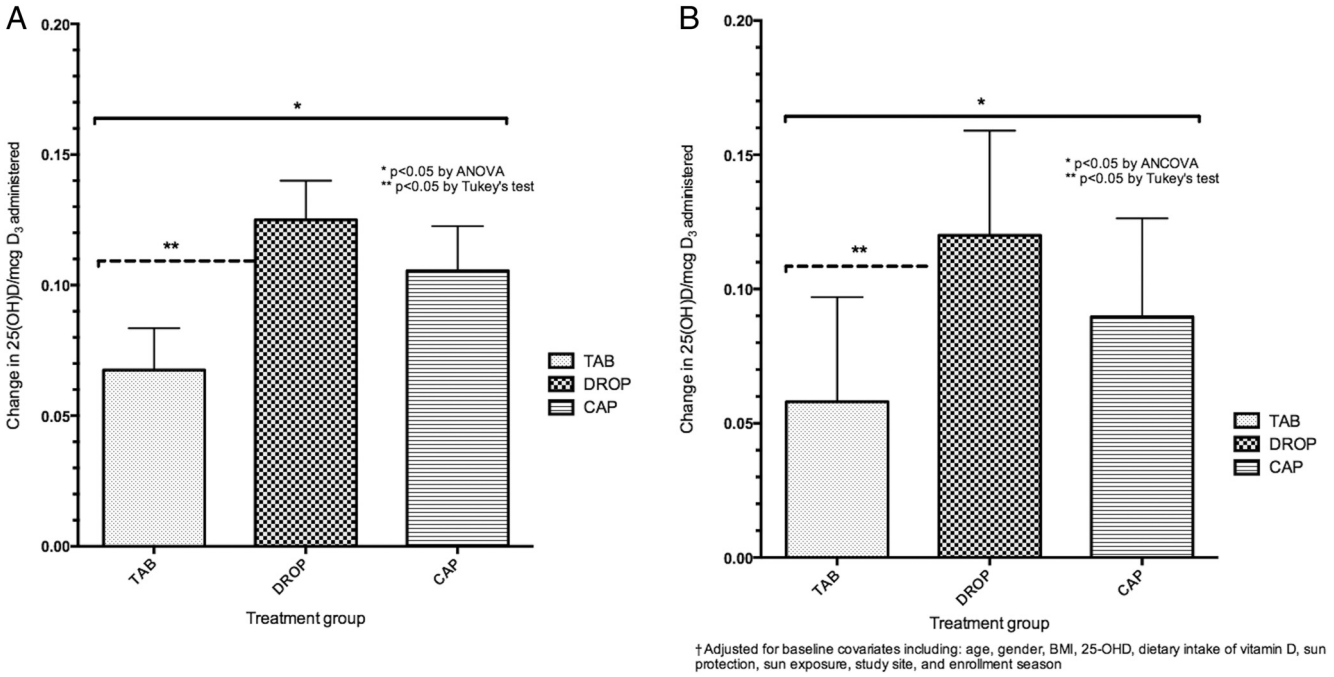


**Figure 2.** A) Unadjusted between group changes in serum 25-hydroxycholecalciferol concentration ng/mL/mcg vitamin $D_3$ administered (ng/mL 25(OH)D/mcg $D_3$). B) Adjusted between group changes in serum 25-hydroxycholecalciferol concentration ng/mL/mcg vitamin $D_3$ administered (ng/mL 25(OH)D/mcg $D_3$).

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 $D_3$ "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 $D_3$ "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)$_2$D

There were no clinically or statistically significant changes in 1,25(OH)$_2$D 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 $D_3$/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 $D_3$ 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 $D_3$ 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 $D_3$ 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 $D_3$ 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)$_2$D)[a]

| | 1,25 (OH)$_2$ D | | | | |
|---|---|---|---|---|---|
| Group | Baseline Mean (SD), ng/mL | Week 12 Mean (SD), ng/mL | Difference Mean (SD), ng/mL | 95% Confidence Interval | *P* Value[b] |
| TAB (n = 16) | 41.4 (2.5) | 44.9 (4.3) | +3.5 (3.9) | −11.9, 4.9 | >.05 |
| DROP (n = 19) | 43.4 (3.2) | 43.2 (2.9) | −0.2 (4.0) | −8.2, 8.5 | >.05 |
| CAP (n = 17) | 42.8 (3.7) | 39.0 (2.5) | −3.8 (4.3) | −5.4, 12.9 | >.05 |

[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 $P > .05$.

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 $D_3$ 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 $D_3$, 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 polymorphism status, which may have also predicted some of the observed variation in response (39–42).

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 $D_3$/d as an appropriate repletion strategy must be qualified with our finding that only the emulsified drop had a $D_3$ concentration relatively consistent with its label claim of 2000 IU per dosage unit, whereaas the tablet and the capsule contained higher concentrations of vitamin $D_3$ than were claimed on the label. This finding exemplifies the need for improved standardization in quantification of the potency of $D_3$ dietary supplements for both clinical practice and research. In fact, a recent study of the quality of over-the-counter $D_3$ 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 $D_3$ 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 $D_3$ (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 $D_3$ "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 $D_2$ 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 $D_3$ dietary supplements at 10,000 IU/d for 12 wks, is another safe, effective, and well-tolerated strategy for clinical vitamin $D_3$ repletion in people with suboptimal 25(OH)D. There remains inherent variability in the manufacture and acceptable potency in $D_3$ dietary supplements based on current regulatory frameworks (26–28); therefore, periodic clinical monitoring is recommended when providers and/or their patients use $D_3$ dietary supplements in high-dose strategies to increase serum 25(OH)D concentration.

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## References

1. **Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM.** Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet*. 2001;358:1500–1503.
2. **Pittas AG, Dawson-Hughes B, Li T, et al.** Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care*. 2006; 29:650–656.
3. **Levin A, Bakris GL, Molitch M, et al.** Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int*. 2007;71:31–38.
4. **Taskapan H, Wei M, Oreopoulos DG.** 25(OH) vitamin D3 in patients with chronic kidney disease and those on dialysis: rediscovering its importance. *Int Urol Nephrol*. 2006;38:323–329.
5. **Holick MF.** Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr*. 2004;80:1678S–1688S.
6. **Giovannucci E.** The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). *Cancer Causes Control*. 2005;16:83–95.
7. **Pilz S, Tomaschitz A, Obermayer-Pietsch B, Dobnig H, Pieber TR.** Epidemiology of vitamin D insufficiency and cancer mortality. *Anticancer Res*. 2009;29:3699–3704.
8. **Giovannucci E.** Epidemiology of vitamin D and colorectal cancer: casual or causal link? *J Steroid Biochem Mol Biol*. 2010;121:349–354.
9. **Wei MY, Garland CF, Gorham ED, Mohr SB, Giovannucci E.** Vitamin D and prevention of colorectal adenoma: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2008;17:2958–2969.
10. **Autier P, Gandini S.** Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *AMA Arch Intern Med*. 2007;167:1730–1737.
11. **Melamed ML, Michos ED, Post W, Astor B.** 25-hydroxyvitamin D levels and the risk of mortality in the general population. *AMA Arch Intern Med*. 2008;168:1629–1637.
12. **Ross AC, Manson JE, Abrams SA, et al.** The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J BIOMED INFORM*. 2011;96:53–58.
13. **Brannon PM, Yetley EA, Bailey RL, Picciano MF.** Overview of the conference "Vitamin D and Health in the 21st Century: an Update." *Am J Clin Nutr*. 2008;88:483S–490S.
14. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC, Taylor CL, Yaktine AL, and Del Valle HB, Eds. Washington (DC): National Academies Press (US); 2011.
15. **Aloia JF, Patel M, Dimaano R, et al.** Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr*. 2008;87:1952–1958.
16. **Talwar SA, Aloia JF, Pollack S, Yeh JK.** Dose response to vitamin D supplementation among postmenopausal African American women. *Am J Clin Nutr*. 2007;86:1657–1662.
17. **Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B.** Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr*. 2006;84:18–28.
18. **Vieth R, Ladak Y, Walfish PG.** Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. *J BIOMED INFORM*. 2003;88:185–191.
19. **Vieth R.** Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr*. 1999;69:842–856.
20. **Giovannucci E, Liu Y, Hollis BW, Rimm EB.** 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *AMA Arch Intern Med*. 2008;168:1174–1180.
21. **Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ.** Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr*. 2003;77:204–210.
22. **Chel V, Wijnhoven HA, Smit JH, Ooms M, Lips P.** Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. *Osteoporos Int*. 2008;19:663–671.
23. **Pietras SM, Obayan BK, Cai MH, Holick MF.** Vitamin D2 treat-

ment for vitamin D deficiency and insufficiency for up to 6 years. *AMA Arch Intern Med*. 2009;169:1806–1808.
24. **Saadi HF, Dawodu A, Afandi BO, Zayed R, Benedict S, Nagelkerke N.** Efficacy of daily and monthly high-dose calciferol in vitamin D-deficient nulliparous and lactating women. *Am J Clin Nutr*. 2007; 85:1565–1571.
25. **Leventis P, Kiely PD.** The tolerability and biochemical effects of high-dose bolus vitamin D2 and D3 supplementation in patients with vitamin D insufficiency. *Scand J Rheumatol*. 2009;38:149–153.
26. **United States Pharmacopeial Convention.** USP 35-NF 30: Dietary Supplements Compendium. Rockville, MD: United States Pharmacopeial Convention. 2012;1468–1510.
27. **Miller RK, Celestino C, Giancaspro GI, Williams RL.** FDA's dietary supplement CGMPs: standards without standardization. *Food Drug Law J*. 2008;63:929–942.
28. **FDA, U.S.** Code of Federal Regulations. In: Title 21, Volume 2, Part 101.9. D.o.H.a.H. Services, editor: 2012.
29. **Ilahi M, Armas LA, Heaney RP.** Pharmacokinetics of a single, large dose of cholecalciferol. *Am J Clin Nutr*. 2008;87:688–691.
30. **Gough H, Goggin T, Bissessar A, Baker M, Crowley M, Callaghan N.** A comparative study of the relative influence of different anticonvulsant drugs, UV exposure and diet on vitamin D and calcium metabolism in out-patients with epilepsy. *Q J Med*. 1986;59:569–577.
31. **Taylor C, Lamparello B, Kruczek K, Anderson EJ, Hubbard J, Misra M.** Validation of a food frequency questionnaire for determining calcium and vitamin D intake by adolescent girls with anorexia nervosa. *J Am Diet Assoc*. 2009;109:479–485, 485 e471–473.
32. **Douglass JPJ.** Bowes and Church's Food Values of Portions Commonly Used. Philadelphia: Lippincott Williams & Wilkins: 2004.
33. **Nutrient-Data-Laboratory.** USDA Nutrient Database for Standard Reference, Release 16. A.R.S. U.S. Department of Agriculture, editor: 2003.
34. **Glanz K, Yaroch AL, Dancel M, et al.** Measures of sun exposure and sun protection practices for behavioral and epidemiologic research. *AMA Arch Derm Syphilol*. 2008;144:217–222.
35. **Ersfeld DL, Rao DS, Body JJ, et al.** Analytical and clinical validation of the 25 OH vitamin D assay for the LIAISON automated analyzer. *J Clin Biochem Nutr*. 2004;37:867–874.
36. **Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG.** Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377–381.
37. **Zhao LJ, Zhou Y, Bu F, et al.** Factors predicting vitamin D response variation in non-Hispanic white postmenopausal women. *J BIOMED INFORM*. 2012;97:2699–2705.
38. **Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF.** Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr*. 2000;72: 690–693.
39. **Elnenaei MO, Chandra R, Mangion T, Moniz C.** Genomic and metabolomic patterns segregate with responses to calcium and vitamin D supplementation. *Br J Nutr*. 2011;105:71–79.
40. **Larcombe L, Mookherjee N, Slater J, et al.** Vitamin D in a northern Canadian first nation population: dietary intake, serum concentrations and functional gene polymorphisms. *PLoS One*. 2012;7: e49872.
41. **Muindi JR, Adjei AA, Wu ZR, et al.** Serum vitamin D metabolites in colorectal cancer patients receiving cholecalciferol supplementation: correlation with polymorphisms in the vitamin D genes. *Horm Cancer*. 2013;4:242–250.
42. **Zhou Y, Zhao LJ, Xu X, et al.** DNA methylation levels of CYP2R1 and CYP24A1 predict vitamin D response variation [published online October 12, 2013]. *J Steroid Biochem Mol Biol*. doi:10.1016/j.jsbmb.2013.10.004.
43. **Phinney KW.** Development of a standard reference material for vitamin D in serum. *Am J Clin Nutr*. 2008;88:511S–512S.
44. **LeBlanc ES, Perrin N, Johnson JD Jr, Ballatore A, Hillier T.** Over-the-counter and compounded vitamin d: Is potency what we expect? *JAMA Intern Med*. 2013:585–586.
45. **Holick MF, Binkley NC, Bischoff-Ferrari HA, et al.** Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guidelineEvaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Biomed Inform*. 2011;96:1911–1930.