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Research Article

Cancer Prevention Research

Effect of Vitamin D_3 Supplementation in Combination with Weight Loss on Inflammatory Biomarkers in Postmenopausal Women: A Randomized Controlled Trial

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Abstract

Obesity and vitamin D deficiency are associated with risk for several cancers, possibly through inflammation and adipokine-related pathways. Two hundred and eighteen postmenopausal women with BMI > 25 kg/m^2 and low serum 25-hydroxyvitamin D (25(OH)D; ≥10–<32 ng/mL), were randomized to 12 months of either (i) weight-loss intervention + 2000 IU/day oral vitamin D_3 or (ii) weight-loss intervention + daily placebo. Serum adiponectin, leptin, TNFα, IL6, IL1β, IL8, and IL10, were measured by immunoassay, and a composite inflammatory biomarker score calculated. Using generalized estimating equations, mean changes in outcomes were compared between arms (intent-to-treat), adjusted for possible confounders. Analyses were also stratified by weight-loss (gained/no weight-loss; <5%; 5% to 10%; ≥10%). At 12 months, there were no significant differences in analyte changes between arms. In stratified analyses, participants randomized to vitamin D_3 who lost 5% to 10% of baseline weight, versus participants who gained weight/had no weight-loss, had significantly greater decreases in levels of IL6 compared with those randomized to placebo: absolute change −0.75 pg/mL (−17.2%), placebo versus −1.77 pg/mL (−37.3%), vitamin D, $P = 0.004$. Similar but attenuated results were observed for participants who lost ≥10% of baseline weight: −0.41 pg/mL (−13.6%), placebo versus −0.67 pg/mL (−17.3%), vitamin D, $P = 0.02$. Effects of vitamin D_3 supplementation on levels of IL1β were inconsistent when stratified by weight loss. There were no intervention effects on IL10, TNFα, IL8, the composite score, adiponectin, or leptin, when stratified by weight-loss. In conclusion, vitamin D_3 supplementation in combination with weight-loss of at least 5% of baseline weight was associated with significant reductions in levels of IL6. *Cancer Prev Res; 8(7); 628–35. ©2015 AACR.*

Introduction

Overweight and obesity are associated with the risk of developing several types of cancer (1–3). The underlying mechanisms linking adiposity to cancer risk are still unknown, but it is likely that the tissue microenvironment in the obese state is involved in dysregulation of a number of systems including inflammatory pathways (4–6), and in the expression of adipokines, secreted by adipose tissue, such as adiponectin and leptin (7). The inflammatory response is mediated via cytokines, proteins that can either promote (e.g., c-reactive protein (CRP), IL1β, TNFα, or suppress inflammation (e.g., IL10). A variety of proinflammatory cytokines including IL1β and IL6 have been implicated in the etiology of cancer (8–12). Adiponectin is a peptide hormone whose levels inversely correlate with body mass index (BMI; ref. 13). It has insulin-sensitizing, antiangiogenic, and anti-inflammatory effects (14, 15). Leptin is a hormone that regulates appetite, food intake, and body weight. Both adiponectin and leptin play a central role in energy homeostasis, metabolism, and regulation of adiposity (16, 17).

The definitions of vitamin D [25-hydroxyvitamin D (25(OH)D)] deficiency and insufficiency have been debated (18), with classifications for "deficiency" ranging from <10 ng/mL to <20 ng/mL (19–21). The Workshop Consensus for Vitamin D Nutritional Guidelines estimated that between 50% to 60% of older populations worldwide are vitamin D deficient (19–21). A recent meta-analysis and systematic review of both observational cohort studies of circulating 25(OH)D, and randomized clinical trials of vitamin D supplementation, reported inverse associations of circulating 25(OH)D with risk of death due to cardiovascular disease, cancer, and other causes, as well as reduced overall mortality with Vitamin D_3 supplementation (22). Furthermore, several investigations suggest that vitamin D deficiency may be associated with increased levels of circulating inflammatory markers (23–26).

Several studies have reported that weight loss is associated with reductions in inflammation and in adipokines: in a previous randomized controlled trial (RCT), a behavioral weight-loss intervention significantly reduced circulating CRP, serum amyloid A, and IL6; increased adiponectin, and decreased leptin (27, 28). However, whether vitamin D administration can increase the effect of weight loss on inflammation-related biomarkers and adipokines is unknown.

This study is ancillary to the Vitamin D diet and Activity (ViDA) clinical trial which randomized overweight/obese, 25(OH)D

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insufficient (i.e., ≥10–<32 ng/mL), postmenopausal, healthy women to one year of 2,000 IU oral vitamin D_3 supplementation daily versus daily placebo. All randomized women underwent a concomitant weight-loss (diet + exercise) program, with a goal of 10% weight loss. We reported previously that levels of CRP decreased significantly with increasing serum 25(OH)D change from baseline to 12 months, adjusted for change in weight, only among the 55% of participants whose study medication adherence data were available (29).

The primary aims of this study are to examine, in the context of a completed RCT, whether 2,000 IU oral vitamin D supplementation in combination with a weight-loss program, reduces serum levels of leptin or the proinflammatory cytokines TNFα, IL6, IL1β, and IL8; or increases levels of adiponectin or the anti-inflammatory marker IL10, compared with a weight-loss program+placebo in postmenopausal overweight or obese women who had insufficient levels of 25(OH)D at baseline. In preplanned analyses, we also stratified analyses by categories of weight change.

Materials and Methods

This study is ancillary to the ViDA study (Trial Registration: www.clinicaltrials.gov Identifier NCT01240213), a randomized clinical trial comparing the effects of 12 months of 2,000 IU/day oral vitamin D (cholecalciferol or vitamin D_3) supplementation versus placebo on weight and other biomarkers of breast cancer risk in overweight and obese postmenopausal women partaking in a lifestyle based weight-loss program.

The study was performed with the approval of the Fred Hutchinson Cancer Research Center (Seattle, WA) Institutional Review Board, in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each participant.

Study population

The study has been described in detail elsewhere (29). Briefly, 218 postmenopausal, overweight or obese (BMI ≥ 25 kg/m^2), ages 50 to 75 years, not taking hormonal therapy, with insufficient serum 25(OH)D concentrations (i.e., ≥10–<32 ng/mL), were enrolled between 2010 and 2012, and randomly assigned to a 1-year intervention arm consisting of 2,000 IU/day of oral vitamin D_3 supplementation + a lifestyle-based weight-loss program (n = 109; `Vitamin D'); or to a control arm of a daily placebo + a lifestyle-based weight-loss program (n = 109; "placebo"). Ineligibility criteria included contraindications to taking >2,000 IU vitamin D_3/day; already participating in a diet or exercise intervention; history of bariatric surgery; use of weight loss medications; or serum 25(OH)D <10 ng/mL (deficient); and ≥32 ng/mL (replete). Randomization was stratified according to BMI (<30 kg/m^2 or ≥30 kg/m^2) and consent for optional breast and abdominal subcutaneous fat biopsies. The number of women randomized to each study arm did not differ by season (χ^2 P > 0.99). All staff except study statisticians were blinded to randomization status. Eighty-six percent of women returned for their 12-month appointment to provide biospecimens and survey data (N = 94, placebo arm; N = 93, vitamin D arm). Of these women, only 55% remembered to return their unused pills. Thus, complete pill counts are available only for a subset of women: 54% of women in the vitamin D arm and 56% of women in the placebo arm. Of these, vitamin D-randomized women consumed 98% of capsules compared with 96% in placebo-randomized women. At baseline, 59 (27.1%) women reported taking vitamin D supplements on a regular basis, compared with 41 (21.8%) at 12 months. The 12-month change in vitamin D intake from supplements did not differ between study arms (P = 0.60).

Weight loss program

The ViDA lifestyle-based weight-loss program was administered to all participants. It included a diet and exercise component, with a weight-loss goal of 10% of participants' baseline weight, which was adapted from a successful intervention that we have previously used in a similar population of overweight and obese postmenopausal women (30), based upon the Diabetes Prevention Program and Look Ahead lifestyle change weight loss programs (31, 32).

The goals of the diet program were: total daily energy intake of 1,200 to 2,000 kcal/day based on baseline weight, less than 30% daily energy intake from fat, and a 10% reduction in body weight by 6 months with maintenance thereafter to 12 months. The nutrition program, led by behaviorally trained registered dieticians, was delivered in groups and individual sessions.

The goal of the exercise program was: ≥45 minutes of moderate-to-vigorous intensity exercise, 5 days per week (225 minutes/week) for 12 months. Women attended two sessions per week at our study facility where they were supervised by an exercise physiologist, and performed their remaining sessions at home. Facility-based exercise consisted of treadmill walking or jogging, stationary bicycling, and use of other aerobic machines, while a variety of home exercises were encouraged including walking/hiking, aerobics, and bicycling.

Blood specimen collection and processing

At baseline and 12 months, participants provided a 12-hour fasting 50 mL sample of blood, which was processed within 1 hour of collection and stored at −70°C. Participants were instructed to refrain from alcohol (48 hours) and vigorous exercise (24 hours) before clinic appointments. Of 218 participants randomized, baseline and 12-month serum was available for 187 (86%) participants (93 control; 94 intervention).

Assays

Serum levels of TNFα, IL6, IL1β, IL8, and IL10 were assayed at the Dept. of Laboratory Medicine, University of Washington (UW), Seattle, WA, using Milliplex Map Human High Sensitivity T Cell Panel, Immunology Multiplex Assay (EMD Millipore Corp.). Internal QA samples containing high and low levels of each analyte were included in each assay to assess the intra-assay coefficient of variation (CV). Baseline and 12-month samples from each individual were included in the same batch, and participants' samples were randomly placed across batches. Laboratory personnel were blinded with regard to participant and QA sample identity. The intra-assay CVs for each assay for high and low concentrations, respectively, were as follows: TNFα, 22.6% and 31.9%; IL1β, 18.7% and 29.4%; IL8, 24.9% and 39.5%; IL10, 29.4% and 20.5%; and IL6, 36.4% and 41.8%. Inter-assay CVs were: TNFα, 14.4%; IL1β, 22.92%; IL8, 24.3%; IL10, 25.91%; and IL6, 25.5%. Serum levels of adiponectin and leptin were measured at the Northwest Lipid Research Laboratory at the UW using commercially immunoassays (Millipore Inc.), with CVs of 8.4% and 8.8% (adiponectin) and 9.1% and 14.3% (leptin), respectively. Serum levels of 25(OH)D were measured as previously described (29).

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Covariates

Demographics, lifestyle behaviors such as sun-exposure and dietary intake, physical activity, medical history, and supplement use were collected by questionnaire; anthropometrics were measured and BMI was calculated as kg/m^2, at baseline and 12 months. Total and percentage body fat and trunk fat mass was measured by a DXA whole-body scanner (GE Lunar). We generated an inflammatory biomarker composite z-score of all of the pro- and anti-inflammatory markers combined as described by Hopkins and colleagues (33). Briefly, a normalized z-score for each biomarker value, with a mean of zero and SD of 1.0, was calculated as $z = (x - \mu)/\sigma$, where x is a participant's biomarker value at a given visit, and μ and σ are the study population mean and SD, respectively, at baseline. The combined z-score for each participant at each trial visit was created by summing the z-scores of each inflammatory marker. IL10 was subtracted as it is classified as an anti-inflammatory cytokine. Inclusion of previously measured CRP (29) in the biomarker score had no effect on the results, and was omitted. Medication use known to have effects on CRP and other inflammatory markers, such as (i) systemic corticosteroids and (ii) either ACE (angiotensin-converting enzyme) inhibitors, statins, or NSAIDs (34), was coded as use/non-use at baseline and 12 months.

Statistical analyses

One participant was omitted from the analysis as she was missing baseline serum measurements. A natural logarithmic transformation was applied to the outcome variables to improve the normality of the distribution.

Mean changes in IL6, IL1β, IL10, IL8, TNFα, the inflammatory composite score, and adiponectin and leptin, from baseline to 12 months, stratified by study arm, were computed. The intervention effect on these variables was examined based on the assigned treatment at randomization, regardless of adherence or study retention (i.e., intent-to-treat). Mean 12-month changes in the vitamin D group were compared with placebo using the generalized estimating equations modification of linear regression to account for intra-individual correlation over time.

Changes in weight were calculated and used to stratify observed changes in analytes between arms at 12 months. Weight-loss was categorized as no change/gained any weight (referent); lost <5% of baseline weight; lost ≥5–<10% of baseline weight; and lost ≥10% of baseline weight.

All models are presented as unadjusted; and adjusting for age, race/ethnicity, baseline serum 25(OH)D, baseline BMI, vitamin D intake (diet+supplement), calcium intake (diet+supplement), average sun exposure and medication use (use of either corticosteroids and/or CRP-lowering agents). Results are presented as (i) percent change, calculated as 100 × (follow-up geometric mean − baseline geometric mean)/baseline geometric mean; (ii) absolute treatment effect, calculated as (absolute change in vitamin D group − absolute change in placebo group); and (iii) relative treatment effect, calculated as (follow-up geometric mean in vitamin D group) × (baseline geometric mean in placebo group)/[(baseline geometric mean in vitamin D group) × (follow-up geometric mean in placebo group)].

All statistical tests were two sided. Statistical analyses were performed using SAS software (version 9, SAS Institute Inc.).

Results

At baseline, participants were on average aged 59.6 years, with a mean BMI of 32.4 kg/m^2 (Table 1). The majority were non-Hispanic white (86.2%), and the average baseline serum 25(OH)D level was 21.4 (SD 6.1) ng/mL. As previously reported, a total of 86% of participants completed their 12-month measurements. Serum 25(OH)D increased by a mean of 13.6 (95% CI, 11.6–15.4) ng/mL in the vitamin D arm while it decreased by a mean 1.3 (95% CI, −2.6 to −0.3) ng/mL in the placebo arm ($P < 0.0001$). Changes in weight, BMI, waist circumference, percentage body fat, insulin, and CRP were similar between groups (all $P > 0.05$; ref. 29).

Although there was a reduction in levels of all analytes comparing baseline and 12 months, there were no significant differences in the magnitude of these changes between arms (Table 2). We examined the effect of removing outlying data points that corresponded to values >99th percentile from the analyses. Three participants had IL1β levels classified as outliers; 3 had IL8 or TNFα classified as outliers (values ≥99th percentile), and 4 had IL6 or IL10 classified as outliers. Removal of these data had no effect on the results (data not shown).

We stratified women by degree of weight-loss, using cut points shown to have clinical significance (ref. 35; loss of <5%; 5%–10%; and ≥10% of baseline weight) compared with participants who experienced no change/gained weight (the referent group; Table 3). Changes in levels of analytes by randomization arm within each weight strata were compared with the referent group, adjusting for age, ethnicity, baseline serum 25(OH)D, vitamin D intake (diet+supplement), calcium intake (diet+supplement), medication use, and average sun exposure.

Participants who lost at least 5% to 10% of their baseline weight in the vitamin D_3 arm, significantly reduced IL6 levels above that of weight-loss alone, compared with the referent group (Table 3): absolute change −0.75 pg/mL (−17.2%), placebo versus −1.77 pg/mL (−37.3%) vitamin D, $P = 0.004$. Similarly, participants who lost ≥10% of their baseline weight randomized to the vitamin D arm, had greater decreases in IL6 compared with those in the placebo arm: −0.41 pg/mL (−13.6%) placebo versus −0.67 pg/mL (−17.3%) vitamin D, $P = 0.02$. Effects of vitamin D on IL1β were inconsistent when stratified by weight loss, but absolute changes across strata were very small. Finally, there were no intervention effects on the anti-inflammatory cytokine IL10, TNFα, IL8, the inflammatory biomarker composite score, adiponectin or leptin, when stratified by weight loss.

We next compared changes in levels of analytes from baseline to 12-month follow-up by intervention arm, stratified by baseline BMI (<30 kg/m^2, ≥30 kg/m^2); no statistically significant differences were observed (all $P > 0.05$; data not shown). Similarly, there were no statistically significant changes in levels of analytes between arms, when stratified by baseline levels of serum 25(OH)D (<20 ng/mL, 20–<32 ng/mL; all $P > 0.05$, data not shown).

In an analysis limited to women randomized to vitamin D, there were no significant differences between levels of analytes comparing the 40 women who did not become replete, with 53 women who became replete [i.e., 25(OH)D ≥32 ng/mL] by 12 months (data not shown). Finally, there were no differences between arms, comparing women with and without complete pill counts (data not shown). Exclusion of outliers from any of these analyses had no effect on results.

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Table 1. Selected baseline characteristics of study participants

	All participants		Intervention arms			
			Placebo		Vitamin D	
Variable	***N***	**Mean (SD)**	***N***	**Mean (SD)**	***N***	**Mean (SD)**
Age, y	218	59.6 (5.1)	109	59.0 (4.7)	109	60.3 (5.3)
Weight (kg)	218	87.7 (16.3)	109	88.1 (17.1)	109	87.4 (15.5)
BMI (kg/m^2)	218	32.4 (5.8)	109	32.5 (6.1)	109	32.3 (5.5)
Waist circumference (cm)	218	100.1 (12.3)	109	100.3 (13.5)	109	100.0 (11.0)
Body fat (%)	215	47.4 (4.9)	107	47.5 (4.5)	108	47.3 (5.2)
Moderate to vigorous physical activity (min/wk)	218	142.2 (143.2)	109	146.6 (140.4)	109	137.9 (146.5)
Caloric intake (kcal/d)[a]	206	2,004 (699.3)	103	1982 (678.4)	103	2,025 (722.3)
Relative % calories from fat[a]	206	33.0 (6.2)	103	32.6 (5.7)	103	33.4 (6.7)
Relative % calories from protein[a]	206	17.6 (3.2)	103	17.9 (3.5)	103	17.2 (2.9)
Relative % calories from carbohydrate[a]	206	48.3 (7.4)	103	48.1 (7.1)	103	48.5 (7.8)
Dietary vitamin D intake (μg)	206	6.6 (4.6)	103	6.9 (5.2)	103	6.3 (4.0)
Vitamin D supplement intake (IU) at baseline	52	280.0 (134.5)	22	303.6 (125.2)	30	262.7 (140.5)
Total calcium intake (mg)	208	1,120 (599.8)	103	1,170 (632.7)	105	1,071 (564.4)
Sun exposure (h/wk)	218	2.4 (1.3)	109	2.2 (1.3)	109	2.5 (1.3)
Serum 25(OH)D (ng/mL)	218	21.4 (6.1)	109	21.4 (6.1)	109	21.4 (6.2)
IL1β (pg/mL)	217	1.5 (10.4)	108	1.0 (3.0)	109	2.0 (14.4)
IL6 (pg/mL)	217	7.2 (19.8)	108	8.6 (27.2)	109	5.7 (7.1)
IL8 (pg/mL)	217	13.5 (64.9)	108	7.6 (5.1)	109	19.4 (91.2)
IL10 (pg/mL)	217	53.7 (386.6)	108	28.9 (33.2)	109	78.3 (544.5)
TNFα (pg/mL)	217	10.4 (4.7)	108	10.4 (4.8)	109	10.4 (4.6)
Adiponectin (μg/mL)	217	12.4 (6.1)	108	12.5 (6.4)	109	12.3 (5.9)
Leptin (ng/mL)	217	40.3 (18.9)	108	40.3 (17.4)	109	40.4 (20.5)
	N (%)		*N* (%)		*N* (%)	
Race/ethnicity						
Non-Hispanic white	188 (86.2)		94 (86.2)		94 (86.2)	
Non-Hispanic Black	13 (6.0)		6 (5.5)		7 (6.4)	
Hispanic	5 (2.3)		4 (3.7)		1 (0.9)	
Other (American Indian, Asian, or Unknown)	12 (5.5)		5 (4.5)		7 (6.4)	
Medication use[b]						
Systemic corticosteroids						
Baseline	11 (5.0)		6 (5.5)		5 (4.6)	
12 mo	7 (3.2)		3 (2.8)		4 (3.7)	
CRP-lowering agents[c]						
Baseline	11 (5.0)		6 (5.5)		5 (4.6)	
12 mo	6 (2.8)		1 (0.9)		5 (4.6)	

[a]Dietary data (FFQs) available for all participants at baseline ($N = 218$) but values derived from FFQ were truncated at <600 kcal and >4,000 kcal.
[b]Only one participant was using both corticosteroid and CRP lowering agent medication simultaneously at baseline. No participant used both medication types simultaneously at 12 months.
[c]Statins, NSAIDs, ACE inhibitors.

Discussion

Here, we report that a 12-month reduced calorie/increased physical activity weight loss intervention in combination with 2,000 IU of vitamin D_3 supplementation per day had no overall effect on circulating levels of inflammatory cytokines compared with the same weight loss intervention + placebo. Participants, who lost at least 5% to 10% of their baseline weight in the vitamin D_3 arm, significantly reduced IL6 levels above that of weight-loss alone. The nonsignificant trend of a reduction in levels of the anti-inflammatory cytokine IL10 with vitamin D_3 supplementation in combination with increased weight-loss is surprising, but may reflect downregulation of this cytokine in response to less localized inflammation. Weight loss in combination with vitamin D_3 supplementation had no effect on levels of IL8, TNFα, the composite biomarker score, adiponectin, or leptin.

Vitamin D deficiency, which has been associated with increased risk for certain cancers (36–40), is associated with higher circulating concentrations of several markers of inflammation, including CRP and IL6 (24–26, 41). It is known that both weight-loss and increased levels of vitamin D_3 are associated with reductions in IL6. We previously reported results from a different RCT in overweight or obese postmenopausal women that used a similar behavioral weight-loss intervention as the present study, and which resulted in significant reductions in IL6 and CRP levels compared with controls (27). A variety of *in vitro* studies have demonstrated that the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D_3) inhibits the production of IL6 (23), possibly by inhibiting p38 via MAPK phosphatase 5, which contains a putative vitamin D response element within its promoter that associated with the vitamin D receptor following treatment with 1,25-(OH)2 D_3 (42). Of interest, a recent study found that 1,25(OH)2D resulted in a dose-dependent downregulation of COX-2 expression in murine macrophages under both basal and lipopolysaccharide-stimulated conditions. It also reduced IL6 and TNFα secretion by suppression of Akt phosphorylation and NF-κB signaling (23). Weight loss and vitamin D_3 supplementation may act synergistically via different mechanisms to reduce levels of IL6. Alternatively, weight loss may increase the bioavailability of vitamin D in different tissues. However, in the present study, achieving 25(OH)D repletion (>32 ng/mL) in women randomized to the vitamin D arm was not associated

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Table 2. Twelve-month changes in serum IL1β, IL6, IL8, IL10,TNFα, BMI, adiponectin and leptin, and in the inflammatory biomarker score, by study arm

	Placebo			Vitamin D			Absolute treatment effect[d]	Relative treatment effect[e]	*P*	
Variable	Baseline (*N* = 109) Geometric mean (95% CI)	12 mo (*N* = 94) Geometric mean (95% CI)	Change Absolute change (%)	Baseline (*N* = 109) Geometric mean (95% CI)	12 mo (*N* = 93) Geometric mean (95% CI)	Change Absolute change (%)	(95% CI)	(95% CI)	P^a	P^b
BMI kg/m^2 (ref. 29; SD)	32.5 (6.1)	29.7 (6.1)	−2.8 (−8.8)	32.3 (5.5)	29.5 (5.6)	−2.8 (−8.7)	0.05 (−0.93–1.03)	1.0 (0.97–1.03)	0.63	0.82
IL1β (pg/mL)	0.14 (0.10–0.22)	0.12 (0.08–0.19)	−0.02 (−15.9)	0.11 (0.08–0.16)	0.09 (0.06–0.15)	−0.02 (−16.7)	0 (−0.04–0.05)	0.99 (0.7–1.4)	0.28	0.12
IL6 (pg/mL)	3.89 (3.15–4.79)	3.52 (2.81–4.42)	−0.36 (−9.3)	3.89 (3.33–4.53)	3.41 (2.82–4.12)	−0.48 (−12.3)	−0.12 (−0.92–0.69)	0.97 (0.78–1.2)	0.71	0.89
IL8 (pg/mL)	6.38 (5.72–7.12)	5.89 (5.12–6.78)	−0.49 (−7.7)	6.64 (5.66–7.80)	6.30 (5.50–7.21)	−0.34 (−5.2)	0.15 (−0.92–1.13)	1.03 (0.87–1.21)	0.93	0.65
IL10 (pg/mL)	16.46 (13.04–20.77)	14.47 (10.87–19.26)	−1.99 (−12.1)	16.74 (13.43–20.87)	15.02 (11.75–19.20)	−1.72 (−10.3)	0.27 (−4.42–4.66)	1.02 (0.76–1.36)	0.60	0.71
TNFα (pg/mL)	9.35 (8.54–10.24)	8.85 (7.94–9.85)	−0.51 (−5.4)	9.30 (8.45–10.24)	9.14 (8.31–10.05)	−0.16 (−1.8)	0.34 (−0.81–1.50)	1.04 (0.92–1.18)	0.41	0.57
Inflammatory biomarker score[c]	1.05 (0.66–1.67)	0.78 (0.47–1.31)	−0.27 (−25.7)	0.96 (0.61–1.51)	0.76 (0.47–1.21)	−0.21 (−21.5)	0.06 (−0.45–0.57)	1.06 (0.62–1.79)	0.79	0.51
Adiponectin (μg/mL)	11.04 (10.0–12.8)	11.85 (10.71–13.1)	0.81 (7.3)	11.11 (10.19–12.14)	12.12 (11.10–13.24)	1.01 (9.1)	0.2 (−0.68–1.07)	1.02 (0.94–1.10)	0.33	0.61
Leptin (ng/mL)	36.17 (32.88–39.78)	24.62 (21.27–28.49)	−11.5 (−31.9)	35.59 (32.27–39.24)	23.5 (20.07–27.61)	−12.0 (−33.9)	0.50 (−4.51–3.45)	0.97 (0.83–1.14)	0.69	0.73

[a]*P* value was obtained from GEE model comparing 12-month change between vitamin D and placebo groups adjusted for baseline BMI.

[b]*P* value was obtained from GEE model adjusting for age, ethnicity, baseline serum 25(OH)D, baseline BMI, vitamin D intake (diet+supplement), calcium intake (diet+supplement), medication use, and average sun exposure.

[c]The inflammatory biomarker score was calculated as described 33 using log-transformed IL1β, IL6, IL8, IL10, and TNFα.

[d]Absolute treatment effect was calculated as (absolute change in vitamin D group − absolute change in Placebo group).

[e]Relative treatment effect was calculated as (follow-up geometric mean in vitamin D group) × (baseline geometric mean in Placebo group)/[(baseline geometric mean in vitamin D group) × (follow-up geometric mean in Placebo group)].

with significant differences in levels of these analytes when compared with women who did not achieve repletion.

A variety of studies have examined the effect of vitamin D supplementation on inflammatory markers in both chronically ill and healthy individuals, with conflicting results.

A 6-month 4-arm RCT randomized 131 healthy women who were deficient in Vitamin D, to receive 60,000 IU/week of vitamin D_3 for 8 weeks followed by 60,000 IU/fortnight; calcium; dual supplementation or placebo. The study examined changes in gene expression of IFNγ, IL4, and its antagonist-IL-4δ2, and a variety of transcription factors involved in their regulation, but reported no significant effect of vitamin D_3 supplementation on their expression (43). Two groups of obese but otherwise healthy adolescents were randomized to receive 4,000 IU of vitamin D_3 daily versus placebo. After 6 months, there was no difference between groups in serum levels of CRP, IL,6 or TNFα, though the authors suggested the study was inadequately powered to detect biologically relevant changes in these three markers (44). A variety of RCTs found no effect on vitamin D_3 supplementation on inflammatory biomarkers. No effect of vitamin D_3 supplementation on CRP or IL6 was observed in a year-long RCT in 305 healthy postmenopausal women ages 60 to 70 years, comparing 400 IU versus 1,000 IU placebo supplementation (26). Similarly no effect on CRP levels were found in RCTs comparing 5,000 IU/day of vitamin D_3 versus vitamin C supplementation on 88 acutely hospitalized patients treated for an average of 8.2 days (45); 7,000 IU of vitamin D_3 daily versus placebo of 52 overweight men (>30 kg/m^2) and women ages 18 to 50 years (26); or in an RCT of 438 overweight/obese subjects, 21 to 70 years old, randomized to one year of either 40,000 IU/week of vitamin D_3, 20,000 IU/week, or placebo for one year. The latter study also found no effect on IL10 (46). Finally, an RCT comparing 3 months of 1,000, 2,000, or 4,000 vitamin D3 supplementation versus placebo in 328 African Americans, found no statistically significant effect of supplementation on changes in CRP, IL6, IL10, or sTNF-R2 (41).

Sixty-three children and adolescents with inflammatory bowel disease were randomized to receive either 400 IU of vitamin D_2/day or 1,000 IU in summer/2,000 IU in winter. Participants who received the lower dose had significantly elevated levels of CRP and IL6 compared with those who received the higher dose CRP 31% versus 10%, $P = 0.04$, and IL6 54% versus 27%, $P = 0.05$; respectively (47). In 267 patients with systemic lupus erythematosus, a chronic multisystem inflammatory autoimmune disease, a year-long supplementation with daily 2,000 IU/day of vitamin D_3 versus placebo for 12 months was associated with a reduction in inflammatory markers (48). Finally, an RCT which randomized 92 patients with colorectal adenoma to receive either 6 months of 2 g/day calcium and/or 800 IU/day of vitamin D_3 versus placebo. The study reported a significant reduction in the combined inflammatory biomarker score (77%, $P = 0.003$) in the vitamin D_3 group compared with placebo (33).

Our previous analysis found no effect of vitamin D_3 supplementation on levels of CRP (either overall or to repletion >32 ng/mL; ref. 29). However, we did observe greater reductions in CRP levels with greater increases in vitamin D_3, suggesting a possible dose-response effect, although this effect was no longer significant after adjusting for percentage weight loss.

Strengths of our study include its prospective design, the use of a double-blind–controlled intervention, the broad set of inflammatory markers that were evaluated, and the year-long timeframe.

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Table 3. Twelve-month changes in serum IL1β, IL6, IL8, IL10, TNFα, an Inflammatory Biomarker Score, and adiponectin and leptin, by study arm, stratified by percent weight loss

	Placebo			Vitamin D						
Weight loss	Baseline ($N = 94$) Geometric mean (95% CI)	12 mo ($N = 94$) Geometric mean (95% CI)	Absolute change (%)	Baseline ($N = 94$) Geometric mean (95% CI)	12 mo ($N = 93$) Geometric mean (95% CI)	Absolute change (%)	Absolute treatment effect[c] (95% CI)	Relative treatment effect[d] (95% CI)	P^a	P^b
IL1β (pg/mL)										
No change/gained weight	0.11 (0.03–0.51)	0.07 (0.03–0.18)	−0.04 (−35.9)	0.10 (0.03–0.27)	0.19 (0.06–0.60)	0.10 (102.5)	0.14 (0.01–0.52)	3.16 (1.06–11.23)	Ref.	Ref.
Lost <5% baseline weight	0.16 (0.06–0.43)	0.13 (0.05–0.35)	−0.03 (−17.9)	0.18 (0.07–0.45)	0.17 (0.08–0.40)	−0.01 (−1.6)	0.02 (−0.09–0.13)	1.2 (0.73–2.01)	0.15	0.03
Lost 5%–10% baseline weight	0.16 (0.07–0.36)	0.10 (0.04–0.27)	−0.06 (−36.4)	0.08 (0.04–0.16)	0.05 (0.02–0.13)	−0.02 (−27.7)	0.04 (−0.02–0.12)	1.14 (0.67–1.98)	0.13	0.02
Lost ≥10% baseline weight	0.15 (0.07–0.30)	0.15 (0.08–0.29)	0.00[e] (1.6)	0.08 (0.04–0.17)	0.08 (0.04–0.17)	−0.01 (−6.0)	−0.01 (−0.07–0.93)	0.93 (0.57–1.49)	0.06	0.008
IL6 (pg/mL)										
No change/gained weight	6.17 (2.53–15.05)	4.30 (2.13–8.70)	−1.87 (−30.3)	3.76 (2.52–5.62)	4.59 (2.88–7.33)	0.83 (22.2)	2.7 (0.03–8.64)	1.75 (1.00–3.19)	Ref.	Ref.
Lost <5% baseline weight	4.43 (2.88–6.82)	4.51 (3.14–6.48)	0.08 (1.9)	3.65 (2.60–5.14)	3.80 (2.66–5.42)	0.14 (3.9)	0.06 (−1.43–1.79)	1.02 (0.70–1.49)	0.12	0.03
Lost 5%–10% baseline weight	4.37 (3.12–6.12)	3.62 (2.25–5.81)	−0.75 (−17.2)	4.76 (3.58–6.32)	2.98 (1.97–4.52)	−1.77 (−37.3)	−1.02 (−2.91–0.54)	0.76 (0.48–1.15)	0.02	0.004
Lost ≥10% baseline weight	2.99 (2.10–4.24)	2.58 (1.74–3.83)	−0.41 (−13.6)	3.89 (2.85–5.30)	3.22 (2.39–4.33)	−0.67 (−17.3)	−0.27 (−1.28–0.65)	0.96 (0.71–1.30)	0.07	0.02
IL8 (pg/mL)										
No change/gained weight	6.26 (4.05–9.66)	5.56 (3.63–8.53)	−0.70 (−11.1)	6.90 (5.35–8.88)	6.54 (5.16–8.30)	−0.35 (−5.1)	0.34 (−1.82–2.70)	1.07 (0.77–1.51)	Ref.	Ref.
Lost <5% baseline weight	7.62 (6.38–9.09)	7.43 (6.03–9.15)	−0.19 (−2.5)	8.62 (5.45–13.62)	7.36 (5.74–9.42)	−1.26 (−14.6)	−1.07 (−5.82–1.35)	0.88 (0.58–1.20)	0.33	0.53
Lost 5%–10% baseline weight	7.19 (5.85–8.84)	5.74 (4.31–7.63)	−1.45 (−20.2)	6.72 (5.31–8.50)	5.57 (4.24–7.32)	−1.15 (−17.1)	0.31 (−1.39–1.95)	1.04 (0.79–1.38)	0.90	0.56
Lost ≥10% baseline weight	5.45 (4.48–6.63)	5.02 (3.86–6.52)	−0.43 (−7.9)	6.75 (4.77–9.56)	6.30 (4.91–8.08)	−0.45 (−6.7)	−0.02 (−1.92–1.46)	1.01 (0.78–1.34)	0.78	0.83
IL10 (pg/mL)										
No change/gained weight	12.68 (6.41–25.05)	9.51 (3.25–27.81)	−3.16 (−25.0)	26.03 (15.89–42.63)	27.82 (18.29–42.30)	1.79 (6.9)	4.95 (−8.31–15.68)	1.42 (0.79–2.78)	Ref.	Ref.
Lost <5% baseline weight	19.88 (13.47–29.33)	14.27 (8.29–24.54)	−5.61 (−28.2)	12.55 (7.37–21.36)	12.77 (7.00–23.28)	0.22 (1.7)	5.83 (−2.27–14.73)	1.42 (0.80–2.64)	0.80	0.79
Lost 5%–10% baseline weight	14.04 (9.23–21.37)	12.28 (6.51–23.15)	−1.77 (−12.6)	18.27 (10.37–32.18)	12.72 (8.02–20.18)	−5.55 (−30.4)	−3.78 (−16.9–2.52)	0.80 (0.44–1.29)	0.18	0.09
Lost ≥10% baseline weight	20.24 (13.33–30.73)	19.46 (13.64–27.76)	−0.78 (−3.9)	17.87 (13.10–24.36)	15.18 (10.11–22.80)	−2.68 (−15.0)	−1.9 (−7.53–4.56)	0.88 (0.62–1.24)	0.13	0.06
TNFα										
No change/gained weight	8.18 (6.16–10.87)	7.94 (6.01–10.50)	−0.24 (−2.9)	7.66 (5.60–10.48)	8.44 (6.15–11.58)	0.78 (10.2)	1.01 (−1.6–4.06)	1.13 (0.82–1.64)	Ref.	Ref.
Lost <5% baseline weight	10.32 (8.65–12.32)	10.53 (8.96–12.38)	0.21 (2.1)	10.56 (8.83–12.62)	11.55 (10.10–13.21)	1.00 (9.4)	0.78 (−0.9–2.46)	1.07 (0.92–1.26)	0.85	0.33
Lost 5%–10% baseline weight	9.69 (8.30–11.31)	7.75 (6.11–9.83)	−1.94 (−20.1)	9.41 (7.66–11.54)	8.74 (7.23–10.58)	−0.66 (−7.0)	1.28 (−1.16–3.87)	1.16 (0.88–1.6)	0.76	0.57
Lost ≥10% baseline weight	9.68 (8.17–11.47)	8.74 (7.17–10.66)	−0.94 (−9.7)	9.60 (8.23–11.19)	8.53 (7.33–9.92)	−1.07 (−11.1)	−0.13 (−1.76–1.41)	0.98 (0.83–1.17)	0.56	0.12
Inflammatory Biomarker Score										
No change/gained weight	1.41	0.80	−0.61 (−43.2)	0.43	0.79	0.36 (84.1)	0.97 (−0.25–6.26)	3.24 (0.71–17.43)	Ref.	Ref.
Lost <5% baseline weight	1.70	2.09	0.39 (−23.1)	2.62	2.58	−0.05 (−1.8)	−0.44 (−5.84–1.54)	0.8 (0.31–1.97)	0.16	0.05
Lost 5%–10% baseline weight	1.81	0.63	−1.18 (−65.2)	0.95	0.45	−0.49 (−52.1)	0.69 (−0.51–2.91)	1.38 (0.48–4.47)	0.45	0.09
Lost ≥10% baseline weight	0.59	0.39	−0.21 (−34.7)	0.87	0.57	−0.30 (−34.3)	−0.09 (−0.99–0.47)	1.01 (0.48–2.06)	0.26	0.06
Adiponectin (μg/mL)										
No change/gained weight	13.35 (10.61–16.80)	13.06 (10.45–16.33)	−0.29 (−2.2)	11.37 (9.24–13.97)	10.78 (8.79–13.23)	−0.58 (−5.1)	−0.29 (−1.27–0.69)	0.97 (0.90–1.04)	Ref.	Ref.
Lost <5% baseline weight	11.61 (9.37–14.39)	11.56 (9.46–14.14)	−0.05 (−0.4)	12.29 (9.73–15.52)	12.62 (10.17–15.65)	0.33 (2.7)	0.38 (−0.73–1.42)	1.03 (0.94–1.12)	0.18	0.38
Lost 5%–10% baseline weight	10.47 (8.68–12.63)	11.34 (9.47–13.59)	0.88 (8.4)	10.84 (9.37–12.54)	11.22 (9.71–12.97)	0.39 (3.6)	−0.49 (−1.67–0.60)	0.96 (0.86–1.06)	0.59	0.36
Lost ≥10% baseline weight	10.29 (8.63–12.26)	12.04 (9.94–14.58)	1.75 (17)	10.57 (8.84–12.65)	13.18 (11.31–15.37)	2.61 (24.7)	0.85 (−0.45–2.15)	1.07 (0.96–1.19)	0.09	0.19
Leptin (ng/mL)										
No change/gained weight	46.16 (35.16–60.61)	46.27 (36.34–58.93)	0.11 (0.2)	38.84 (29.00–52.01)	44.24 (31.92–61.31)	5.40 (13.9)	5.29 (−2.27–14.19)	1.14 (0.95–1.35)	Ref.	Ref.
Lost <5% baseline weight	34.15 (27.24–42.82)	31.24 (25.77–37.89)	−2.91 (−8.5)	29.67 (23.41–37.61)	29.61 (23.88–36.73)	−0.05 (−0.2)	2.86 (−2.39–8.31)	1.09 (0.92–1.30)	0.60	0.98
Lost 5%–10% baseline weight	34.33 (27.99–42.12)	25.34 (19.46–33.00)	−9.00 (−26.2)	35.77 (29.24–43.75)	26.24 (20.29–33.94)	−9.53 (−26.6)	−0.53 (−4.15–3.05)	0.99 (0.86–1.14)	0.15	0.25
Lost ≥10% baseline weight	34.32 (29.56–39.84)	15.22 (11.72–19.77)	−19.1 (−55.7)	36.18 (30.25–43.27)	14.85 (11.18–19.72)	−21.3 (−59.0)	−2.23 (−7.81–2.82)	0.93 (0.72–1.19)	0.13	0.22

[a]GEE model, *P* value comparing differences between vitamin D and Placebo arms, within each weight strata, compared with those participants who had no change/gained weight after 12 months (referent), adjusted for baseline BMI.

[b]GEE model, *P* value comparing differences between vitamin D and Placebo arms within each weight strata, compared with those participants who had no change/gained weight after 12 months (referent)., adjusting for age, race/ethnicity, baseline serum 25(OH)D, vitamin D intake (diet+supplement), calcium intake (diet+supplement), medication use, and average sun exposure.

[c]Absolute treatment effect was calculated as (absolute change in vitamin D group − absolute change in Placebo group).

[d]Relative treatment effect was calculated as (follow-up geometric mean in vitamin D group) × (baseline geometric mean in Placebo group)/[(baseline geometric mean in vitamin D group) × (follow-up geometric mean in Placebo group)].

[e]Absolute changes appear as 0.00 due to rounding.

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Limitations include the fact that we did not include women with a 25(OH)D concentration <10 ng/mL, and that complete pill counts were only available for 55% participants over the course of the study. All participants received a weight-loss intervention, thus we were unable to determine independent effects of vitamin D_3 supplementation. Finally our study population was relatively homogeneous, and these results may not be generalizable to other racial/ethnic groups. We used multiplex immunoassays to measure concentrations of these cytokines, and inter- and intra-assay CVs were high. A number of studies have cited this issue as a potential limitation of bead-based multiplex immunoassays, compared with ELISAs (49, 50). However, these and other reports state that despite high CV values, excellent correlations were observed between ELISAs for IL1β, IL10, IL6, and TNFα when compared with multiplex assays including Milliplex (49–54), and have demonstrated that these immunoassays correlated well with ELISA measurements in serum (53, 54). Finally a study examining the utility of a fluorescent bead-based assay to simultaneously measure a variety of cytokines reported that inter-individual differences outweighed substantial assay variation for these assays (55).

In conclusion, 12 months of vitamin D_3 supplementation in combination with a weight-loss program in overweight or obese postmenopausal women, was not associated with overall reductions in inflammatory cytokines compared with women undergoing a weight-loss program alone. However, participants who lost at least 5% of their baseline body-weight, and who were randomized to the vitamin D arm, had significantly greater reductions in IL6, compared with those randomized to the placebo arm. Further investigations into the relationship between weight-loss, vitamin D_3 supplementation, and inflammation are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Cancer Prevention Research

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