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Vitamin D status and impact of vitamin D_3 and/or calcium supplementation in a randomized pilot study in the southeastern U.S.*

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Abstract

Objective—Vitamin D supplementation may be required for certain subgroups in the U.S. in whom status and intake is inadequate, but the impact of various doses, and whether calcium administration jointly or independently influences vitamin D metabolite levels, is unclear.

Methods—In a pilot chemoprevention trial of biomarkers of risk for colorectal adenoma, we measured the impact of vitamin D supplementation, and/or calcium supplementation, on plasma vitamin D metabolite concentrations. Ninety-two adult men and women living in the southeastern U.S. were randomized to either 800 IU vitamin D_3, 2,000 mg elemental calcium, both, or placebo daily for six months. We examined vitamin D status at baseline and post-intervention, and compared the change in plasma 25(OH)D and $1,25(OH)_2D$ levels by intervention group using general linear models.

Results—Eighty-two percent (%) of the study population had insufficient plasma 25(OH)D concentrations (< 75 nmol/L) at baseline, with lowest levels among African American participants. Vitamin D supplements, with or without calcium supplementation, raised plasma 25(OH)D concentrations, on average, 25 - 26 nmol/L. Half of study participants were classified as having sufficient 25(OH)D status after six months of 800 IU vitamin D_3 daily. Calcium alone did not influence 25(OH)D concentrations.

Conclusion—In this southeastern U.S. population, half of the study participants receiving 800 IU vitamin D_3 daily had blood 25(OH)D concentrations of ≤ 75 nmol/L after a six-month

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intervention period, supporting higher vitamin D dose requirements estimated by some groups. More research is needed to identify the optimal vitamin D dose to improve 25(OH)D status in various at-risk populations.

Introduction

With several recent studies linking higher vitamin D status to prevention of osteoporosis [1], cancer [2], diabetes [3, 4], hypertension [5], and immunological disorders [6, 7], much discussion has centered on whether the recommended vitamin D intake should be increased [8-11]. The current daily recommended intake defined by the Institute of Medicine (IOM) to avoid deficiency range from 200-600 IU, depending on age [12]. These recommendations are based on various hormonal indicators and a minimum blood 25-hydroxyvitamin D (25(OH)D) (vitamin D metabolite reflecting vitamin D status) cut-point of approximately 30 nmol/L. Scientific experts estimate that target concentrations of 25(OH)D for prevention of chronic diseases are in the range of 70-90 nmol/L [9], and estimate that to reach these 25(OH)D concentrations, vitamin D intake requirements are closer to 1,000 IU/day [9].

Although vitamin D is efficiently synthesized in the skin with exposure to UVB radiation between 290-315 nm [13], latitude, season and time of day can affect the quantity of ultraviolet photons that reach the earth's surface. Melanin in skin, and clothing, sunscreen, aging skin and other factors also limit production [13]. In addition, excessive UV exposure can lead to certain skin cancers and cataracts [14], complicating the practicality of recommending UVB as a source of vitamin D. Studies have shown that a substantial proportion of the U.S. population does not meet the current dietary vitamin D intake recommendations of 200 - 600 IU/day [15]. In the U.S. and Canada, hypovitaminosis D is common, especially among dark skinned individuals, the elderly, and those living in northern climates [16-18]. Because food contains relatively small amounts of vitamin D, discussion has centered on the optimal dose for vitamin D supplementation in at-risk individuals or enhanced fortification of the food supply [16, 19].

Vitamin D and calcium recommendations usually coincide because these nutrients are metabolically interrelated to serve endocrine functions [12]. Their inter-relationship may also apply to their potential roles in prevention of other chronic diseases, including cancer [20]. Vitamin D regulates calcium absorption and utilization[12], and the potential for calcium to influence circulating vitamin D metabolite concentrations has also been raised [21-23]. For example, dietary calcium may spare 25(OH)D from conversion to $1,25(OH)_2D$, thus maintaining higher 25(OH)D concentrations [23]. A recent comprehensive review identified 74 randomized controlled trials that evaluated the effect of vitamin D supplements with or without calcium supplementation on 25(OH)D concentrations, with 30 of these specific to postmenopausal women or older men [24]. None of the studies evaluated independent and combined effects of vitamin D and calcium on blood vitamin D metabolites using a 2 × 2 factorial design.

Herein we describe the impact of 800 IU vitamin D_3 and/or 2,000 mg elemental calcium on plasma vitamin D metabolites using a randomized, 2 × 2 factorial study design provided over a 6-month period in a study population of older adults residing in the southeastern U.S. Specifically, we examined whether 800 IU vitamin D would raise 25(OH)D levels to sufficient status in the majority of the population, and whether calcium intake contributed to any changes observed in vitamin D metabolites.

Materials and Methods

Population

Adult men and women living in Georgia participated in a pilot study – "Calcium, Vitamin D and Markers of Adenomatous Polyps" (CaD v MAP), a randomized, double-blind, placebo-controlled six-month clinical trial utilizing a 2 × 2 factorial design to test the effects of calcium and/or vitamin D on biomarkers of risk for colon cancer. Ninety-two adults, aged 30 - 74 were recruited from the patient population attending the Digestive Diseases Clinic of the Emory Clinic at Emory University, Atlanta, GA. Eligibility criteria included a history of at least one pathologically-confirmed adenomatous colonic polyp within the previous 36 months and a physician's assessment of general good health. Exclusion criteria included an inability to understand informed consent and to cooperate with study procedures, major changes in diet within the previous six months, supplemental intake of calcium or vitamin D greater than 100% DRI (Dietary Reference Intake) recommendations [12], unwillingness to forego the use of usual calcium or vitamin D supplements during the study, and failure to take ≥ 80% of their study pills during a one-month placebo run-in period. One randomized participant did not complete study questionnaires and a total of seven participants were lost to follow-up; thus, complete baseline data on 91 participants, and complete follow-up data on 85 (92%) participants were available for this analysis.

At baseline, eligible subjects were randomized, stratified by sex and nonsteroidal anti-inflammatory drug (NSAID) use, to each of four groups: placebo, 2,000 mg elemental calcium daily (as two 1.25 g calcium carbonate pills taken twice daily with meals), 800 IU vitamin D daily (as 400 IU cholecalciferol (D_3) taken twice daily with meals), or 2,000 mg calcium *plus* 800 IU vitamin D daily. Study tablets were custom manufactured by Tishcon Corporation, NY, USA. The corresponding supplement and placebo pills were identical in size, appearance, and taste. The placebo was free of calcium, magnesium, vitamin D, and chelating agents. The rationale for choosing a dose of 800 IU vitamin D was based on suboptimal 25(OH)D status in the U.S. population with lower intakes at the time of study initiation [25], and safety of this dose [12]. Calcium supplements containing 2,000 mg elemental calcium is in the upper range of normal intake, closer to estimated intake levels from the Paleolithic diet [26] and was shown to more favorably impact the distribution of proliferating colorectal cells compared to 1,000 mg in previous investigations [27]. Adherence to study medication was assessed by pill counts at visit 1 (placebo run-in), and at visit 2 (randomization, one month after visit 1), visit 3 (1-month follow-up), and visit 4 (6-month follow-up). All participants completed questionnaires on diet, medical history and lifestyle, and provided a 4-week sun exposure history, and had blood drawn at baseline (randomization) and at study completion. The food frequency questionnaire (FFQ) included questions on dietary intake and supplemental sources of calcium and vitamin D, and has been validated elsewhere [28, 29]. No treatment-related adverse events occurred. This study was approved by the Emory University Institutional Review Board. Written informed consent was obtained from each study participant.

Laboratory analysis

Plasma—The outcomes of interest included plasma 25(OH)D (indicator of status) and 1,25-dihydroxy-vitamin D ($1,25(OH)_2D$, active hormone) concentrations at baseline and at six-months follow-up, and the change in these concentrations during follow-up. Plasma $1,25(OH)_2D$ and 25(OH)D concentrations were determined by radioimmunoassay (RIA) as described elsewhere [30, 31]. The mean intra-assay coefficient of variation from 10 blinded quality control replicate samples for plasma 25(OH)D was 2.3% and for $1,25(OH)_2D$ was 6.2%.

Genotyping—Genotyping for vitamin D receptor (VDR) polymorphisms was conducted on all participants, using a Taqman allelic discrimination assay for the VDR polymorphism *BsmI* (rs 1544410) SNP typing. The assay (Assay ID: C_8716062_10) was validated and inventoried by Applied Biosystems (Foster City, CA). Two samples from each of the three genotypes (*BB, Bb* and *bb*) were randomly selected to be validated on a different platform, i.e. automated sequencing. Sequencing was carried out for both the forward and reverse strands on an ABI 3100 Genetic Analyzer (Applied Biosystems). For all samples assayed (representing 6.2% of the total sample population), the concordance rate between the two platforms was 100%.

Statistical analysis

Blood concentrations of vitamin D metabolites were normally distributed and were therefore not transformed. We examined vitamin D status using the following plasma 25(OH)D cut-points: hypovitaminosis D (< 37.5 nmol/L) [12] and vitamin D sufficiency (≥ 75 nmol/L) [1]. Concentrations intermediate between 37.5 and < 75 nmol/L were termed “insufficient”. We examined the distribution of 25(OH)D status according to various demographic factors at baseline, including age, sex, race, and season. Baseline sun exposure is defined as the sum of hours of usual sun exposure, exposure during travel or vacation to a sunny location, and tanning booth use over the past month.

We used analysis of covariance (ANCOVA) to examine continuous patient characteristics by treatment group and chi-square or Fisher's exact test, as appropriate, to compare categorical variables across treatment groups. All analyses were conducted with SAS 9.1 software (SAS Institute, Cary, NC). Dietary variables at baseline were energy-adjusted using the residual method of Willett & Stampfer [32].

To examine the impact of 800 IU vitamin D and/or 2,000 mg calcium on plasma vitamin D metabolites, we used general linear models to compare blood concentrations at baseline and follow-up. We included an interaction term for time (baseline vs. follow-up visit) and treatment group to account for the change in the placebo group when calculating mean differences in blood concentrations for each of the three active treatment arms. We used the Tukey-Kramer method [33] to adjust for multiple comparisons (placebo vs. each of the active treatment arms) and applied Bonferroni corrections to multiple pair-wise comparisons among the three active treatment arms. Our primary model was based on intention to treat, and did not adjust for other factors expected to be balanced by the randomized design. However, because of the expected strong effect of season at baseline on vitamin D concentrations, we also assessed a model adjusted for season of blood draw. Adjustment for additional stratification factors (e.g. sex and NSAID use) had little influence on results and, therefore, was not included. We further examined whether response to treatment varied by baseline 25(OH)D status (<55 nmol/L or ≥ 55, median 25(OH)D concentration). Two-sided tests were statistically significant if $p<0.05$.

Results

Men comprised 64% of the study population. Mean (± S.D.) ages were 58.4 ± 6.7 and 61.7 ± 8.4 years among men and women, respectively. Approximately 70% of the participants were Caucasian, 20% African-American, 4% Asian and 3% other (1 Native American, 1 Hispanic, 1 Pacific Islander). Baseline blood draws occurred throughout the year: fall (39%), summer (25%), winter (26%) and only one person in the spring. On average, 93% of all participants in each group took at least 80% of their pills at the first follow-up visit and 84% at the final follow-up visit. Baseline plasma 25(OH)D measurements in the study population are provided in **Table 1**. Only 18% of the population had 25(OH)D concentrations in the sufficient range (≥ 75 nmol/L). Of the 82% of individuals with 25(OH)D concentrations <

75 nmol/L, 28% were classified as having hypovitaminosis D. African-Americans had a higher prevalence of hypovitaminosis D (p=0.002). Individuals who reported lower sun exposure the month prior to blood draw had a higher prevalence of deficient levels (p=0.07). Vitamin D status was also lowest during the winter months and among those with less than 400 IU vitamin D intake daily, or those who had low sun exposure over the previous month, but these differences were not statistically significant. At the end of the six-month pilot trial, 50% of individuals randomized to 800 IU vitamin D (with or without calcium) were classified as having sufficient 25(OH)D concentrations, and only 4.7% had hypovitaminosis D (**Table 2**). The different proportion of individuals in the "sufficient" group was not significant overall, nor was the pair-wise comparison on vitamin D alone vs. calcium plus vitamin D (p=0.20, Chi-square test).

The baseline characteristics of the subjects were not significantly different by treatment group, as expected due to the randomization scheme (**Table 3**). Although season of initial blood draw appeared somewhat different according to intervention group, these differences were not statistically significant.

The changes in plasma metabolites according to intervention arm are presented in **Table 4**. Individuals receiving supplemental vitamin D, with or without calcium, had an average increase in 25(OH)D of 25 and 26 nmol/L, respectively, relative to the placebo group. These estimates became only slightly stronger when controlling for season of blood draw, and were not influenced when adjusted for genotype (data not shown). Plasma concentrations of 1,25(OH) $_2$D were lower in the calcium only intervention group although this was not statistically significant. Because baseline 25(OH)D may influence the quantity of change in 25(OH)D from supplementation, we also stratified results at the median of baseline blood concentrations (55 nmol/L). In crude analyses (uncontrolled for placebo effect), it did appear that individuals with a higher baseline 25(OH)D status experienced a smaller rise in plasma vitamin D; however, after accounting for the change in the placebo group, participants receiving vitamin D had a similar rise in 25(OH)D concentrations regardless of baseline status (not shown).

Discussion

In this population of adult men and women residing in the southeastern U.S., insufficient 25(OH)D concentrations at baseline were common, especially among African Americans, and regardless of time of year of blood draw. The 25(OH)D concentrations in individuals randomized to 800 IU vitamin D daily for six months (with or without calcium) increased by 25-26 nmol/L regardless of baseline status. By study completion, half of the participants randomized to 800 IU supplemental vitamin D reached sufficient 25(OH)D concentrations, and only one participant in each vitamin D arm had concentrations less than 37.5 nmol/L.

The high prevalence of inadequate vitamin D intake [34] and status [17] in the U.S. has been emphasized recently because of the emerging potential for vitamin D in preventing several chronic diseases in addition to its well-known role in bone health. Current dietary recommendations for vitamin D of 200-400 IU/day up to age 70 and 600 IU/day for over age 70 are based on hormonal markers of bone metabolism with the criteria for adequacy of blood 25(OH)D concentrations approximately 30 nmol/L [12], but most U.S. residents do not even reach these dietary intake levels [15, 35]. This is disconcerting because a recent meta-analysis of vitamin D and osteoporosis prevention trials found that 25(OH)D blood concentrations of 70-90 nmol/L were associated with prevention of this disease characterized by bone loss; the authors estimated that 1,000 IU of daily vitamin D was required to maintain these blood concentrations [9]. Further, a recent study found that average 25(OH)D concentrations appear to be decreasing, likely due to a combination of

lower milk consumption and UVB exposure and greater body weight [17]. Optimal 25(OH)D concentration and intake level for prevention of other diseases are still unknown; however, 75 nmol/L has been suggested as a minimum concentration for preventing certain cancers [2, 36]. Approximately three-quarters of U.S. whites and ninety percent or greater of non-Hispanic black and Mexican American men have 25(OH)D blood concentrations that are <75 nmol/L [17].

UVB exposure provides the major source of circulating 25(OH)D, although diet and supplements are also important sources, especially during winter months and among groups at high risk of suboptimal vitamin D status. Populations at especially high risk include pregnant and breast feeding mothers, infants, children, the elderly, dark-skinned individuals, those with little exposure to the sun, and those living in northern climates during the winter. At 33 degrees latitude, Atlanta, Georgia is considered a geographic area where UVB synthesis is possible during most times of the year. Nevertheless, the majority of this study population had suboptimal 25(OH)D concentrations at baseline regardless of season, and 23% were classified as having hypovitaminosis D, despite the fact that over 38% of the current intervention study participants reported consuming greater than 400 IU vitamin D daily (from diet and supplements reported on their baseline FFQ).

Baseline prevalence of low 25(OH)D concentrations was greater among African Americans than whites in this study (55% vs 14.1% <37.5 nmol/L, respectively). These figures are generally similar to, if not slightly greater than, estimates from two NHANES III studies [34, 37] and recent data from NHANES 2000-2004 [17]. Nesby-O'Dell, et al [34] reported a greater prevalence of hypovitaminosis D (<37.5 nmol/L) in African American (42.4%) compared to white U.S. women (4.3%) of childbearing age. Data from NHANES 2000-2004 indicate a greater prevalence of hypovitaminosis D (<37.5 nmol/L) among both non-Hispanic black and Mexican American, compared to non-Hispanic white adult men and women (approximately 35-60%, 15-30% and 10-20%, respectively) [17]. A lower 25(OH)D status among African Americans is presumed due to a higher concentration of melanin in the skin, which reduces cutaneous photosynthesis of vitamin D [38]. A recent hypothesis points to higher urinary excretion of vitamin D binding protein among African Americans on a high-salt diet [39].

The vitamin D dose required to raise 25(OH)D concentrations to an optimal range depends on several factors, including initial blood concentration [1], duration of supplement use, UVB exposure and cutaneous synthesis of vitamin D depending on geographic region, season, skin color, and cultural practices. Because this was a randomized trial, most factors that could influence 25(OH)D concentrations were spread equally across intervention groups. In the current study, 800 IU vitamin D taken for six months raised blood concentrations of 25(OH)D by 25 - 26 nmol/L on average, shifting the status of the study population toward a greater proportion (50%) achieving concentrations considered sufficient. These findings are generally similar to other randomized, placebo-controlled [40, 41] or non placebo-controlled [21, 42] trials of the same vitamin D dose, which reported increases of 16-45 nmol/L. Some variability in these findings may be due to different assays used [24]. Likewise, trials that provided 1,000 IU vitamin D per day for eight weeks [43] and three years [44] reported increases in 25(OH)D concentrations of 24-29 nmol/L. Changes in 25(OH)D concentrations with supplementation are also known to vary by starting concentration of 25(OH)D, with more dramatic increases seen among deficient individuals and more blunted responses among individuals with 25(OH)D concentrations greater than 75 nmol/L [1]. In the current study, the increase in 25(OH)D did not vary among those above or below the median starting 25(OH)D concentration (55 nmol/L).

While vitamin D (primarily $1,25(OH)_2D$ but also 25(OH)D in much higher concentrations) is known to be involved in active calcium absorption, the influence of calcium on vitamin D metabolism is less clear. Calcium intake is thought to down-regulate circulating $1,25(OH)_2D$ concentrations [22] through subtle influences on serum calcium concentrations, and it has been suggested that it spares 25(OH)D from conversion to the active metabolite [21, 23]. Because both nutrients have a role in bone mineralization and are metabolically interdependent, they are often provided simultaneously in intervention studies [44, 45]; thus, few studies have examined their independent impact on vitamin D metabolites. In an intervention study of 800 IU vitamin D_3/day, Goussous et al [21] randomized participants to receive vitamin D alone or vitamin D with 1,000 mg calcium/day over a 3-month period. The authors found that 500-1500 mg calcium/day did not substantially affect the rise in blood 25(OH)D in response to 800 IU vitamin D_3 among adult men and women [21]. During the six-month intervention in the current study, the intervention group receiving both calcium (2,000 mg) and vitamin D experienced a similar rise in 25(OH)D concentrations compared to vitamin D alone, and the change in 25(OH)D concentrations among those on placebo vs calcium-only was also similar. Consistent with a down-regulating effect on $1,25(OH)_2D$ synthesis, individuals in the calcium-only group had a non-statistically significantly lower concentration of this metabolite after the intervention period. However, this was not observed in the calcium and vitamin D arm. The difference in pre-post intervention $1,25(OH)_2D$ levels comparing the calcium only and vitamin D only groups was borderline statistically significant ($p=0.07$). Whether administration of vitamin D mitigates the effect of calcium on $1,25(OH)_2D$ was not a hypothesis in this study. We focused primarily on 25(OH)D response, as this is a more stable metabolite compared to $1,25(OH)_2D$, and reflects vitamin D status. The half-life of the active vitamin D metabolite ($1,25(OH)_2D$) is approximately four hours [46]; thus, short duration intervention studies should further examine these relationships.

Limitations of this pilot trial include the relatively small sample size, which may have limited our ability to observe significant differences in some intervention groups. Although not population-based, the study population represented an otherwise healthy group of men and women with a history of sporadic colorectal adenomas. Strengths of this analysis include the randomized, double-blind, placebo-controlled, 2×2 factorial design, the high levels of adherence to pill-taking and attending study visits, and the low drop-out rate of the participants. The availability of various measures of sun exposure and dietary intake enabled us to determine that randomization was effective.

Conclusion

In this study, 800 IU vitamin D, with or without calcium supplementation, increased 25(OH)D concentrations by 25-26 nmol/L, on average, which was enough to achieve sufficient 25(OH)D concentrations in half the population. Thus, our findings suggest that for half of adults living in the southeastern U.S., a vitamin D dose of 800 IU/day may be insufficient to reach suggested 25(OH)D concentrations for chronic disease prevention. Calcium supplementation did not impact 25(OH)D concentrations. More research on the optimal concentration of 25(OH)D for health effects, and the optimal dose of vitamin D to improve vitamin D status, is needed.

Literature Cited

1. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int. 2005; 16
2. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review. Cancer Causes and Control. 2005; 16:83–95. [PubMed: 15868450]

3. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care. 2006; 29:650–656. [PubMed: 16505521]

4. 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. [PubMed: 11705562]

5. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007; 49:1063–1069. [PubMed: 17372031]

6. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr. 2004; 80(suppl):1678S–1688S. [PubMed: 15585788]

7. Cannell JJ, Vieth R, Umhau JC, Holick M, Grant WB, Madronich S, Garland CF, Giovannucci E. Epidemic influenza and vitamin D. Epidemiol Infect. 2006:1–12.

8. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr. 2005; 135:317–322. [PubMed: 15671234]

9. 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. [PubMed: 16825677]

10. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006; 81:353–373. [PubMed: 16529140]

11. Vieth R, Bischoff-Ferrari HA, Boucher BJ, Dawson-Hughes B, Garland CF, Heaney RP, Holick MF, Hollis BW, Lamberg-Allardt C, McGrath JJ, Norman AW, Scragg R, Whiting SJ, Willett WC, Zittermann A. The urgent need to recommend an intake of vitamin D that is effective. Am J Clin Nutr. 2007; 85:649–650. [PubMed: 17344484]

12. Institute of Medicine. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. National Academy Press; Washington, D.C.: 1997.

13. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr. 1995; 61:638S–645S. [PubMed: 7879731]

14. World Health Organization. Global disease burden from solar ultraviolet radiation. 2008. http://www.who.int/mediacentre/factsheets/fs305/en

15. Moore CE, Murphy MM, Holick MF. Vitamin D Intakes by Children and Adults in the United States Differ among Ethnic Groups. J Nutr. 2005; 135:2478–2485. [PubMed: 16177216]

16. Calvo M, S, Whiting SJ. Public health strategies to overcome barriers to optimal vitamin D status in populations with special needs. J Nutr. 2006; 136:1135–1139. [PubMed: 16549495]

17. Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. Am J Clin Nutr. 2008; 88:1519–1527. [PubMed: 19064511]

18. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 1988; 67:373–378. [PubMed: 2839537]

19. Calvo M, S, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr. 2004; 80:1710S–1716S. [PubMed: 15585792]

20. Grau MV, Baron JA, Sandler RS, Haile RW, Beach ML, Church TR, Heber D. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. J Natl Cancer Inst. 2003; 95:1765–1771. [PubMed: 14652238]

21. Goussous R, Song L, Dallal GE, Dawson-Hughes B. Lack of effect of calcium intake on the 25-hydroxyvitamin d response to oral vitamin D3. J Clin Endocrinol Metab. 2005; 90:707–711. [PubMed: 15562015]

22. Sanchez M, de la Sierra A, Coca A, Poch E, Giner V, Urbano-Marquez A. Oral calcium supplementation reduces intraplatelet free calcium concentration and insulin resistance in esstential hypertensive patients. Hypertension. 1997; 29:531–536. [PubMed: 9039155]

23. Heaney RP. Vitamin D and calcium interactions: functional outcomes. Am J Clin Nutr. 2008; 88:541S–544S. [PubMed: 18689398]

24. Cranney, A.; Horsley, T.; O'Donnell, S.; Weiler, H.; Puil, L.; Ooi, D.; Atkinson, S.; Ward, L.; Moher, D.; Hanley, D.; Fang, M.; Yazdi, F.; Garritty, C.; Sampson, M.; Barrowman, N.; Tsertsvadze, A.; Mamaladze, V. Effectiveness and safety of vitamin D in relation to bone health. Evidence Report/Technology Assessment No. 158 (Prepared by the University of Ottawa Evidence-based Practice Center (UO-EPC) under Contract No. 290-02-0021. AHRQ Publication No. 07-E013. Agency for Healthcare Research and Quality; Rockville, MD: Aug. 2007
25. Semba RD, Garrett E, Johnson BA, Guralnik JM, Fried LP. Vitamin D deficiency among older women with and without disability. Am J Clin Nutr. 2000; 72:1529–1524. [PubMed: 11101482]
26. Eaton, SB.; Shostak, M.; Konner, M. The Paleolithic Prescription, Book The Paleolithic Prescription. Harper and Row; New York: 1988.
27. Bostick RM, Fosdick L, Wood JR, Grambsch P, Grandits G, Lilliemoe JT, Louis TA, Potter JD. Calcium and colorectal epithelial cell proliferation in sporadic adenoma patients. J Natl Cancer Inst. 1995; 87:1307–1315. [PubMed: 7658483]
28. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of a expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol. 1992; 135:1114–1126. [PubMed: 1632423]
29. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol. 1989; 18:858–867. [PubMed: 2621022]
30. Hollis BW, Kamerud JQ, Salvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an 125 I-labelled tracer. Clin Chem. 1993; 39:529–533. [PubMed: 8448871]
31. Hollis BW, Kamerud JQ, Kurkowski A, Beaulieu J, Napoli JL. Quantification of circulating 1,25-dihydroxyvitamin D by radioimmunoassay with 125 I-labeled tracer. Clin Chem. 1996; 42:586–592. [PubMed: 8605676]
32. Willett W, Stampfer MJ. Total energy intake: Implications for epidemiologic analyses. Am J Epidemiol. 1986; 124:17–27. [PubMed: 3521261]
33. Tukey JW. The philosophy of multiple comparisons. Statist. Sci. 1991; 6:100–116.
34. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, Allen C, Dougherty C, Gunter EW, Bowman BA. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr. 2002; 76:187–192. [PubMed: 12081833]
35. Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: a global perspective of current status. J Nutr. 2005; 135:310–316. [PubMed: 15671233]
36. Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, Newmark HL, Giovannucci E, Wei M, Holick MF. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. Am J Prev Med. 2007; 32:210–216. [PubMed: 17296473]
37. Looker AC, Dawson-Hughes B, Calvo M, S, Gunter EW, Sahyoun NR. Serum 25-hyroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. bone. 2002; 30:771–777. [PubMed: 11996918]
38. Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hyddroxyvitamin D concentrations of young American black and white women. Am J Clin Nutr. 1998; 67:1232–1236. [PubMed: 9625098]
39. Thierry-Palmer M, Henderson VM, Hammali RE, Cephas S, Palacios C, Martin BR, Weaver CM. Black and white female adolescents lose vitamin D metabolites into urine. Am J Med Sci. 2008; 335:278–283. [PubMed: 18414066]
40. Brazier M, Grados F, Kamel S, Mathieu M, Morel A, Maamer M, Sebert JL, Fardellone P. Clinical and laboratory safety of one year's use of a combination calcium + vitamin D tablet in ambulatory elderly women with vitamin D insufficiency: results of a multicenter, randomized, double-blind, placebo-controlled study. Clin Ther. 2005; 12:1885–1893. [PubMed: 16507374]
41. Patel R, COllins D, Bullock S, Swaminathan R, Blake GM, Fogelman I. The effect of season and vitamin D supplementation on bone mineral density in healthy women: a double-masked crossover study. Osteoporos Int. 2001; 12:319–325. [PubMed: 11420782]

42. 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. [PubMed: 18065583]

43. Barger-Lux MJ, Heaney RP, Dowell S, Chen TC, Holick MF. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporos Int. 1998; 8:222–230. [PubMed: 9797906]

44. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007; 85:1586–1591. [PubMed: 17556697]

45. Wactawski-Wende J, Kotchen JM, Anderson GL, Assaf AR, Brunner RL, O'Sullivan MJ, Margolis KL, Ockene JK, Phillips L, Pottern L, Prentice RL, Robbins J, Rohan TE, Sarto GE, Sharma S, Stefanick ML, Van Horn L, Wallace RB, Whitlock E, Bassford T, Beresford SA, Black HR, Bonds DE, Brzyski RG, Caan B, Chlebowski RT, Cochrane B, Garland C, Gass M, Hays J, Heiss G, Hendrix SL, Howard BV, Hsia J, Hubbell FA, Jackson RD, Johnson KC, Judd H, Kooperberg CL, Kuller LH, LaCroix AZ, Lane DS, Langer RD, Lasser NL, Lewis CE, Limacher MC, Manson JE. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Eng J Med. 2006; 354:684–696.

46. Gray AW, Caldas AE, Wilz DR, Lemann J, Smith GA. Excretion of ^{3}H-1,25-dihydroxyvitamin D_3 in healthy adults. J Clin Endocrinol & Metab. 1978; 46:756–765. [PubMed: 263717]

Table 1

Baseline plasma 25(OH)D levels in the CaD v MAP trial (n = 91), by selected participant characteristics

Characteristics	Deficient <37.5 nmol/L *n (%)*	Insufficient 37.5 to <75 nmol/L *n (%)*	Sufficient ≥ 75 nmol/L *n (%)*	*P*[1]
All	21 (23.1)	54 (59.3)	16 (17.6)	0.001
Treatment group				0.26
Placebo (n=23)	5 (21.7)	16 (69.6)	2 (8.7)	
Calcium (n=23)	2 (8.7)	15 (65.2)	6 (26.1)	
Vitamin D (n=22)	6 (27.3)	13 (59.1)	3 (13.6)	
Calcium + Vitamin D (n=23)	8 (34.8)	10 (43.5)	5 (21.7)	
Age				0.54
< 60 yrs (n=47)	13 (27.7)	27 (57.5)	7 (14.9)	
≥ 60 yrs (n=44)	8 (18.2)	27 (61.4)	9 (20.5)	
Sex				0.18
Men (n=64)	12 (18.8)	42 (65.6)	10 (15.6)	
Women (n=27)	9 (33.3)	12 (44.4)	6 (22.2)	
Race				0.002
White (n=64)	9 (14.1)	40 (62.0)	15 (23.4)	
African American (n=20)	11 (55)	8 (40.0)	1 (5.0)	
Other (n=7)	1 (14.3)	6 (85.7)	0.0	
Body mass index (BMI, kg/ m^2)				0.89
< 25 (n=16)	3 (18.8)	9 (56.2)	4 (25.0)	
25-< 30 (n=43)	7 (20.6)	21 (61.8)	6 (17.7)	
≥ 30 (n-41)	11 (26.8)	24 (58.5)	6 (14.6)	
Sun exposure (hr/week)				0.07
< 1 (n=13)	5 (38.5)	7 (53.9)	1 (7.7)	
1-4 (n=36)	10 (27.8)	23 (63.9)	3 (8.3)	
≥ 4 (n=42)	6 (14.3)	24 (57.1)	12 (28.6)	
Total vitamin D intake (IU/d)				0.35
≤ 400 (n=55)	16 (28.1)	32 (56.1)	9 (15.8)	
> 400 (n=34)	5 (14.7)	22 (64.7)	7 (20.6)	
Date of blood draw:				0.26
Fall (Sep-Nov) (n=39)	6 (15.4)	23 (59.0)	10 (25.6)	
Winter (Dec-Feb) (n=26)	9 (34.6)	15 (57.7)	2 (7.7)	
Summer (May-Aug)[2] (n=26)	6 (23.1)	16 (61.5)	4 (15.4)	

[1] P-value calculated using the Chi-square or Fisher's exact test, as appropriate

[2] One person had a baseline blood draw in the Spring (May), and was included with Summer

Table 2

Number and percentage of subjects with plasma 25(OH)D concentrations above or below defined sufficiency cut-points at six-month follow-up, CaD v MAP pilot trial, n=85

Treatment group	Deficient <37.5 (nmol/L) *n (%)*	Insufficient 37.5 to <75 (nmol/L) *n (%)*	Sufficient ≥75 (nmol/L) *n (%)*
Placebo (n=21)	6 (28.6)	15 (71.4)	0.0
Calcium[1] (n=21)	5 (23.8)	11 (52.4)	5 (23.8)
Vitamin D[2] (n=22)	1 (4.6)	7 (31.8)	14 (63.6)
Calcium + Vitamin D[3] (n=21)	1 (4.8)	12 (57.1)	8 (38.1)
P[4]	*0.09*	*0.41*	*0.10*

[1]Calcium: 2,000 mg elemental calcium daily given as 1.25 g calcium carbonate twice daily with meals

[2]Vitamin D: 800 IU vitamin D_3 given as 400 IU twice daily with meals

[3]Calcium plus vitamin D as in footnotes 1 and 2 given together

[4]Fisher's exact test for difference across treatment group within strata of vitamin D status; overall chi-square test for treatment group by status =$p<0.001$

Table 3

Selected baseline characteristics of study participants by treatment group

	Treatment Group				
Characteristic[1]	**Placebo (n=23)**	**Calcium (n=23)**	**Vitamin D (n=22)**	**Calcium + Vitamin D (n=23)**	***P***[2]
Age (yrs)	58.5 ± 1.7	62 ± 1.7	60.2 ± 1.7	62.1 ± 1.7	*0.39*
Male, n (%)	16 (69.6)	16 (69.6)	16 (72.7)	16 (69.6)	*0.99*
Caucasian, n (%)	17 (73.9)	19 (82.6)	14 (63.6)	14 (60.9)	
African-American, n (%)	2 (8.7)	4 (17.4)	6 (27.3)	8 (34.8)	*0.12*
Body Mass Index (kg/m^2)	30.6 ± 1.3	29.4 ± 1.3	29.2 ± 1.3	31.6 ± 1.3	*0.51*
Total vitamin D intake[3] (IU/day)	279.0 ± 56.5	326.0 ± 57.7	347.7 ± 57.7	401.5 ± 57.7	*0.50*
Total calcium intake[3] (mg/day)	625.3 ±90.6	677.6 ± 92.7	753.3 ± 92.7	733.7 ± 92.7	*0.74*
Sun exposure (hrs/wk)	4.0 ± 0.6	4.2 ± 0.6	4.9 ± 0.6	3.8 ± 0.6	*0.61*
Travel to warm/sunny location, n (%)	6 (26.1)	10 (43.48)	4 (18.2)	3 (13.0)	*0.10*
Suncreen use, n (%)					
Never	16 (30.2)	9 (17.0)	12 (22.6)	16 (30.2)	
Rarely	4 (21.1)	6 (31.6)	5 (26.3)	4 (21.0)	
Sometimes to Always	3 (15.8)	8 (42.1)	5 (26.3)	3 (15.8)	*0.30*
Date of blood draw, n (%)					
Fall (Sep-Nov)	9 (23.1)	10 (25.6)	12 (30.8)	8 (20.5)	
Winter (Dec-Feb)	9 (34.6)	6 (23.1)	3 (11.5)	8 (30.8)	
Summer (May-Aug)[4]	5 (19.2)	7 (26.9)	7 (26.9)	7 (26.9)	*0.60*
Plasma 1,25(OH)$_2$D levels (pmol/L)	94.0 ± 11.4	108.9 ± 11.4	106.7 ± 11.6	90.9 ± 11.4	*0.60*
Plasma 25(OH)D levels (nmol/L)	51.1 ± 4.3	64.2 ± 4.3	52.6 ± 4.4	52.3 ± 4.3	*0.12*

[1] Continuous variables presented as means ±SEM; categorical variables as n (%)

[2] ANOVA for mean differences for continuous variables; Fisher's exact test for categorical variables

[3] Total intake (diet plus supplements), derived from baseline FFQ, energy-adjusted

[4] One person had a baseline blood draw in the Spring (May).

Table 4

Changes in plasma vitamin D metabolites over six months, by treatment group (mean ± SEM)

	Baseline	Follow-up	Change[1]	P[1]	Change2[2]	P[2]	Change3[3]	P[3]
Placebo (n=21)								
25(OH)D (nmol/L)	51.1 (3.9)	44.7 (3.8)	−6.3 (5.6)	*0.30*	−4.9 (3.1)	*0.10*	−4.6 (3.2)	*0.10*
$1,25(OH)_2D$ (pmol/L)	94.0 (6.1)	89.1 (5.6)	−4.9 (8.3)	*0.60*	−5.3 (15.5)[7]	*0.70*	0.5 (16.7)[7]	*0.97*
	Baseline	**Follow-up**	**Change[1]**	**P[1]**	**Change2[2]**	**P[2]**	**Change3[3]**	**P[3]**
Calcium[4,5] (n=21)								
25(OH)D (nmol/L)	64.1 (4.0)	58.0 (4.8)	−6.2 (6.2)	*0.30*	−0.7 (4.4)	*0.90*	0.8 (4.2)	*0.90*
$1,25(OH)_2D$ (pmol/L)	108.9 (17.8)	76.0 (6.5)	−32.9 (19.5)	*0.09*	−27.8 (22.0)[7]	*0.20*	−23.9 (22.5)[7]	*0.30*
	Baseline	**Follow-up**	**Change[1]**	**P[1]**	**Change2[2]**	**P[2]**	**Change3[3]**	**P[3]**
Vitamin D[4,6] (n=22)								
25(OH)D (nmol/L)	52.6 (4.4)	73.7 (3.8)	21.1 (5.9)	*0.001*	26.0 (4.4)	*0.001*	28.9 (4.3)	*0.001*
$1,25(OH)_2D$ (pmol/L)	106.7 (11.6)	113.8 (14.3)	7.0 (18.4)	*0.70*	12.3 (21.7)[7]	*0.60*	13.3 (22.6)	*0.60*
	Baseline	**Follow-up**	**Change[1]**	**P[1]**	**Change2[2]**	**P[2]**	**Change3[3]**	**P[3]**
Calcium+Vitamin D[5,6] (n=21)								
25(OH)D (nmol/L)	52.3 (5.0)	71.3 (4.3)	19.0 (6.7)	*0.007*	25.4 (4.4)	*0.001*	25.7 (4.2)	*0.001*
$1,25(OH)_2D$ (pmol/L)	90.9 (6.31)	88.1 (15.1)	−2.8 (15.8)	*0.90*	0.9 (22.0)[7]	*0.97*	2.9 (22.3)[7]	*0.90*

[1] Difference between follow-up and baseline in each treatment group (without any adjustment); corresponding p-value adjusted for multiple comparisons (Tukey-Kramer)

[2] Difference in treated groups adjusted for placebo effect, i.e. difference between follow-up and baseline in treated group compared with the corresponding difference in placebo group (time*RX interaction term in model); corresponding p-value adjusted for multiple comparisons (Tukey-Kramer)

[3] Difference in treated group (as in Change[2]) additionally adjusted for season of blood draw; corresponding p-value adjusted for multiple comparison (Tukey-Kramer)

[4] Difference (Change[2,3]) in pre-post intervention 25(OH)D in calcium only vs vitamin D only group $p<0.008$; Bonferroni correction for multiple comparisons

[5] Difference (Change[2,3]) in pre-post intervention 25(OH)D in calcium only vs. calcium plus vitamin D group $p<0.008$; Bonferroni correction for multiple comparisons

[6] Difference (Change[2,3]) in pre-post intervention 25(OH)D in vitamin D only vs. calcium plus vitamin D group not significant; Bonferroni correction for multiple comparisons

[7] Difference (Change[2,3]) in pre-post intervention $1,25(OH)_2D$ not significant; Bonferroni correction for multiple comparisons.