Strategies for Repletion of Vitamin D Nutritional Deficiency: A Comparative Effectiveness Trial of High-quality Vitamin D_3 Nutritional Supplements to Replete Serum Vitamin D

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Abstract

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Background: Vitamin insufficiency and deficiency may be associated with increased risk for cardiovascular disease, diabetes, some cancers and all cause mortality. Practice guidelines define sufficient vitamin D status as a serum 25-hydroxycholecalciferol (25-OHD) concentration >30 ng/mL (70 nmol/L). Supplementation strategies to safely achieve vitamin D sufficiency are needed. **Objective:** We evaluated the change in serum 25-OHD concentrations after supplementation with three commercially available vitamin D_3 products with different vehicles of delivery: a chewable tablet; a liquid drop; and a encapsulated powder. **Methods:** The clinical trial design was a prospective, randomized, pragmatic, comparative effectiveness study of vitamin D_3 supplementation. We

conducted third-party analysis of the three commercially available vitamin D_3 supplements used in this trial. Subjects were provisioned informed consent and randomized to one of the three vitamin D_3 supplements, dosed at 10,000 IU per day for 12 weeks, per label claim. **Results:** Eligible subjects (n=66) were enrolled in the study, of which 11 subjects were lost to follow up (17%). 100% of the subjects in the tablet and capsule allocation arms attained sufficient vitamin D status, compared to 80% in the oil drop arm. The mean (±SD) change in 25-OHD (ng/mL) from baseline to week 12 reached the level of statistical significance ($p<0.001$) for each of the three allocation groups: tablet (n=18); 31.8 (7.6); oil drop (n=20); 32.2 (8.0); and capsule (n=17); 48.1 (8.1). Between group changes in serum 25-OHD were also significant (ANOVA; $p<0.05$). While pairwise-comparisons of changes in serum 25-OHD showed a statistically significant difference, distinguishing Group C from Groups A and B (Tukey's Test; $p<0.05$). Three non-serious adverse events occurred during the study, without observed hypercalcemia or hypervitaminosis D. **Discussion:** We observed variability in vitamin D_3 concentration in the three supplements, with the tablet and capsule showing increased D_3 concentration, compared to label claim. The majority of subjects (93%) achieved sufficient 25-OHD status at the end of the study. We conclude that 10,000 IU vitamin D_3 per day for 12-weeks may be a safe and effective strategy for vitamin D supplementation to correct nutritional deficiency in 25-OHD insufficient populations. This trial was registered at clinicaltrials.gov as NCT01524874.

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3 Introduction

3.1 Background

Vitamin D (calciferol) is a fat-soluble vitamin first described by Sir Edward Mellanby in 1919 for its role in the etiology of rickets.[1] Vitamin D has since been described as a secosterol, which acts as a prohormone on the vitamin D receptors (VDR) in tissues throughout the body. Vitamin D was originally identified as a factor needed to treat rickets and is now known to play an important role in the regulation of the metabolism of calcium, phosphate and magnesium, and bone metabolism.[1-3] Vitamin D is also thought to exhibit pleotropic effects on tissues throughout the body, through control of over 200 genes involved in the promotion of cellular apoptosis, terminal cellular differentiation, angiogenesis, and immunomodulation.[4,5]

3.1.1 Sources of Vitamin D

In humans, vitamin D_3 (cholecalciferol) is primarily made endogenously via cutaneous sunlight exposure.[6,7] Vitamin D_2 (ergocalciferol) is formed upon exposure of ergosterol, a component of the cell wall of in yeast or fungi, to sunlight or ultraviolet (UV) light.[8-10]

The primary food sources of vitamin D include: fatty fish, liver and egg yolks (vitamin D_3); and mushrooms and yeast (vitamin D_2).[11,12] Vitamin D_3 and D_2 may be used interchangeably to fortify foods in the United States.[10] Oral ingestion, via diet or supplementation, is an increasingly important source of vitamin D in populations at northern latitudes, with inadequate sun exposure or food

fortification, and with darker skin pigmentation.[12] There is disagreement on the biological equivalence of vitamin D_3 and D_2 forms.[9,13] As well, there are still questions looming about the optimal vehicle of delivery of vitamin D, whether from the sun,[14] or in food or nutritional supplements.[15]

3.1.2 Vitamin D Nutritional Deficiency

Vitamin D deficiency is prevalent in children, adults, elderly and nursing mothers worldwide. Vitamin D deficiency is defined as serum 25-hydroxycholecalciferol (25-OHD) concentrations less than 20 ng/mL (50 nmol/mL) and insufficiency as less than 30 ng/mL (75 nmol/ml).[5,11] Risk factors known to influence vitamin D status include: advanced age, sun exposure, use of sunscreen (SPF >15), skin pigmentation, air pollution, prolonged breast feeding of a child, northern latitudes, smoking status, obesity, malabsorption, and medication use.[16,17]

There is a growing body of research showing associations between 25-OHD insufficiency and risk for cardiovascular disease,[18-21] diabetes,[22-24] colon, prostate and breast cancers,[25-28] and all cause mortality.[29,30] One study suggested an inverse association between serum vitamin D levels and risk of pancreatic cancer.[31] There seems to be no clear association between vitamin D status and atrial fibrillation but a suggestion that vitamin D toxicity may result in atrial fibrillation.[32]

The prevalence of vitamin D deficiency and insufficiency increased from 1998-2004.[33] Vitamin D deficiency is recognized as a readily treatable nutritional

deficiency that affects between 30% and 50% of the U.S. population.[11,34] To ensure that the majority of individuals maintain sufficient vitamin D status, the Institute of Medicine set a recommended daily allowance (RDA) of vitamin D of 600 IU/d for children and 800 IU/d for adults, with respective upper limits (UL) set at 2,500 IU/d and 4,000 IU/d.[2]

3.1.3 Strategies to Correct Vitamin D Deficiency

Supplemental dosages from 400 IU per day to a single time administration of 300,000 IU have been reported in the literature.[13,35-39] The safety of taking vitamin D_3 dosages of up to 10,000 IU/day have been well established, but dosages over 40,000 IU/d have been associated with vitamin D toxicity.[18,40,41]

Table 3.1: Studies of effectiveness of vitamin D supplementation to correct nutritional deficiency

Study	N	Female (%)	Mean Age	Vit D Form	Dosage	Duration	Baseline 25-OHD (ng/mL)	Follow-up 25-OHD (ng/mL)	Difference (p-value)	Optimal* (%)
Elderly										
Chel, 2007[37]	55	83.6	84.3 (6.3)	Oral $Vit D_3$	600 IU/day	4 mos	23.0 (8.3)	69.9 (17.8)	p<0.001	37%
	54	72.2	84.3 (6.4)		4200 IU/wk		27.3 (12.7)	67.2 (14.0)	p<0.001	28%
	57	73.7	83.9 (6.9)		18,000 IU/mos		23.8 (8.0)	53.1 (15.9)	p<0.001	5%
Adults										
Pietras, 2009[13]	86	79	61 (18-91)	Oral $Vit D_2$	50,000 IU/wk	8 wks	19.3 (6.2)	37.2 (13.0)	p<0.001	86%
Saadi, 2007[38]	25	100	29.2 (5.5)	Oral $Vit D_2$	2,000 IU/day	3 mos	27.3 (10.4)	42.2 (13.9)	p<0.01	36%[a]
	25	100	29.9 (6.7)	Oral $Vit D_2$	60,000 IU/mos	3 mos	23.2 (10.7)	37.6 (10.5)	p<0.01	33%[a]
Leventis, 2009[39]	50	86	53 (29-82)	I.M. $Vit D_2$	300,000 IU dose	single	20.3 (17–35)	32.3 (17–49)	p>0.05	0%[b]
	19	79	43 (23-72)	Oral $Vit D_3$	300,000 IU dose	single	27.0 (5–40)	81.8 (47–119)	p<0.001	89%[b]

* percent (%) reaching optimal vitamin D levels, defined as >30 ng/mL (75nmol/L); [a]optimal defined as >20 ng/mL (50 nmol/L); [b]optimal defined as >16 ng/mL (40 nmol/L) I.M. intra-muscular

A representative sample of clinical trials evaluating the effectiveness of supplementation strategies to correct vitamin D nutritional insufficiency are reported in Table 3.1. The total IU administered per duration included: 72,000 IU vitamin D_3 over 4 months;[37] 400,000 IU vitamin D_2 over 8 weeks;[13] 180,000 IU vitamin D_2 over three months;[38] and single I.M. dose of vitamin D_2 (300,000 IU) or a single oral dose of vitamin D_3 (300,000 IU).[39] No incidences of hypercalcemia or vitamin D toxicity were observed in these study participants. Efficacy, reported as percentage of study participants achieving "optimal" vitamin D status ranged from 33% to 86% in the participants receiving oral vitamin D_2 and from 5% to 89% in the participants receiving oral vitamin D_3.

Safe and effective strategies are needed to replenish vitamin D deficiency and insufficiency in at-risk populations. However, there remains much debate around the optimal strategy to correct vitamin D nutritional deficiencies.

3.2 Objective

The Institute of Medicine report on the Dietary Reference Intakes for Calcium and Vitamin D suggests substantial gaps in vitamin D research in hazard identification and characterization, including: determining the quality of available vitamin D supplements, structured analysis of adverse events associated with high-dose vitamin D supplementation, and the assessment of the effectiveness of high-dose supplementation strategies.[1,42]

Our study will conduct a interventional study of three vitamin D_3 supplements to evaluate product quality and evaluate the effectiveness and safety of a high-dose vitamin D intervention to correct nutritional deficiencies in vitamin D insufficient populaitons.

3.2.1 Phase I – Determination of Quality of Vitamin D Supplements

Phase I of this study will establish the variability in quality of three vitamin D_3 supplements commonly prescribed: a lipid-emulsified drop; a encapsulated powder; and a chewable tablet.

3.2.2 Phase II – Comparative Effectiveness Trial of Vitamin D

Phase II of this study is an open-label, intent-to-treat comparative effectiveness trial designed to determine the effects of random assignment of one of three vitamin D_3 supplements to correct vitamin D insufficiency. The outcome measures will be comparison of serum 25-OHD and 1,25-dihydroxycholecalciferol (1,25-OH_2D) between the three arms at baseline and after random administration of one of the three vitamin D_3 preparations for 12-weeks at a dosage of 10,000 IU vitamin D per day.

4 Phase I – Determination of Quality of Vitamin D Supplements

4.1 Methods

4.1.1 Specific Aims

4.1.1.1 Primary Aim

To evaluate the vitamin D concentration and variability in each of three vitamin D_3 supplements.

4.1.1.2 Secondary Aim

To evaluate the percent label claim of vitamin D present in each of three vitamin D_3 supplements.

4.1.2 Vitamin D_3 Supplements and Manufacurer Specifications

Commonly used Vitamin D_3 nutritional supplements were donated by three different manufacturers: a lipid emulsified drop, by Biotics Research Corporation, Rosenberg, Texas; an encapsulated powder, by Integrative Therapeutics Incorporated, Green Bay, Wisconsin; and a chewable tablet, by Vital Nutrients Inc., Middletown, Connecticut. The label claim of each product was 2,000 IU per dosage unit (i.e., one drop, one tablet or one capsule). The results of the manufacturers' internal Certificates of Analysis (CoA) are represented below in Table 4.1.

Based on the manufacturers' internal CoA, the drops contained 92 to 114%; the tablets contained 134%; and the capsules contained 154% of the label claim (2,000 IU per dosage unit), respectively (Table 4.1).

Table 4.1: Manufacturers' internal certificates of analysis of vitamin D_3 concentration of three supplements

Product Supplier	Matrix	Lot No.	Exp. Date	Value (IU)	% Label Claim	Preparation/ Method
Biotics Research Corporation	Drop-1 (liquid)	34030	05/2011	1,849	92 %	1 drop (0.04 mL)/ HPLC
	Drop-2 (liquid)	34875A	01/2012	2,288	114 %	1 drop (0.04 mL)/ HPLC
Integrative Therapeutics Inc.	Tablet (chewable)	97982	07/2011	2,670	134 %	20-sample composite/LCMS
Vital Nutrients	Capsule (powder)	9E12	07/2012	3,072	154 %	20-sample composite/LCMS

Note: high-performance liquid chromatography (HPLC); liquid chromatography mass spectrometry (LCMS)

4.1.3 Sample Preparation and Extraction Procedures

Vitamin D_3 is degraded by acid, light, and water. It is important to keep samples away from light exposure and minimize air exposure from the time that sample matrix is composited forward.

4.1.3.1 Tablet and Capsule Preparation

The tablet and capsule study samples were taken from a 20-unit composite of the tablet or capsule, respectively, ground by hand with a mortar and pestle and prepared under minimal light and air exposure. The mass of one dosage unit (based on the mean mass of twenty dosage units) was obtained from the each 20-unit composite, respectively. Each sample was analyzed in triplicate, with an additional spike recovery, and the results were then summarized by descriptive statistics including the mean, standard deviation (SD), residual standard deviation (RSD), and percent spike recovery. Comparisons were then made to the original label claim and the respective CoA provided by each manufacturer.

4.1.3.2 Liquid Drop Preparation

The liquid drop study samples were prepared in triplicate, with one additional spike sample, made from a single dispensed drop unit per sample. The presence of the micro-emulsion of vitamin D_3 required modified sample preparation, to dissolve the microencapsulating shell. Drops containing a micro emulsion of D_3 were placed directly into a 50mL volumetric flask made form low actinic glass. The product containers were well shaken to mix thoroughly and immediately dispensed. Additional preparation of the drop samples included adding 1mL of dimethyl sulfoxide (DMSO; Fluka, USA Biochemical Grade) to dissolve the encapsulation coating on the D_3 contained in the drop so that it could be extracted into solution. The solution containing the drop and solvate was swirled to mix completely.

4.1.3.3 General Sample Preparation

To each study sample and spike sample, we added 10mL of 1mg/mL aqueous ascorbic acid solution (ascorbic acid Fluka, St. Louis, MO, water Barnstead Type 1 Water Generator) and sonicated for 5 minutes covered (Equatherm Sonicator, Fisons, USA); we then added 5mL of acetone (Chromasolv, Sigma-Aldrich, St. Louis, MO) swirled to mix and sonicated again for 5 minutes covered. After allowing the flasks to equilibrate to room temperature, the contents were diluted to volume with acetontrile (EMD, USA HPLC Grade) containing 0.5mg/mL Butylated hydroxyanisole and butylated hydroxytoluene (BHT/BHA; reagent grade Sigma-Aldrich St. Louis, MO). The ascorbic acid in water and the BHT/BHA in the acetonitrile are present to help prevent decomposition of the D_3

during sample preparation. Each sample was capped and mixed by inverting at least three times and allowed to settle for 10 minutes. Each sample was then filtered through 0.45µM x 25mm PVDF filters (Macherney-Nagel, USA), discarding first 2 mL, and collecting next 1mL in amber auto-sampler vials with Teflon-lined red rubber-septum snap cap (National Scientific, USA).

4.1.3.4 Spike Preparation

The spike sample was prepared by adding 50 µL of 1000 µg/mL D_3 standard (D_3 standard prepared from neat vitamin D_3 supplied by Accustandard, USA diluted in acetonitrile solution containing BHA/BHT). The spike was added to the spike replicate of each sample matrix analyzed (i.e. drop, capsule and tablet).

4.1.4 Instrumentation and Parameters

Samples were introduced for analysis on Ion-Trap Liquid Chromatograph Mass Spectrophotometer (Agilent 500 Ion-Trap LCMS) with APCI Source equipped with 212 Binary Pumps (Agilent), column heater with a thermostat (Meta-Therm), and 430 Peltier Controlled Autosampler (Agilent). We used a Dominik Hunter N2 Generator (Parker, USA) for gas supply. The column used for the analysis was a PursuitXRs C8 150mm x 4.6mm, equipped with guard cartridge (both Agilent).

4.1.4.1 Liquid Chromatograph (LC)

The LC flow rate was 1.0 mL/min, with a 15 min run time, using a mobile phase of 100% methanol (EMD, USA HPLC grade). The injection volume was 10µL. The calibration curve was established from 1.0 µg/mL to 15.0 µg/mL using $1/x^2$ linear regression with triplicate injections at each calibration point.

4.1.4.2 Liquid Chromatograph Mass Spec (LCMS) Conditions

The LCMS instrument was run under APCI positive mode conditions. The spray chamber was maintained at a temperature of 50 degrees Celcius (ºC), with nitrogen nebulizer gas at 50 pounds per square inch (psi). The drying gas was nitrogen at 350ºC and 30psi. We used vaporizer gas nitrogen at 500ºC and 60psi. The spray shield voltage was set at +600V; the corona discharge needle was set at +5µAmps; and the capillary voltage was set at +70V.

4.1.4.3 Mass Spectrometer (MS)

The Precursor ion 385.3 m/z, isolation window 3 m/z, resonant waveform, excitation storage level 118.2 m/z, excitation amplitude 1.56V, product ion scan range 118-395 m/z, RF loading 90%, quantitative Ion 367.3 m/z, qualifier ion 259.5 m/z.

4.2 Results

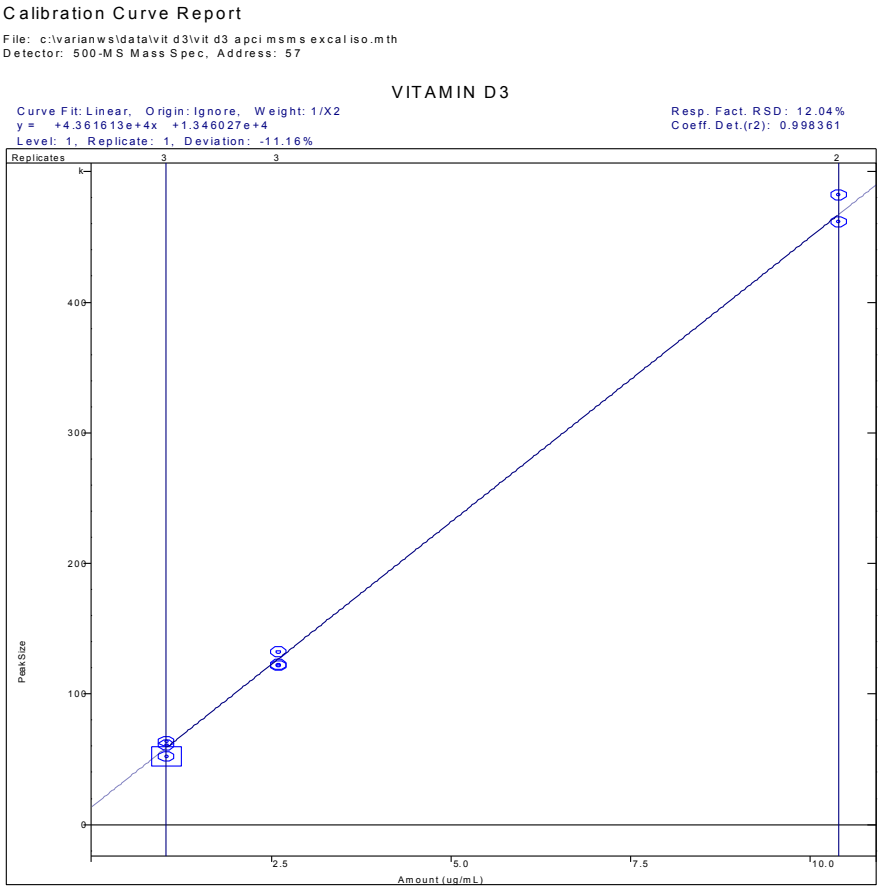
4.2.1 Quantification of Vitamin D3

The standard calibration curve for the Vitamin D_3 reference standard showed linearity (r^2 >0.998 using a 1/X2 weighted regression), with linearity established from 1.04 µg/mL to 10.4 µg/mL (Figure 4.1).

Previous experiments to determine extraction efficiency using various solvent volumes and extraction times (data not shown) demonstrated that a 50mL volume per dosage unit was adequate to extract the D_3 from the matrix. Previous experiments showed that DMSO was needed to solvate the encapsulated D_3 in the micro-emulsion. LCMS HPLC conditions were optimized

to allow sufficient time to elute matrix from each run (this is the reason for the extended run time beyond the D_3 peak).

Figure 4.1: Linear calibration curve for vitamin D_3 standard in blank reagent



4.2.2 Sample Analysis

Vitamin D_3 supplements showed wide variation in measured dose compared to label claim (Figure 4.2, Figure 4.3, Table 4.2). Replicate injections of a mid-calibration standard showed excellent precision. We suspect that the measured intra-sample variability were due to variability in the distribution of the active ingredient in the solid matrices, not due to variability in the analytical method.

Figure 4.2: Agilent LCMS chromatograms and mass spectra from 1st analytical run: A-1) drop-1; A-2) drop-2; B) tablet; C) capsule

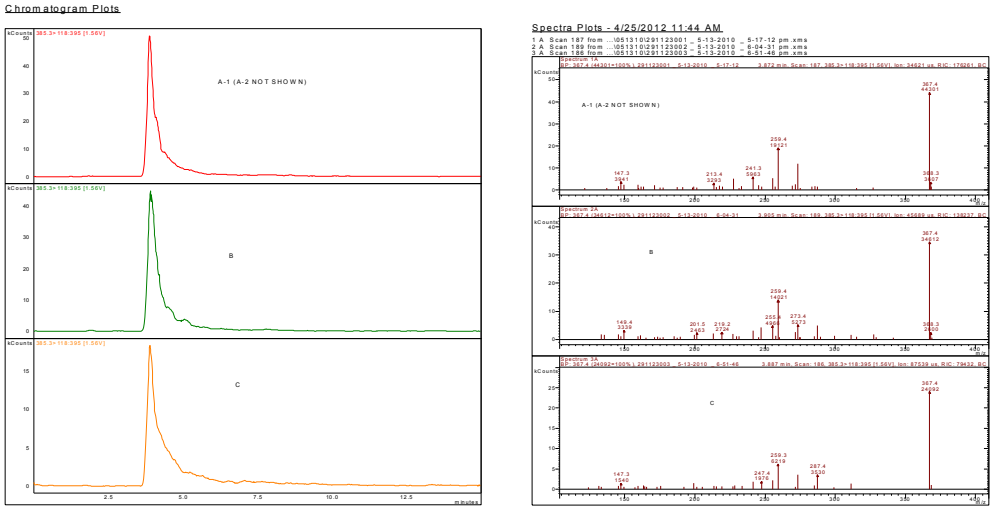


Figure 4.3: Agilent LCMS chromatograms and mass spectra from 2nd analytical run: A-2) drop-2; B) tablet; C) capsule

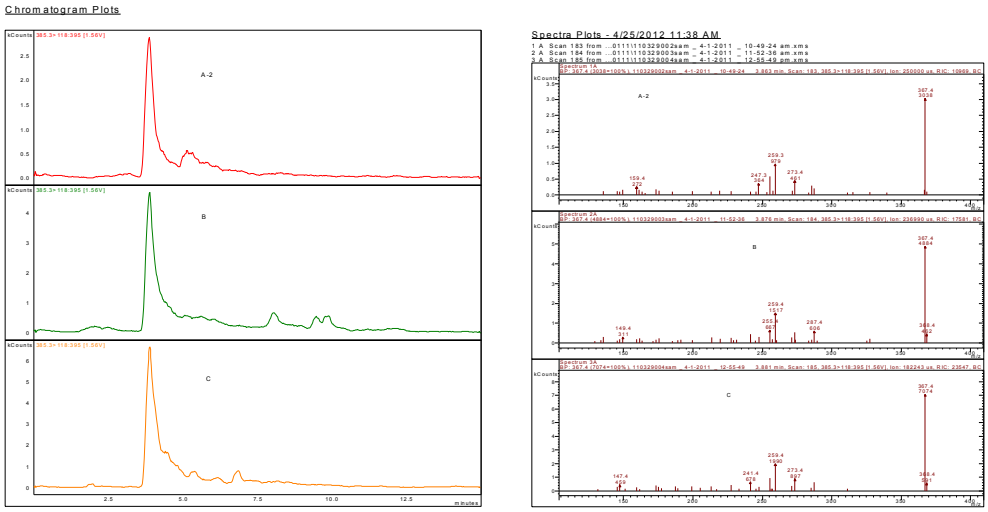


Table 4.2: Individual vitamin D_3 concentrations, in triplicate, with spike

Matrix	1st Run (IU per unit)	2nd Run (IU per unit)
Drop-1 (SAM)	1,841	2,514
Drop-1 (DUP)	2,674	2,228
Drop-1 (TRP)	1,795	2,472
Drop-1 (SPK)	3,068 (96.5%)	3,844 (72.0%)
Drop-2 (SAM)	-	1,668
Drop-2 (DUP)	-	2,852
Drop-2 (TRP)	-	1,832
Drop-2 (SPK)	-	3,800 (84.1%)
Tablet (SAM)	4,516	3,198
Tablet (DUP)	5,312	3,332
Tablet (TRP)	3,787	3,510
Tablet (SPK)	6,590 (205.2%)	8,078 (236.6%)
Capsule (SAM)	4,245	4,418
Capsule (DUP)	3,267	4,256
Capsule (TRP)	3,822	4,776
Capsule (SPK)	4,638 (86.0%)	8,850 (218.3%)
Blank	0	0

IU - International Units
SAM - sample; DUP - duplicate; TRP - triplicate; SPK - spike

4.2.3 Spike Recovery and Relative Standard Deviation

Vitamin D_3 sample preparations were spiked each with an equivalent of one dosage unit, per label claim of 2,000 IU. Spike recoveries ranged from 72% to 237%. The spike recoveries were 72 to 97% for the drops; 205% to 237% for the tablet; and 86% to 218% for the capsule (Table 4.2). It should be noted that the spike recovery is the calculated percent (%) recovery, based on the average concentration of vitamin D_3 in the three individual samples (SAM/DUP/TRP) prepared for each matrix, respectively (Table 4.2).

Table 4.3: Mean analytical results of vitamin D_3 analysis

Product	Value (IU)	% Label Claim	RSD (%)	SD (IU)	Spike Recovery (%)
		1st Analytical Run			
Drop-1	**2,103**	105 %	19.2	404	96.5
Tablet	**4,538**	227 %	13.7	623	205.2
Capsule	**3,778**	189 %	10.6	401	86.0
		2nd Analytical Run			
Drop-1	**2,405**	106 %	5.2	126	72.0
Drop-2	**2,117**	120 %	24.7	524	84.1
Tablet	**3,347**	167 %	6.5	217	236.6
Capsule	**4,483**	224 %	4.8	217	218.3

IU - international units
RSD - residual standard deviation (%)
SD - standard deviation (IU)

All three vitamin D_3 supplements measured by our research team exceeded the 2,000 IU per unit dosage claimed by the product label. The tablets and capsules were super-potent (exceeding 165% of the label claim of 2,000 IU per dosage unit), and the drops were only slightly (5 to 20%) above than the label claim (Table 4.3).

Figure 4.4: Comparison of certificate of analysis to results of third party analysis

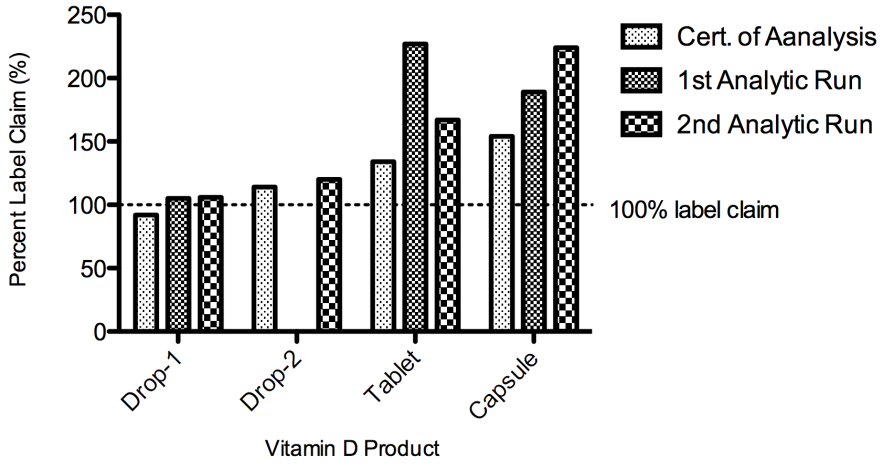
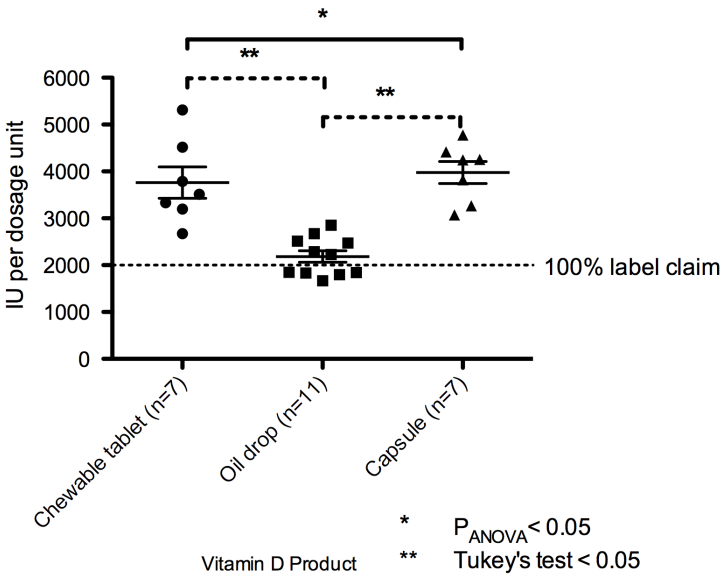


Figure 4.5: Variability of mean concentration of vitamin D_3 (IU) per dosage unit, measured by internal manufacturers' certificate of analysis and our third-party analysis



Our TPA of the vitamin D_3 concentration compared to label claim of 2000 IU per dosage unit using the Student's T-test, suggest that the mean IU in the tablet ($p<0.05$) and capsule ($p<0.001$) are significantly different from the label claim, while we were unable to reject the null hypothesis that the oil drop was different from label claim. Significance was maintained, even when the manufacturers' internal CoA were taken into account. The mean vitamin D_3 concentration per dosage unit, measured by CoA and TPA (Figure 4.5) demonstrate all three nutritional supplements tested, on average, exhibited statistically significant differences in concentration ($P<0.0001$ for within group concentration for all three products using ANOVA). However, only respective pair-wise comparisons of the

oil drop to the tablet, or the oil drop to the capsule, maintained statistical significance ($p<0.05$) using Tukey's Test (Figure 4.5).

4.3 Discussion

4.3.1 Quality of Vitamin D Supplements

Previous analyses conducted on the quality of commercially available vitamin D supplements showed that 8 of 28 (29%) failed to meet quality standards of ConsumerLab due to the incorrect amount of vitamin D (n=5), heavy metal contamination (n=2), and labeling infarctions (n=2).[43]

4.3.2 Analytical Methodologies for Vitamin D Determination

Analytical methodologies developed for homogenous food products, such as milk products and baby foods, have been applied to nutritional supplements with mixed success.[44] The reported challenges and differing opinions in the quantification of vitamin D in food products include: matrix-dependent interference;[45-47] non-homogeneity of product matrices;[45] selection of an appropriate reference standard.[48] Increasingly, methodologies using tandem mass spectroscopy are preferred for the detection of vitamin D_3, because of the sensitivity and specificity needed to quantify fat-soluble vitamins in multi-component food products.[49,50]

We developed an analytical method to determine vitamin D_3 concentrations in commercially available supplements of heterogeneous sample matrix (liquid drop; chewable tablet; encapsulated powder). Out TPA method seemed to be well suited to detecting vitamin D_3 in multi-component sample matrices. The

results suggest that there may be higher variability in solid sample matrices of the tablet and capsule, compared to the liquid drop.

4.3.3 Matrix Interference

A limitation in our methodology is the potential for nonhomogeneous distribution of active ingredient within the sample matrix, nonspecific matrix interferences and utilization of non-isotopic vitamin D_3 as the reference standard. Homogeneity and matrix effects may be more pronounced in solid matrices, compared to liquid matrices.[44,51,52] Non-homogeneity phenomena have been observed with other lipophilic vitamins in multi-component foods and nutritional supplements.[44]

We were unable to fully account for potential ion-suppression or non-homogeneity of the sample matrix, which may have influenced our analytical results for the tablet and capsule. A strategy in future studies may be to prepare calibrated spiked samples, using an isotopic standard, to control for nonspecific matrix effects in the analysis of solid matrix samples.

4.3.4 Standard Reference Materials

The cost of deuterated (isotopic) vitamin D_3 does not allow its use as an internal standard (ISTD) in routine sample preparation. One (1) mg of deuterated vitamin D_3 ISTD at ~$500-600 would only allow for about 10 sample preparations adding $50-$60 per sample in cost of materials for each test. Attempts to utilize vitamin K_3 as an ISTD were abandoned due to the fact that it eluted with the BHA/BHT.

In this study, we opted to run the samples using external standard (ESTD) curve, with vitamin D_3 as the reference standard. The RSD, recovery and other factors support the validity of this method. It is hoped that costs will come down in the future to allow for use of the deuterated vitamin D_3 material in routine use, which would provide for greater precision and correction of ion-suppression of signal from matrix effects.

4.3.5 Conclusions

Based on our independent TPA, the oil drop was the product most consistent with the label claim of 2000 IU per dosage unit, with respect to both the mean concentration and concentration variance. The tablet and capsule, while mutually consistent, showed supra-potency (i.e. higher mean IU vitamin D_3 per dosage unit), which was significantly different from both the label claim and the oil drop. Our results may not be generalizable to all lots of these vitamin D_3 supplements, as the concentration of vitamin D_3 in commercially available supplements may be variable.

Product potency and variability is a critical measure that deserves accurate reporting, especially in products at or near the upper end of the accepted dosing range for daily dosing. In future studies, we recommend the use of isotope standards to better correct for matrix interference. Our data highlight the need for more development in the analytical methodologies used in the determination of vitamin D in multi-component nutritional supplements.

5 Phase II – Comparative Effectiveness Trial of Vitamin D Supplementation

5.1 Methods

This research study is a 12-week randomized, pragmatic comparative effectiveness trial of vitamin D_3 supplementation to replenish nutritional deficiency.

5.1.1 Specific Aims

5.1.1.1 Primary Aim

To evaluate the change in serum 25-OHD and 1,25-OH_2D concentrations between treatment arms

5.1.1.2 Secondary Aim

To compare the proportion of participants reaching "optimal" 25-OHD concentration (i.e. ≥33 ng/ml) between treatment arms

5.1.1.3 Tertiary Aim

To evaluate the change in serum 25-OHD concentrations between treatment arms by actual IU administered, compared to label claim, based on results of our third-party analysis of vitamin D_3 supplements used in the study

5.1.2 Study Setting

The study was carried out at three clinical settings: Bastyr Center for Natural Health: Seattle, Washington; Bastyr Clinical Research Center: Kenmore, Washington; and Lokahi Health Center: Kailua Kona, Hawaii. Recruitment included patients at Lokahi Health Center and Bastyr University, as well as outreach to the greater community of Seattle, Washington.

5.1.3 Study Subjects

For both clinical sites, patients who were screened for 25-OHD status as an element of their routine clinical care were invited to participate. Candidate participants were informed about the ongoing study and asked if they want to be screened for participation, at the discretion of their physician. The target enrollment was N=45 for the Bastyr University sites and; n=15 for the Lokahi site. The study was approved to recruit N=90 to provision for unexpected withdrawal or loss to follow up.

5.1.4 Inclusion and Exclusion Criteria

5.1.4.1 Inclusion Criteria

Study participants agreed to be provisioned informed consent in English. We recruited subjects 18-65 years of age; since there is reported age-related decline in the absorption, transport or liver hydroxylation of orally-consumed vitamin D,[36] adults older than 65 were excluded. Study participants agreed to perform baseline screening and 12-week follow-up tests to measure serum 25-OHD and 1,25-OH_2D, a comprehensive metabolic panel and complete blood count, and were willingly randomized to one of three active treatments for 3 months. Screening serum 25-OHD <33 ng/ml (82.5 nmol/ml).

5.1.4.2 Exclusion Criteria

- Study participants were excluded upon screening if they had a baseline serum 25-OHD >=33 ng/ml (82.5 nmol/ml) or had extra-dietary use of vitamin D (>1,000 IU per day) currently or for the previous 3 months. Study participants were also excluded if their laboratory results met the following criteria: aspartate

transaminase (AST)>60 U/L; alanine transaminase (ALT)>65 U/L; alkaline phosphatase >120 U/L; total bilirubin>1.5 mg/dL; serum creatinine>1.4 mg/dL; or blood urea nitrogen (BUN) >25 mg/dL. Subjects were also excluded if they were pregnant, or could become pregnant, unless they are using regular birth control, in the form of oral contraceptives, condoms, or intra-uterine device). Subjects with the following medical conditions were also excluded: established osteoporosis; parathyroid disorder; difficulty swallowing pills; psychological conditions or substance abuse that may make the subject non-adherent; heart arrhythmia; or other severe illness. Study participants were excluded from enrollment if, over the 3 months previous to the study, they were taking medications that interfere with the metabolism of vitamin D (anti-convulsants, anti-coagulants, oral corticosteroids, or barbiturates).[35,53] Study participants were excluded if they were unwilling to use sunscreen, have an adverse reaction to sunscreen, or have an allergy to sesame oil.

5.1.5 Data collection

The clinical trial enrollment (n=66) occurred between August 2010 and August 2011. The trial was approved by the Bastyr University Institutional Review Board (#09A-1241). The trial was registered at clinicaltrials.gov as NCT01524874.

Study participants were administered hard copy informed consent materials and HIPAA authorization by the study physician or study coordinator. Study data were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at the University of Washington.[54] REDCap is a secure, web-based

application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. During each study visit, all patient data, medical history, vitals and physical exam data, and questionnaires were recorded directly into the REDCap database via an iPad or laptop computer.

Table 5.1: REDCap data entry event grid

Data Collection Instrument	**Screen** (1)	**Study Consent Randomization** (2)	**Telephone Contact** (8)	**Final Screening Lab** (9)
Study Id Entry	X			
Telescreen	X			
Demographics	X			
Screening Checklist	X			
Health History Questionnaire	X			
Food Frequency Questionnaire	X			X
Sun Exposure Questionnaire	X			X
Vitals Exam	X			X
Physical Exam	X			
Sample Collection	X			X
Full Study Consent and Randomization		X		
Telephone Contact			X	
Adverse Event Reporting Form				
Study Completion				X

The following data was collected during the course of the study: informed consent; medical history and physical exam; height, weight, heart rate and blood

pressure; 25-OHD; 1,25-OH_2D; hemoglobin A1c (HbA1c); total cholesterol; low-density lipoprotein (LDL); high-density lipoprotein (HDL); triglycerides; calcium; fasting insulin and glucose; comprehensive metabolic panel. Pacific Physicians' Laboratory, Inc. (PPL), Lynnwood, WA, conducted the laboratory analysis for the study.

The analytical method used for serum 25-OHD determination was the LIAISON 25-OH Vitamin D Total Assay – 310600 (DiaSorin Inc. Stillwater, MN) a chemi-luminescence immunoassay.[55] Specifications of the LIAISON assay reported by DiaSorin include: a detection limit of 4.0 to 150 ng/mL; functional sensitivity defined as the concentration at which the coefficient of variance exceeds 20%, was ≤4 ng/mL. The assay was linear on dilution. The correlation coefficient, when compared to radioimmunoassay (RIA) was r=0.936. The analytical method used for serum 1,25-OH_2D determination was liquid chromatography tandem mass spec, using the extraction, chromatography, radioreceptor assay (Quest Diagnostics, Madison, NJ). Specifications of the 1,25-OH_2D assay reported by Quest include: analytical sensitivity of 5 pg/mL.

Participants were also asked to answer questions about dietary intake and supplementation of vitamin D using a standardized food frequency questionnaire (FFQ),[56-58] and to answer questions about sun protection and exposure using a standardized sun exposure questionnaire (SEQ).[59]

Table 5.2: Food frequency questionnaire IU vitamin D per serving

Vitamin D IU	Food	Serving Size
92.4	Milk (whole, low-fat, skim, chocolate, soy) [a]	8 oz
46.4	Milk over cereal [a]	4 oz
11.6	Milk, cream in coffee [a]	1 oz
30.4	Ravioli or quiche [a]	1 cup
44.4	Pudding or custard made with milk [a]	0.5 cup
40.0	Cream soup, chowders, cream sauces [a]	1 cup
11.6	Muffins [a]	1 medium
20.4	Biscuit of cornbread [a]	2 inch cube
12.8	Pancakes or waffles (frozen) [a]	4 inch
26.0	Eggs [a]	1 each
663.0	Canned salmon with bones [a]	3.75 oz
250	Canned sardines in oil [b]	3.75 oz
360	Cooked Salmon [b]	3.5 oz
345	Cooked Mackerel [b]	3.5 oz
200	Canned tuna in oil [b]	3 oz
12	Cheese [b]	1 oz
40	Ready-to-eat cereal [b]	1 cup milk
15	Cooked liver or beef [b]	3.5 oz
Self-Reported	IU Vitamin D3 in dietary Supplements	Per dosage

[a] Taylor, C. et al. (2009) [56]
[b] Douglass, JS. et al. (2004)[57]; Nutrition Coordinating Center, U. Minn. (2003)[60]; USDA Nutrient Database for Standard Reference (2003) [58]

5.1.6 Adverse Event Reporting

Study participants were instructed to report adverse events (AE) to the study physician, via phone or email, and participants were contacted for a standardized 6-week telephone interview for AE reporting.

5.1.7 Randomization

Enrollees were randomized to one of three vitamin D_3 supplements: a lipid emulsified drop (Biotics Research Corporation, Rosenberg, TX); an encapsulated powder (Integrative Therapeutics Incorporated, Green Bay, WI); and a chewable

tablet (Vital Nutrients Inc., Middletown, CT). Randomization was achieved using a plan with 30 blocks of 3 (n=90) to allow for overage to mitigate unexpected withdrawal or loss to follow up; the plan was created on June 20, 2010 11:50:15 PM PDT using seed 15524 at http://www.randomization.com. The dosage administered was 10,000 IU of vitamin D_3 per day for a period of 12 weeks.

5.1.8 Data Analysis

The following participant characteristics were collected at screening and at 12-week follow-up: age, gender, blood pressure, height, weight, body mass index, study site, study arm, estimate of dietary vitamin D intake, estimate of sun exposure and sun protection; 25-OHD, 1,25OH_2D.

Table 5.3: Description of study variables

Name	Meaning	Significance	Units	Data Type	Other
Age	Age in years	Potential confounder	Years	Ordinal	
Gender	Sex	Potential confounder	M=1/F=0	Binary	
Height	Length	Potential Confounder	Inches	Continuous	
Weight	Mass	Potential Confounder	Pounds	Continuous	
BMI	Body Mass Index	Potential Confounder	kg/m2	Continuous	
SBP/DBP	Blood Pressure	Potential Confounder	mmHg	Continuous	
Arm	A, B, C	Potential Effect Modifier	A=1; B=2; C=3	Categorical	
Study Site	Seattle, WA; Lokahi Kona, HI	Potential Confounder	WA=1/HI=0	Categorical	
Food Frequency	Total IU vitamin D per servings food per week	Potential Confounder	IU/wk	Continuous	Composite from FFQ
Sun Exposure	Frequent sun exposure	Potential Confounder	Y=1/N=0	Binary	Composite from SEQ
25-OHD	25-hydroxycholecalciferol	Outcome	ng/mL	Continuous	
1,25OH_2D	1,25-dihydroxycholecalciferol	Outcome	pg/mL	Continuous	

Two models were created to explore the effect of adjustment for potential confounders on the point estimates.

- Model-1 adjusted for: gender, study site and age
- Model-2 adjusted for: gender, study site, age, BMI and baseline 25-OHD

To determine the mean change between groups per product administered (based on label claim administration of 10,000 IU/day), we conducted analysis of variance (ANOVA), H_0= difference in mean change between groups=0. If the null hypothesis was not accepted, we determined the final distributions of the difference in 25-OHD/1,25-OH_2D concentrations by doing pairwise comparison using Tukey's Test. Proportion by group assignment reaching 25-OHD >33 ng/dL were compared using chi^2 test.

We also used ANOVA, H_0= difference in mean change between groups, by vitamin D_3 IU administered based on the results of the certificates of analysis and third party analysis of the vitamin D_3 supplements, compared to label claim. If the null hypothesis was not accepted, we determined the final distributions of the mean difference in 25-OHD/1,25-OH_2D concentrations by doing pairwise comparison using Tukey's Test.

5.1.9 Study Power

Our power calculations for this pilot study were based on our hypothesis that the non-emulsified vitamin D_3 supplement would result in 50% or less of the response (change in serum 25-OHD concentration) observed with the emulsified supplement. Based on our preliminary observations of an average change of 25

ng/mL in serum 25-OHD using the emulsified product, a 12.5 ng/mL difference in means equates to the non-emulsified vitamin D supplement resulting in 50% of the expected change in serum 25-OHD concentration of the emulsified supplement. We estimated that our sample size of 60 total participants would allow us to detect a between-group difference of 12.5 ng/mL (or greater), assuming a standard deviation of response up to 10 ng/mL, with 80% power at a significance level of alpha=0.05. See Tables 5.4 & 5.5 for these calculations.

Table 5.4: Determination of estimated effect size

% response of emulsified product	Greatest Expected Difference in Means[1]	StdDev	Delta (Largest Mean-Smallest Mean/SD)
10	22.5	5	4.5
10	22.5	10	2.25
10	22.5	20	1.125
25	18.75	5	3.75
25	18.75	10	1.875
25	18.75	20	0.9375[2]
50	12.5	5	2.5
50	12.5	10	1.25
50	12.5	20	0.625[2]
75	6.25	5	1.25
75	6.25	10	0.625[2]
75	6.25	20	0.3125[2]

1. Based on mean 25 ng/mL response
2. Likely inadequate power to detect difference in mean change due to high variability relative to mean response.

Table 5.5: Power table for given sample size for 3-Arm ANOVA

DELTA (in units of sigma=Std. Dev.)

N	0.500	1.000	1.500	2.000	2.500	3.000	3.500	4.000	4.500
2	0.058	0.082	0.125	0.185	0.260	0.349	0.444	0.541	0.634
3	0.068	0.126	0.232	0.380	0.551	0.712	0.839	0.922	0.967
4	0.078	0.173	0.343	0.559	0.761	0.898	0.966	0.991	0.998
5	0.088	0.221	0.449	0.701	0.883	0.968	0.994	0.999	0.999
6	0.099	0.269	0.545	0.805	0.946	0.991	0.999	0.999	0.999
7	0.110	0.318	0.631	0.877	0.976	0.997	0.999	0.999	0.999
8	0.121	0.365	0.704	0.924	0.990	0.999	0.999	0.999	0.999
9	0.132	0.412	0.766	0.954	0.996	0.999	0.999	0.999	1.000
10	0.143	0.457	0.817	0.973	0.998	0.999	0.999	0.999	1.000
12	0.166	0.542	0.891	0.991	0.999	0.999	0.999	1.000	1.000
14	0.189	0.619	0.937	0.997	0.999	0.999	1.000	1.000	1.000
16	0.213	0.686	0.965	0.999	0.999	0.999	1.000	1.000	1.000
18	0.237	0.744	0.980	0.999	0.999	1.000	1.000	1.000	1.000
20	0.261	0.793	0.989	0.999	0.999	1.000	1.000	1.000	1.000
25	0.321	0.882	0.998	0.999	0.999	1.000	1.000	1.000	1.000
30	0.380	0.936	0.999	0.999	1.000	1.000	1.000	1.000	1.000
35	0.437	0.966	0.999	0.999	1.000	1.000	1.000	1.000	1.000
40	0.492	0.982	0.999	1.000	1.000	1.000	1.000	1.000	1.000
50	0.593	0.995	0.999	1.000	1.000	1.000	1.000	1.000	1.000

The sample size values given are those for each of the 3 levels of the factor called 'Factor A'.

5.2 Results

5.2.1 Approach

Study participants were not blinded to their product assignment in this open-label pragmatic trial. The principal investigator of this trial was blinded to product assignments of the allocation groups throughout the study. Unblinding of the allocation groups occurred once the study data collection was completed, the analytical plan was implemented and the analytical results were considered finalized, by the principal investigator and the co-Investigators.

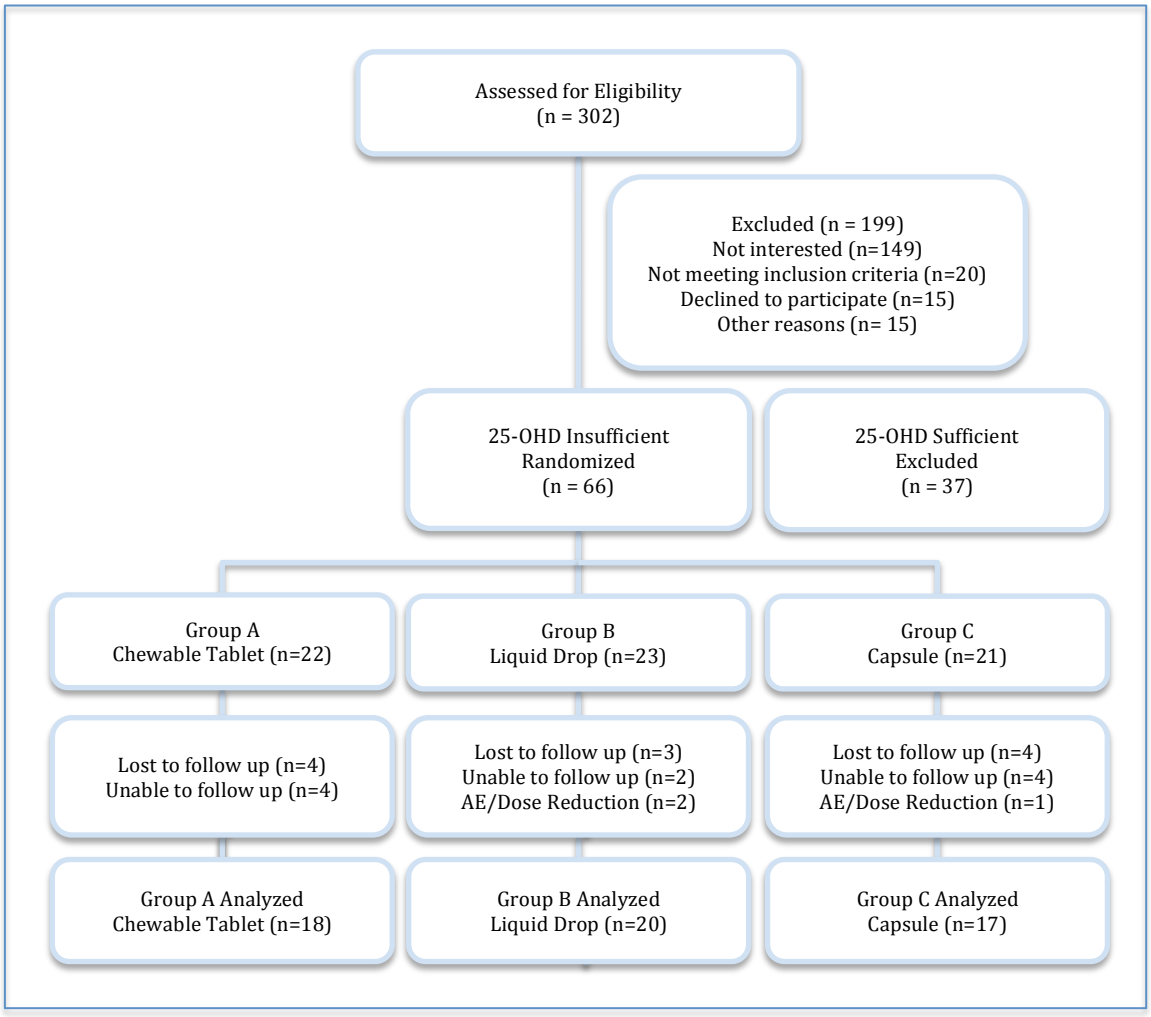
5.2.2 Screening and Enrollment

Enrollment occurred from August 2010 to August 2011, and all data collection activities were completed in November of 2011. A total of 302 individuals were contacted based on their interest in the trial (Figure 5.1). N=199 were excluded for the following reason: not interested (n=149); not meeting inclusion criteria (n=20); eligible but declined to participate (n=15); and other reasons (n=15). Of

the 103 participants screened, (n=37) were sufficient in serum 25-OHD.

Vitamin D insufficient, age-eligible participants were enrolled in the study. A total of N=66 participants were found to be deficient in vitamin D, defined as <33 ng/mL serum 25-OHD, met the inclusion and exclusion criteria and were enrolled in the study. Participants were randomized to one of three vitamin D_3 supplements: group A (chewable tablet); group B (oil drop); or group C (capsule).

Figure 5.1: Flow diagram of three-arm comparative effectiveness trial



5.2.3 Baseline Characteristics

Baseline characteristics (Table 5.6) did not differ significantly between groups (all p>0.05), suggesting that randomization was effective. While height and weight varied slightly between allocation groups, BMI were similar. Of interest is that group B had slightly higher baseline serum 25-OHD and lower usage of sun protection, compared to the other allocation groups (all p>0.05).

Table 5.6: Baseline Characteristics

Variable N (number)	All 55	Group A 18	Group B 20	Group C 17
Study Site (Seattle)	75%	72%	75%	76%
Age (years)	39.9 (13.7)	40.3 (13.5)	39.5 (13.6)	39.9 (14.7)
Gender (female)	85%	83%	85%	88%
Systolic BP (mmHg)	111.3 (12.7)	112.4 (11.2)	107.7 (9.8)	114.4 (16.4)
Diastolic BP (mmHg)	73.1 (9.5)	75.9 (9.4)	69.8 (9.9)	73.8 (8.5)
Height (inches)	65.8 (3.1)	65.5 (3.0)	65.5 (3.1)	66.4 (3.4)
Weight (pounds)	161.5 (39.6)	168.9 (46.9)	154.8 (27.6)	161.6 (44.1)
Body Mass Index (kg/m^2)	26.1 (5.3)	27.4 (6.5)	25.2 (4.0)	25.6 (5.2)
Food Frequency (IU vitamin D/wk)	1525 (1826)	1545 (1933)	1502 (1869)	1530 (1770)
Sun Exposure (Hours/wk)	9 (10)	10 (13)	10 (10)	8 (5)
Sun Protection (Y=1/N=0)	76%	83%	60%	88%
Baseline Vitamin D (ng/mL)	22.6 (6.7)	21.9 (7.8)	24.2 (5.1)	21.5 (7.2)

*Data are means (SD) or numbers (%)

5.2.4 Adverse Events and Loss to Follow Up

Three mild AEs occurred, including tachycardia, anxiety, itching, muscle cramping and nausea - all without hypervitaminosis D or hypercalcemia, verified by laboratory tests. Two dose reductions to 6,000 IU vitamin D_3/day were required per protocol, and all AE cases resolved. Overall loss to follow up was n=11 (16.7%). Changes in serum calcium levels, from baseline to 12 weeks, were not significant within or between groups (all p>0.05).

5.2.5 Primary Aim: Change in Vitamin D Status

5.2.5.1 Change in serum 25-OHD between treatment arms

All allocations groups showed a mean increase in serum 25-OHD concentrations from baseline to 12-week follow-up (Table 5.7 & Table 5.8).

Table 5.7: Summary of 25-hydroxycholecalciferol (25-OHD) levels at baseline and at 12 weeks

Study Arm	Product	Baseline	12-weeks
Units	Matrix	ng/mL (SD)	ng/mL (SD)
Group A (n=18)	Tablet	21.9 (1.8)	55.1 (3.6)
Group B (n=20)	Oil Drop	24.2 (1.1)	58.6 (6.1)
Group C (n=17)	Capsule	21.5 (1.7)	75.1 (5.5)

Table 5.8: Summary of changes in serum 25-hydroxycholecalciferol (25-OHD)

Study Arm	Product	Difference	95% Confidence Intervals	
Units	Matrix	ng/mL (SD)	lower limit	upper limit
Group A (n=18)	Tablet	33.3 (5.7)	[21.9	44.6]
Group B (n=20)	Oil Drop	34.4 (5.4)	[23.6	45.1]
Group C (n=17)	Capsule	53.6 (5.8)	[41.9	65.2]

Two adjustment models were created to explore the potential confounding of the point estimates. Model-1 adjusted for gender, study site, and age (Table 5.9). However reductions in the point estimates for the change of 25-OHD status in all allocation groups did not reach the level of significance (all $p>0.05$).

Table 5.9: Model-1: summary of changes in serum 25-hydroxycholecalciferol (25-OHD), adjusted for gender, study site, and age

Study Arm	Product	Difference	95% Confidence Intervals	
Units	Matrix	ng/mL (SD)	lower limit	upper limit
Group A (n=18)	Tablet	31.5 (8.4)	[14.6	48.5]
Group B (n=20)	Oil Drop	32.2 (8.3)	[15.6	49.0]
Group C (n=17)	Capsule	51.3 (8.7)	[33.8	68.8]

Model-2 also adjusted for gender, study site, and age, with the addition of BMI and baseline 25-OHD levels (Table 5.10). Model-2 showed greater reductions in the point estimates for 25-OHD, than did Model-1, and both BMI and baseline 25-OHD status significantly affected the point estimates ($p<0.001$).

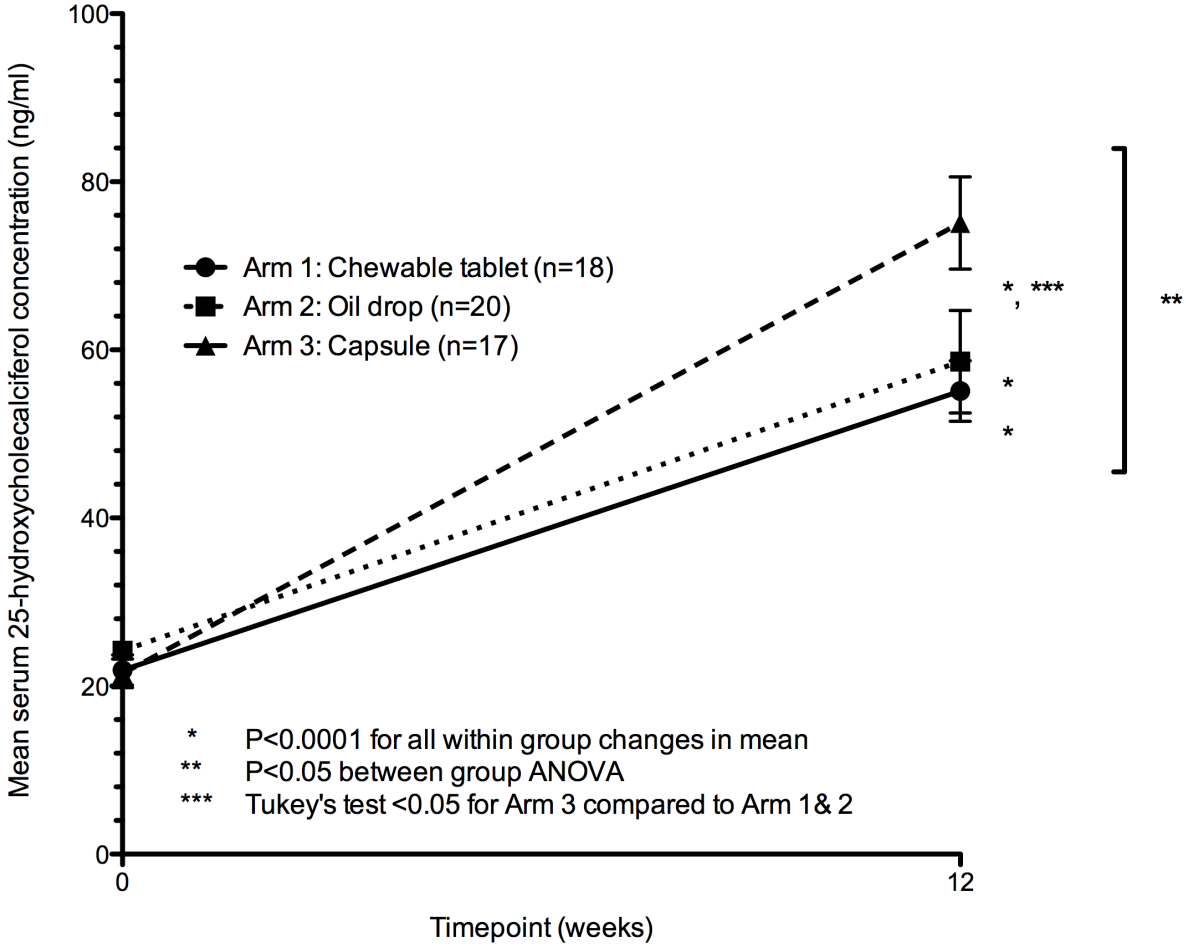
Table 5.10: Model-2: summary of changes in serum 25-hydroxycholecalciferol (25-OHD), adjusted for gender, study site, age, BMI and baseline 25-OHD status

Study Arm	Product	Difference	95% Confidence Intervals	
Units	Matrix	ng/mL (SD)	lower limit	upper limit
Group A (n=18)	Tablet	31.8 (7.6)	[16.5	47.2]
Group B (n=20)	Oil Drop	32.16 (7.98)	[16.1	48.2]
Group C (n=17)	Capsule	48.1 (8.08)	[31.8	64.4]

Within group changes in serum 25-OHD levels, from baseline to 12 weeks, were significant for each of the three allocation groups ($p<0.001$), as represented in Figure 5.2. Changes in serum 25-OHD were also significant between groups (between group ANOVA $p<0.05$). Pairwise-comparisons of changes in serum

25-OHD from baseline to 12-week follow-up, showed similar increases between Group A and B, while Group C showed a statistically significant difference from Group A and B (pairwise comparison using Tukey's Test $p<0.05$), respectively.

Figure 5.2: Change in mean serum 25-hydroxycholecalciferol (25-OHD) concentration by treatment arm



5.2.5.2 Change in serum 1,25-OH_2D between treatment arms

There were no statistically of clinically significant changes in 1,25-OH_2D status for any of the allocation groups. Allocations groups did not show consistent changes in serum 1,25-OH_2D concentrations from baseline to 12-week follow-up (Table 5.8). Changes in serum 1,25-OH_2D levels, from baseline to 12 weeks, were not significant within or between groups (all $p>0.05$).

Table 5.11: Summary of changes in serum 1,25-dihydroxycholecalciferol (1,25-OH_2D)

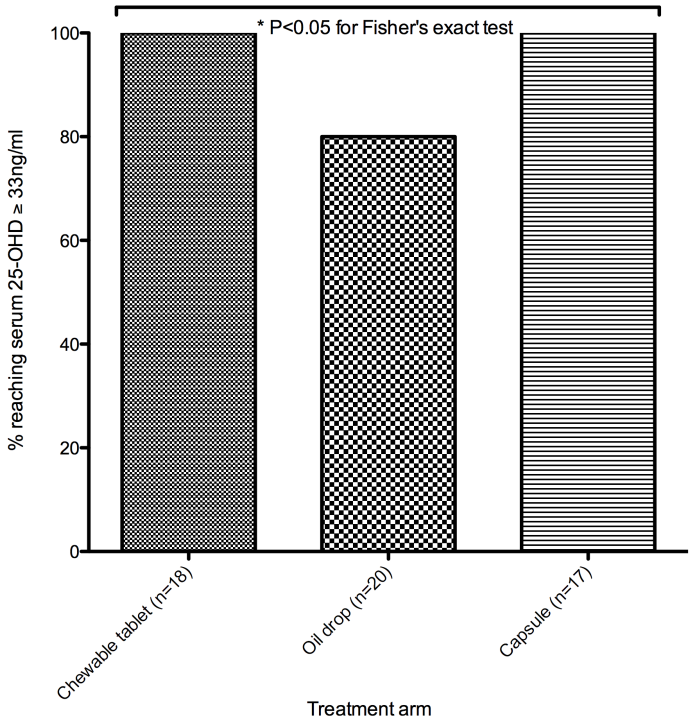
Study Arm	Product	Baseline	12-weeks	Difference
Units	Matrix	ng/mL (SD)	ng/mL (SD)	ng/mL (SD)
Group A (n=16)*	Tablet	41.4 (2.5)	44.9 (4.3)	3.5 (3.9)
Group B (n=19)*	Oil Drop	43.4 (3.2)	43.2 (2.9)	- 0.2 (4.0)
Group C (n=17)	Capsule	42.8 (3.7)	39.0 (2.5)	- 3.8 (4.3)

* missing values: Group A (n=2); Group B (n=1)

5.2.6 Secondary Aim: Proportion Attaining serum 25-OHD Sufficiency Status

The proportion of participants reaching “optimal” 25-OHD concentration ≥33 ng/ml between treatment arms (Figure 5.3) was 100% for the group A (tablet) and C (capsule), and 80% for group B, the oil drop. (comparison of proportions $p<0.05$).

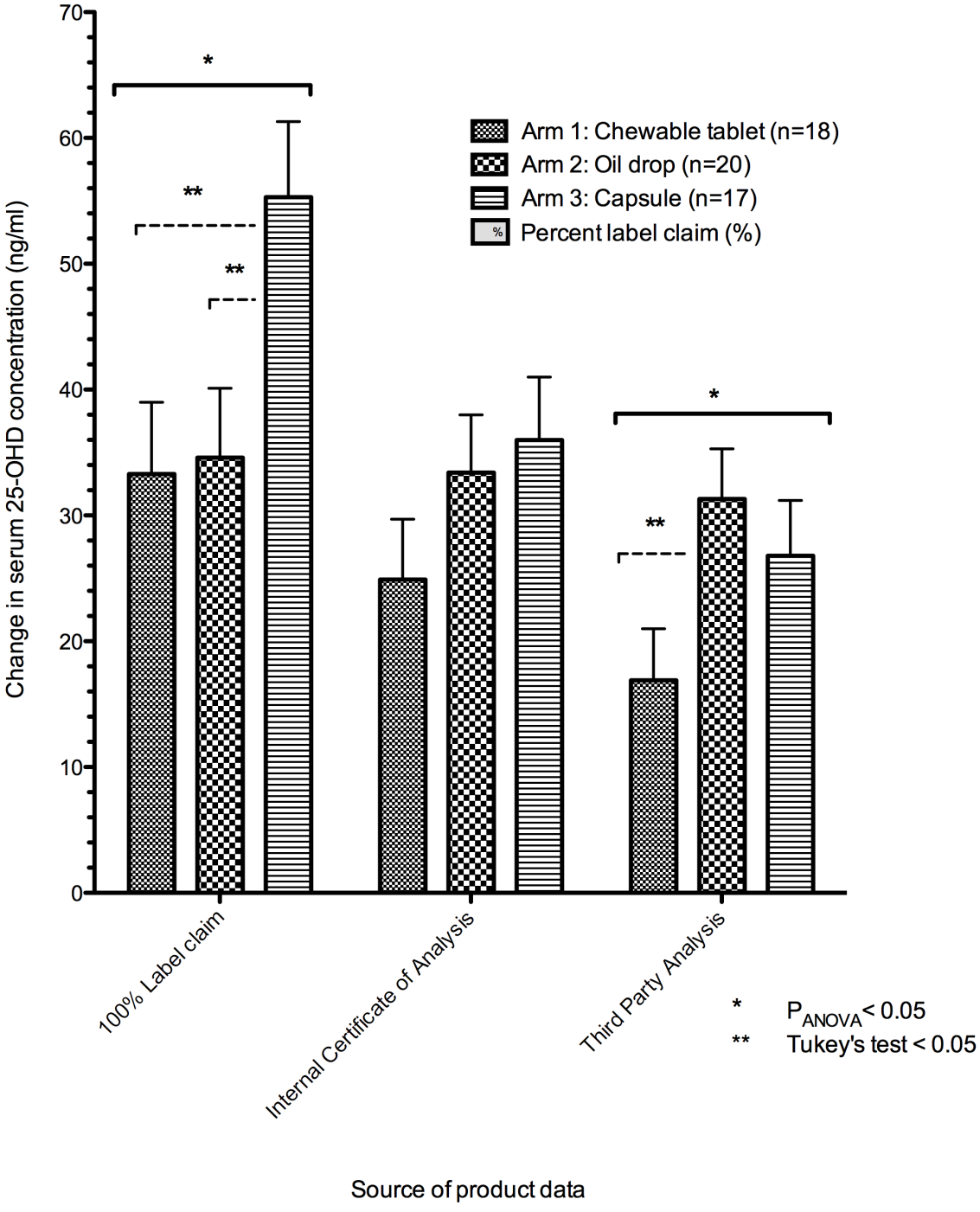
Figure 5.3: Proportion reaching serum 25-hydroxycholecalciferol (25-OHD) ≥33 ng/mL by treatment arm



5.2.7 Tertiary Aim: Changes in 25-OHD Status by IU Vitamin D3 Administered

The change in serum 25-OHD between treatment arms by actual IU administered, compared to label claim, was calculated based on results of the internal manufacturers' certificates of analysis (CoA) and the third-party analysis (TPA) of vitamin D3 supplements used in the study.

Figure 5.4: Change in mean serum 25-hydroxycholecalciferol (25-OHD) concentration per standardized dosing unit by treatment arm



5.3 Discussion

5.3.1 Strengths

The study design was a pragmatic, open-label, randomized, comparative effectiveness, clinical trial of vitamin D repletion strategies, which assessed and controlled for potential confounders, including BMI, dietary intake of vitamin D, sun exposure, and sunscreen use. The study was appropriately powered to measure 25% differential effect between groups for change in mean for 25-OHD. The study was preceded by our third party analysis of supplement potency, for each of three products used in the trial. Structured AE reporting, clinical laboratory monitoring and dose reduction protocols were also in place and utilized to ensure the safety of study participants.

5.3.2 Limitations

All subjects were treated with one of three active vitamin D_3 supplements with the intent to treat nutritional deficiency. There was not a conventional control or placebo group assignment in this study. While the patients and the study physicians were not blinded to treatment assignment, the principal investigator, was blinded to assignment arm.

There remains considerable debate about the “gold standard” for the determination of serum 25-OHD, and observed serum 25-OHD levels may differ from actual blood-serum levels. The majority of clinical laboratories currently use the DiaSorin method that was used in this study; however, improvements to the measures of serum 25-OHD, such as using liquid chromatography, tandem mass

spectroscopy (LC/MS/MS) may result in improved sensitivity and specificity in future vitamin D clinical trials.[48]

Race and ethnicity data were not collected during the study and subjects were not included or excluded based on race or ethnicity. The degree of skin pigmentation may be related to individual ability to produce endogenous vitamin D upon exposure to sunlight. Current research has not definitively demonstrated that the level of skin pigmentation effects changes in serum 25-OHD induced by administration of exogenous sources of vitamin D. A limitation of this study is that we are unable to explore the potential confounding by race or ethnicity.

5.3.3 Significance

In this study, we conducted an independent TPA of three commercially available vitamin D_3 nutritional supplements. The results of the TPA showed super-potency in the tablet and capsule, compared to the oil drop; however the results may have been confounded by matrix signal interference evidenced by increased recovery in the tablet and capsule spike samples.

It should be noted that our study assessed the effectiveness of a high-dose supplementation strategy, with per protocol administration of 840,000 IU of vitamin D over 12 weeks. The results of this pragmatic comparative effectiveness trial of vitamin D supplementation to correct nutritional deficiency and insufficiency showed greater effectiveness (93%) compared to other supplementation strategies reported in the literature (5% to 89%), shown in

Table 3.1. All three vitamin D_3 nutritional supplements were safe to administer for 12 weeks at 10,000 IU/day, without hypercalcemia or other significant adverse events, and effectively returned the majority of participants to vitamin D sufficiency status.

Physicians in clinical practice routinely recommend or prescribe nutritional supplements to correct vitamin D nutritional deficiency. The quality of commercially available vitamin D nutritional supplements is an important consideration, which may influence dosing and prescribing patterns. Physicians might be more confortable recommending high-dose vitamin D repletion strategies, were the quality of commercially available products reproducibly verifiable. Therefore, we believe that improvements to currently methodologies are needed to establish the quality of commercially available vitamin D supplements delivered in multi-component matrices.

It is important that practice guidelines are set based on the preponderance of evidence. The Endocrine Society Clinical Practice Guidelines recommends a prescribed dosage of 50,000 IU per week for 8 weeks as a safe, effective and well tolerated method to correct vitamin D deficiency.[61] We conclude that the strategy used in this pragmatic clinical trial, dosing commercially available vitamin D_3 dietary supplements at 10,000 IU/day for 12 weeks, is another safe, effective, and well tolerated strategy for clinical vitamin D_3 repletion in people with vitamin D_3 insufficiency.

6 List of References

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8 Dedication

To my Father and my dear Aunt

Jerry Wayne Finnell

and

Nelda Kay Finnell