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Research Article

Cancer Prevention Research

Impact of Vitamin D Supplementation on Inflammatory Markers in African Americans: Results of a Four-Arm, Randomized, Placebo-Controlled Trial

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Abstract

African Americans have a disproportionate burden of inflammation-associated chronic diseases such as cancer and lower circulating levels of 25-hydroxyvitamin D [25(OH)D]. The effect of vitamin D_3 (cholecalciferol) supplementation on inflammatory markers is uncertain. We conducted a randomized, double-blind, placebo-controlled trial of supplemental oral vitamin D (placebo, 1,000, 2,000, or 4,000 IU/day of vitamin D_3 orally for 3 months) in 328 African Americans (median age, 51 years) of public housing communities in Boston, MA, who were enrolled over three consecutive winter periods (2007–2010). Change from 0 to 3 months of plasma levels of 25(OH)D, high-sensitivity C-reactive protein (CRP), interleukin (IL)-6, IL-10, and soluble TNF-α receptor type 2 (sTNF-R2) in 292 (89%) participants were measured. Overall, no statistically significant changes in CRP, IL-6, IL-10, and sTNF-R2 were observed after the vitamin D supplementation period. Baseline CRP was significantly inversely associated with the baseline 25(OH)D level ($P < 0.001$) in unadjusted and adjusted models. An interaction between baseline 25(OH)D and vitamin D supplementation was observed for outcome change in log CRP (month 3–month 0; P for interaction = 0.04). Within an unselected population of African Americans, short-term exposure to vitamin D supplementation produced no change in circulating inflammatory markers. This study confirms the strong independent association of CRP with 25(OH)D status even after adjusting for body mass index. Future studies of longer supplemental vitamin D_3 duration are necessary to examine the complex influence of vitamin D_3 on CRP and other chronic inflammatory cytokines for possible reduction of cancer health disparities in African Americans. *Cancer Prev Res; 7(2); 218–25. ©2013 AACR.*

Introduction

African Americans have a disproportionate elevation in chronic inflammation (1) after accounting for differences in body mass index (BMI) and other potential confounding factors (2, 3). Vitamin D deficiency may contribute to higher levels of inflammation (4). Chronic inflammation has been associated with a number of health outcomes including increased risk of cancer, cardiovascular disease, and diabetes (5–9). Previous observational and intervention studies have suggested that supplemental vitamin D may reduce circulating C-reactive protein (CRP) levels as well as other plasma inflammatory cytokines; nonetheless, results across all completed randomized trials have been inconsistent (10, 11). Of note, the inconsistency in the results of these trials may be related to the dose of vitamin D administered as well as the baseline plasma level of 25(OH)D. Furthermore, none of these trials enrolled a sufficient number of African American participants to examine the specific effects of supplementation on inflammation in this population.

In humans, cytokines such as CRP, interleukin (IL)-6, IL-10, and soluble TNF-α receptor type 2 (sTNF-R2) not only mediate the inflammatory response, but also serve as potential biomarkers of inflammation-related chronic diseases (9, 12, 13). If vitamin D supplementation reduces chronic inflammation among African Americans, its widespread use may have major public health impact on the reduction of cancer health disparities given the disproportionate prevalence of vitamin D deficiency in African Americans (14–16).

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Thus, we examined whether oral vitamin D supplementation reduces proinflammatory factors CRP, IL-6, and sTNF-R2 or increases the anti-inflammatory marker IL-10 within a randomized, double-blind, placebo-controlled trial designed to evaluate the effect of vitamin D supplementation on circulating 25(OH)D levels.

Materials and Methods

Study design

This is a prospective, randomized, double-blind, placebo-controlled clinical trial of oral cholecalciferol (vitamin D_3) in a community-based healthy black population (ClinicalTrials.gov NCT00585637). The protocol has been described in detail elsewhere (17). The primary goal of the trial was to examine the effect of daily supplementation of 1,000 international units (IU) of vitamin D_3, 2,000 IU of vitamin D_3, and 4,000 IU of vitamin D_3 and placebo on plasma 25(OH)D levels. Participants were drawn from Open Doors to Health (ODH), a colorectal cancer prevention intervention study conducted in 12 public housing communities in the Boston metropolitan area (16). All participants provided written informed consent. The project was approved by the Institutional Review Boards of Harvard School of Public Health and the Dana-Farber Cancer Institute. All procedures were followed in accordance with the institutional guidelines.

Recruitment and randomization

Participants in ODH were invited to participate if they were 30 to 80 years old, understood written and spoken English, self-identified as black (18–20), and had permission from their primary care doctors. A total of 328 individuals were enrolled into the parent trial (Fig. 1). Exclusion criteria included pregnancy, renal disease, preexisting parathyroid, thyroid, or calcium metabolism disorders, sarcoidosis, requirement for calcium channel blockers, type I diabetes, and active malignancies (other than nonmelanoma skin cancer). Those taking vitamin D supplementation were enrolled if they agreed to discontinue these medications for 6 months before enrollment and during the study.

Treatment

Participants were randomly assigned to four treatment arms: placebo, 1,000 IU/day, 2,000 IU/day, or 4,000 IU/day (Pharmavite LLC) of vitamin D_3 for 3 months. All capsules also contained 200 mg of calcium carbonate. All capsules were indistinguishable, and both participants and research staff were blinded to the treatment assignment. Study medications were started in early winter (November or December) and were taken orally once daily for 3 months (completed in February or March).

Endpoints and follow-up

The primary endpoints of the study were the changes in plasma inflammatory marker levels, IL-6, IL-10, sTNF-R2, and CRP, from baseline to the initial 3-month follow-up at the conclusion of supplement administration. Inflammatory markers were also measured at the 6-month follow-up. Individuals attended study visits at baseline, 3 months (at the end of randomized treatment), and 6 months (3 months after treatment discontinuation).

Compliance and safety

All participants were assessed for adverse events by study staff over the phone at week 2 of each month and in-person at the beginning of each month when the next month's supply of vitamins was provided. Participants were educated on the warning signs and symptoms of hypercalcemia. Any subject found to have serum calcium >10.5 mg/dL was immediately discontinued from the study and the primary care provider was notified.

Plasma vitamin D levels

Blood samples collected at baseline, 3 months, and 6 months were separated and plasma was stored in liquid nitrogen in the Dana-Farber Cancer Institute Clinical Research Laboratory. Once the study was completed, all plasma samples were sent as a single batch to the laboratory of Dr. Bruce Hollis (Medical University of South Carolina, Charleston, SC), where 25(OH)D concentrations were measured using the Diasorin (DiaSorin, Inc.) radioimmunoassay (21). Masked quality control samples were interspersed among the cases and all laboratory personnel were blinded. The mean coefficient of variation of 25(OH)D measurements was 9%.

Plasma inflammatory biomarker levels

Blood samples from women were collected in tubes treated with liquid sodium heparin, and those from men were collected in EDTA-treated tubes. The tubes were then placed on ice packs, stored in Styrofoam containers, returned to our laboratory by overnight courier, centrifuged, and divided into aliquots for storage in liquid-nitrogen freezers (−130°C or colder).

The levels of CRP were determined by means of a highly sensitive immunoturbidimetric assay with the use of reagents and calibrators from Denka Seiken; this assay has a day-to-day variability of 1% to 2%. Levels of sTNF-R2, IL-6, and IL-10 were measured by means of ELISA (R&D Systems), which have a day-to-day variability of 3.5% to 9.0%. Levels of sTNF-R2 show a strong correlation with TNF-α mRNA expression in human adipose tissue (8). The mean intra-assay coefficients of variations from blinded quality control samples for each analyte were as follows: CRP, 2.6%; IL-6, 3.8%; IL-10, 10.3%; and sTNF-R2, 3.3%.

Statistical analyses

The trial was designed with a statistical power of 80% to detect differences in the plasma 25(OH)D level of 5.3 ng/mL between treatment groups. On the basis of this planned sample size, we estimated that the trial would have 80% power to detect a 25% change in inflammatory biomarkers IL-6, IL-10, sTNF-R2, and CRP per 1,000 IU/day of vitamin D_3. All statistical analyses were performed using

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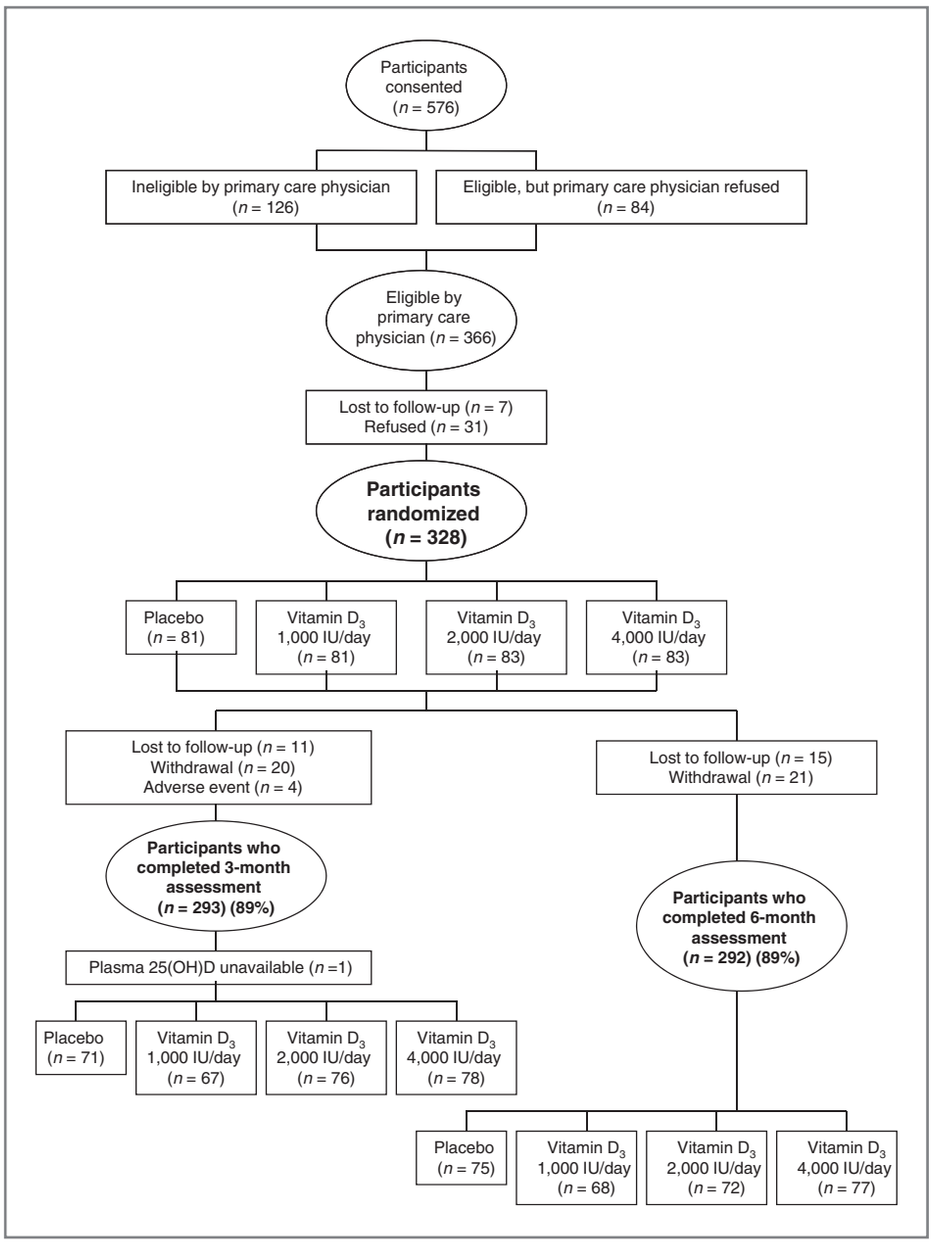

Figure 1. Consort diagram.

SAS version 9.2 (SAS Institute). Baseline characteristics of the study population were compared between supplementation arms using the χ^2 test for categoric variables and the Kruskal–Wallis test for continuous variables.

The primary endpoints of the study were the changes in plasma inflammatory marker levels, IL-6, IL-10, sTNF-R2, and CRP, from baseline to the initial 3-month follow-up. For our primary analysis, we used linear regression with the dose of vitamin D_3 (per 1,000 IU/day) as the independent variable and the log 3-month change in inflammatory marker as the dependent variable. The inflammatory biomarkers had skewed distributions so the data were natural log transformed.

We performed a number of *a priori* secondary analyses. We evaluated the independent association between baseline 25(OH)D, baseline BMI, smoking status, and inflammatory markers in univariate and multivariable linear regression. The covariates for the multivariable linear regression model included age, gender, and BMI as a continuous variable. Then, we analyzed the change in inflammatory markers according to the change in plasma 25(OH)D levels. Second, we analyzed the effect of any vitamin D supplementation (all 3 treatment groups combined) compared with placebo on inflammatory markers. Finally, to assess whether the effect of supplementation with vitamin D on the primary endpoint, 3-month change in inflammatory

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markers, varied according to baseline 25(OH)D or baseline BMI, we tested for an interaction between the treatment group and the baseline 25(OH)D level or baseline BMI.

Results

Subject characteristics according to the supplementation arm

Among the 328 eligible participants, baseline characteristics were relatively well balanced with an overall median age of 51 years and a median BMI of 31 kg/m^2 (Table 1). Slightly more participants in the placebo and 1,000 IU/day arms had a past history of cancer than those assigned to 2,000 or 4,000 IU/day. Otherwise, there were no significant differences in any of the subject characteristics between the supplementation arms. The compliance rate with study medication in the entire cohort was 96.6%. The 3-month follow-up plasma measurements were completed in 292 of the 328 participants (89%).

Baseline inflammatory marker predictors

At baseline, obese participants (BMI $\geq$ 30 kg/m^2) had significantly higher proinflammatory marker levels, IL-6, sTNF-R2, and CRP, than nonobese participants (Table 2). We also assessed the influence of smoking history on baseline circulating inflammatory markers, IL-6, IL-10, sTNF-R2, and CRP. Current smokers had higher CRP levels than nonsmokers, although the finding did not reach the level of statistical significance ($P > 0.05$).

Finally, we assessed the relation between baseline inflammatory markers, IL-6, IL-10, sTNF-R2, and CRP and baseline plasma 25(OH)D levels. We stratified participants by 25(OH)D <20 ng/mL and 25(OH)D $\geq$20 ng/mL (Table 2). No difference in baseline inflammatory markers was noted between the two groups except that participants with 25(OH)D <20 ng/mL had a statistically significant higher CRP level ($P < 0.0001$; Table 2). Even in models adjusted for BMI as a continuous variable, participants with 25(OH)D <20 ng/mL had a statistically significant higher CRP level ($P < 0.0001$; Table 3).

Response to vitamin D_3 supplementation by the treatment arm

The primary endpoint of the study was change in the plasma inflammatory marker level from baseline to the 3-month follow-up according to treatment arms. Overall, there was no statistically significant change in circulating levels of IL-6, IL-10, sTNF-R2, and CRP associated with treatment arms (Table 4) even after adjustment for BMI as a continuous variable (data not shown). Furthermore, there was no statistically significant change with any vitamin D supplementation (all 3 treatment groups combined) compared with placebo on inflammatory markers (data not shown).

Effect of vitamin D_3 supplementation on plasma 25(OH)D

Among 328 participants, the median 25(OH)D level at baseline was 15.3 ng/mL. Plasma 25(OH)D did not differ significantly between treatment arms ($P = 0.63$; Table 4). Circulating 25(OH)D levels at 3 months differed significantly by the vitamin D_3 supplementation arm, with a median of 13.7, 29.7, 34.8, and 45.9 ng/mL for the placebo, 1,000 IU/day, 2,000 IU/day, and 4,000 IU/day arms, respectively ($P = 0.001$). Notably, plasma 25(OH)D decreased at 3 months among participants treated with placebo (Table 3).

Interaction analyses

Interaction analyses were performed to determine the treatment effects of vitamin D by baseline 25(OH)D levels. With the exception of CRP, no interaction between baseline 25(OH)D and vitamin D treatment was found. For CRP, the interaction remained after adjustment for age, sex, BMI, and log baseline CRP. For BMI, no interaction between baseline BMI and vitamin D treatment for change in inflammatory markers at 3 months was observed.

Discussion

To our knowledge, this is the largest randomized placebo-controlled trial to examine the impact of oral vitamin D supplementation for 3 months on circulating inflammatory markers in an African American cohort. Overall, vitamin D_3 supplementation did not reduce proinflammatory markers CRP, IL-6, and sTNF-R2 or increase anti-inflammatory marker IL-10. Despite a clear trend in the change of follow-up serum 25(OH)D concentrations with increasing doses of supplemental vitamin D_3, we did not observe a significant association between supplemental vitamin D_3 dose and change in the measured inflammatory markers, IL-6, IL-10, sTNF-R2, and CRP, even after adjustment for BMI.

Our null finding is similar to Jorde and colleagues, who also found no overall change in inflammatory markers with vitamin D supplementation (D3 20,000 IU/week or D3 40,000 IU/week; ref. 22). No gender differences between 25(OH)D baseline levels and inflammatory markers of inflammation were observed. These plasma inflammatory markers may be a relatively nonspecific measurement of short-term changes in the tissue-specific inflammatory pathways relevant to inflammatory chronic diseases such as diabetes and cardiovascular disease. Several researchers report significant favorable effects of vitamin D supplementation in randomized control trials on proinflammatory cytokines such as IL-6, sTNF-R2, and CRP, but only in strictly selected groups of patients such as type 2 diabetics (23) and patients with congestive heart failure (24).

Baseline 25(OH)D may be a good predictor of long-term vitamin D status. Baseline inflammatory marker CRP adjusted for BMI was inversely associated with the baseline 25(OH)D level with a significantly lower CRP level noted in participants with 25(OH)D $\geq$20 ng/mL compared with 25(OH)D <20 ng/mL. This inverse relationship is consistent with prior observational studies that reported an inverse relationship between CRP and 25(OH)D in patients with colorectal adenoma (24) and in overweight and obese individuals (25). These results were different from a non–African American cohort. The Framingham Offspring Cohort study also found no correlation between 25(OH)D

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Table 1. Subject characteristics by supplementation arm[a]

	Vitamin D_3 dose assignment (IU/d)				
Characteristic	**Placebo ($n = 81$)**	**1,000 ($n = 81$)**	**2,000 ($n = 83$)**	**4,000 ($n = 83$)**	**P[b]**
Median age, years (IQR)	50.7 (44.1–58.0)	51.1 (43.4–60.1)	50.3 (43.5–58.3)	51.3 (44.1–59.7)	0.98
Sex, No. (%)					0.72
Male	27 (33.3)	22 (27.2)	28 (33.7)	29 (34.9)	
Female	54 (66.7)	59 (72.8)	55 (66.3)	54 (65.1)	
Median BMI, kg/m^2 (IQR)	31.2 (26.5–35.9)	30.5 (27.0–37.5)	31.9 (26.2–36.9)	31.4 (27.4–35.7)	0.82
Inflammatory markers					
IL-6 (pg/mL)	2.21 (1.32–3.42)	2.38 (1.53–4.02)	2.30 (1.19–4.17)	2.24 (1.09–3.84)	0.71
IL-10 (pg/mL)	0.55 (0.46–0.68)	0.51 (0.47–0.58)	0.53 (0.48–0.69)	0.52 (0.47–0.61)	0.31
sTNF-R2 (pg/mL)	2.047.3 (1.631.0–2,670.5)	1,984.8 (1,727.2–2,342.9)	2,143.2 (1,685.4–2,652.5)	2,011.5 (1,713.1–2,513.7)	0.93
CRP (mg/L)	2.74 (1.14–4.56)	1.95 (0.79–5.02)	2.18 (0.60–6.74)	2.23 (0.66–5.35)	0.88
25 (OHD) ng/mL	15.1 (10.4–23.6)	16.2 (11.0–22.7)	13.9 (9.5–22.3)	15.7 (11.0–23.3)	0.63
Smoking status, No. (%)					0.26
Never	33 (40.7)	36 (44.4)	33 (39.8)	44 (53.0)	
Past	20 (24.7)	16 (19.8)	27 (32.5)	20 (24.1)	
Current	28 (34.6)	29 (35.8)	23 (27.7)	19 (22.9)	
Frequency of exercise, days per week[c], median (IQR)	3.0 (0.5–5.0)	3.0 (1.0–5.0)	3.0 (0–5.0)	3.0 (0–5.0)	0.99
Dietary vitamin D intake, median (IQR)					
Baseline ($n = 328$)	147.3 (71.4–262.8)	162.5 (92.6–295.5)	144.0 (58.0–265.1)	198.1 (83.2–306.4)	0.41
Regular multivitamin use[d], No. (%)	10 (12)	18 (22)	15 (18)	22 (27)	0.16
Regular vitamin D supplement use[d], No. (%)	8 (10)	6 (8)	2 (2)	8 (10)	0.45
Postmenopausal hormone use, No. (%)[e]	0	0	0	1 (0.5)	0.72
Yes					
Regular calcium supplement use[f], No. (%)	7 (8.7)	9 (11.1)	7 (8.4)	9 (10.8)	0.49
Regular aspirin use[g], No. (%)	4 (4.9)	10 (12.3)	5 (6.0)	8 (9.6)	0.23
Regular NSAID use[h], No. (%)	6 (7.4)	10 (12.3)	10 (12.0)	7 (8.4)	0.73
Regular acetaminophen use[g], No. (%)	6 (7.4)	6 (7.4)	5 (6.0)	5 (6.0)	0.96
Marital status, married, No. (%)	23 (28.4)	30 (37.0)	23 (27.7)	24 (28.9)	0.58
Median household income, No. (%)					0.92
<$10,000	33 (40.8)	23 (28.4)	27 (32.5)	27 (32.5)	
$10,000–19,999	15 (18.5)	17 (21.0)	17 (20.5)	17 (20.5)	
$20,000–29,999	11 (13.6)	10 (12.3)	16 (19.3)	10 (12.0)	
$30,000–39,999	4 (4.9)	9 (11.1)	4 (4.8)	9 (10.8)	
$40,000–49,999	4 (4.9)	5 (6.2)	4 (4.8)	4 (4.8)	
≥$50,000	9 (11.1)	11 (13.6)	11 (13.3)	11 (13.3)	
History of cancer[i], No. (%)	6 (7.4)	0	3 (3.6)	15 (4.6)	0.032
History of hypertension, No. (%)	35 (43.2)	35 (43.2)	36 (43.3)	35 (42.1)	0.99

Abbreviations: IQR, interquartile range; No., number; NSAID, non-steroidal anti-inflammatory drug.

[a]Data are No. (%) unless otherwise indicated. The numbers do not always sum to group totals due to missing information for some variables.

[b]The Kruskal–Wallis test was used to calculate P values for continuous variables. All statistical tests were two-sided.

[c]Exercise defined as moderate to vigorous physical activity for at least 30 minutes, resulting in a faster-than-normal heart rate, sweating, and deep breathing.

[d]Refers to intake during preceding month.

[e]Percentages calculated from a total of 222 females.

[f]Defined as supplement use for 7 days per week during preceding month.

[g]Defined as 3 or more pills per week during the past week.

[h]Defined as 3 or more pills per week during the past week. Types of NSAIDs included salsalate, diflunisal, ibuprofen, ketoprofen, nabumetone, piroxicam, naproxen, diclofenac, indomethacin, sulindac, tolmetin, etodolac, ketorolac, and oxaprozin.

[i]Reported cancers include breast cancer, cervical cancer, uterine cancer, lung cancer, prostate cancer, and sarcoma.

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Table 2. Baseline inflammatory markers, median (IQR), stratified by potential predictors[a]

Variable	*N*	CRP (mg/dL)	IL-6 (pg/mL)	sTNF-R2 (pg/mL)	IL-10 (pg/mL)
25(OH)D <20 ng/mL	215	2.61 (0.89–4.49)[b]	2.38 (1.32–3.83)[c]	2031.4 (1687.7–2460.8)[c]	0.53 (0.46–0.67)[c]
25(OH)D ≥20 ng/mL	113	1.68 (0.48–4.19)[b]	2.10 (1.16–3.88)[c]	2139.0 (1732.8–2610.0)[c]	0.52 (0.46–0.63)[c]
BMI[d] <30 kg/m^2	146	1.19 (0.38–2.74)[b]	1.68 (0.99–2.73)[b]	1882.8 (1585.1–2316.5)[b]	0.52 (0.46–0.65)[c]
BMI[d] ≥30 kg/m^2	182	3.84 (1.51–7.21)[b]	3.00 (1.78–4.62)[b]	2186.7 (1815.7–2721.7)[b]	0.53 (0.46–0.65)[c]
Smoker	99	2.76 (0.87–5.51)[c]	2.30 (1.60–4.17)[c]	2047.3 (1763.1–2728.6)[c]	0.52 (0.45–0.64)[c]
Nonsmoker	223	2.07 (0.67–4.88)[c]	2.30 (1.17–3.81)[c]	2045.9 (1658.5–2481.3)[c]	0.53 (0.46–0.65)[c]

[a]*P* value calculated using the Kruskal–Wallis test.
[b]$P < 0.0001$.
[c]$P > 0.05$.
[d]BMI missing for 3 patients.

and CRP (26). The lack of correlation between 25(OH)D and CRP in the Framingham Offspring Cohort study may be related to that fact that it is a leaner cohort with few African Americans and less confounding by BMI. Factors that contribute to ongoing inflammation and immune activation in these chronic diseases are incompletely understood. In human and *in vitro* studies, vitamin D exerts a diverse array of immunomodulatory effects by interacting with the vitamin D system in a complex pathway that includes precursors, active metabolites, enzymes, and receptors (27, 28).

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 collection of cytokine data in community dwelling adults. Moreover, the precision of inflammatory marker measurement seemed adequate with low coefficients of variation for each marker: CRP, 2.6%; IL-6, 3.8%; IL-10, 10.3%; and sTNF-R2, 3.3%.

Some limitations should also be considered. Given the 3-month duration of vitamin D supplementation, the endpoint of the study was not long-term benefit of supplementation on inflammation. In this heterogeneous convenience sample of healthy African American participants, exogenous factors may bias the trial to null, and this trial may be underpowered to detect clinically significant effects of vitamin D on inflammatory markers. Although CRP is a reliable marker of systemic inflammation (29), we cannot exclude the possibility that the inflammatory markers chosen for the analysis may not have adequately captured systemic inflammation.

Furthermore, we selected a target 25(OH)D of ≥33 ng/mL based on prospective observational studies (30), but clinical trials assessing the effect of achieving this level are not available. Finally, because the highest dose of vitamin D_3 in our trial was 4,000 IU/day, we were not able to evaluate the influence of higher doses on inflammatory markers. Given the variation of these inflammatory markers that may occur with acute infection and other conditions, a large-scale longitudinal study of longer duration, such as the VITamin D and OmegA-3 TriaL (VITAL) with over 20,000 participants and oversampling of African Americans (31, 32), would be a good way to evaluate the effect of vitamin D on these inflammatory markers.

The theory of vitamin D playing a role in cancer prevention is biologically plausible (15). Studies in cell culture and

Table 3. Baseline mean adjusted and unadjusted CRP levels stratified by the baseline 25(OH)D level and the baseline BMI level, mean (95% confidence interval)

	Unadjusted mean (95% confidence interval)			Adjusted mean (95% confidence interval)		
Variable	*N*	CRP (mg/L)	*P*	*N*	CRP (mg/L)	*P*[a]
25(OH)D <20 ng/mL	212	2.15 (1.77–2.60)	0.03	212	2.01 (1.66–2.43)[b]	0.03
25(OH)D ≥20 ng/mL	107	1.49 (1.14–1.96)		107	1.42 (1.08–1.88)[b]	
BMI <30 kg/m^2	140	0.99 (0.79–1.23)	<0.0001	140	1.01 (0.81–1.27)[c]	<0.0001
BMI ≥30 kg/m^2	179	3.17 (2.61–3.84)		179	3.23 (2.60–4.02)[c]	
Smoker	99	1.83 (1.51–2.21)	0.33	97	1.69 (1.43–2.04)[c]	0.06
Nonsmoker	223	2.16 (1.63–2.87)		222	2.25 (1.67–2.81)[c]	

[a]*P* value was computed by the linear regression model, PROC GLM; unadjusted and adjusted means are geometric mean of log CRP.
[b]Adjusted for age, BMI (as continuous variable), sex, smoking status.
[c]Adjusted for age, sex, smoking status.

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Table 4. Change in inflammatory markers and 25(OH)D by treatment arm, median (IQR)

	Vitamin D_3 dose assignment (IU/d)				
Time point/variable	**Placebo**	**1,000**	**2,000**	**4,000**	***P*[a]**
3 months					
No. of participants	71	67	76	78	
25(OH)D (ng/mL)	13.7 (7.2–18.6)	29.7 (25.6–32.9)	34.7 (28.8–41.0)	45.9 (39.4–55.2)	0.001
IL-6 (pg/mL)	2.25 (1.30–3.46)	2.53 (1.57–4.16)	2.24 (1.29–4.28)	1.97 (1.09–3.78)	0.42
IL-10 (pg/mL)	0.51 (0.47–0.63)	0.49 (0.45–0.57)	0.52 (0.45–0.59)	0.52 (0.45–0.61)	0.63
sTNF-R2 (pg/mL)	2033.4 (1595.4–2542.1)	2061.4 (1670.5–2586.8)	2198.6 (1851.0–2632.3)	1946.0 (1674.5–2537.9)	0.32
CRP (mg/L)	2.46 (1.27–4.06)	2.21 (0.92–4.70)	2.39 (0.75–5.29)	1.98 (0.49–5.96)	0.98
Δ3 month–baseline					
No. of participants	71	67	76	78	
25(OH)D ng/mL	−2.3 (−5.4–1.7)	10.8 (2.5–18.9)	19.2 (11.5–26.2)	30.2 (21.5–37.6)	<0.001
IL-6 (pg/mL)	−0.03 (−0.96–0.88)	−0.07 (−1.07–0.93)	0.01 (−0.57–0.89)	0.08 (−0.61–0.71)	0.84
IL-10 (pg/mL)	0 (−0.06–0.07)	−0.01 (−0.08–0.02)	−0.02 (−0.09–0.02)	0.00 (−0.06–0.04)	0.40
sTNF-R2 (pg/mL)	−0.89 (−187.75–160.10)	−9.88 (−130.28–149.19)	59.16 (−132.66–214.59)	13.29 (−137.03–144.32)	0.35
CRP (mg/L)	−0.05 (−1.13–0.98)	0.07 (−0.58–0.86)	0.02 (−0.68–1.21)	0.03 (−0.68–0.79)	0.91

Abbreviations: No., number; IQR, interquartile range.

[a]*P* value calculated using the Kruskal–Wallis test.

experimental models show that calcitriol may promote cell differentiation, inhibit cancer-cell proliferation, and exhibit anti-inflammatory, proapoptotic, and antiangiogenic properties (33, 34). A compartmental model such as colonic mucosa may be a better way to evaluate the local effect of vitamin D on inflammatory cytokines because blood dilutes the inflammatory cytokines. Yet, this study does validate the association of low plasma 25(OH)D with elevated CRP (24, 35) and provide evidence that vitamin D supplementation does not increase the production of proinflammatory cytokines.

In conclusion, we failed to find an influence of relatively short-term supplementation with vitamin D_3 on inflammatory markers in a cohort of African Americans. For analyses stratified by baseline 25(OH)D status, we did observe a significant interaction of baseline 25(OH)D and vitamin D supplementation on change in log CRP at 3 months. This study contributes to the growing body of literature evaluating the impact of vitamin D_3 supplementation on inflammation for cancer risk reduction and confirms the strong independent association of CRP with obesity (36) and CRP with 25(OH)D status even after adjusting for BMI. Nonetheless, future studies with larger sample sizes and longer durations of supplemental vitamin D_3 intervention are necessary to examine the influence of vitamin D_3 on CRP and other inflammatory cytokines for reduction of cancer disparities in African Americans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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References

1. Martinez Cantarin MP, Keith SW, Deloach S, Huan Y, Falkner B. Relationship of adipokines with insulin sensitivity in African Americans. Am J Med Sci 2011;342:192–7.
2. Albert MA, Glynn RJ, Buring J, Ridker PM. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). Am J Cardiol 2004;93:1238–42.
3. Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB Jr, et al. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. Am Heart J 2006;152:593–8.
4. Akin F, Ayca B, Kose N, Duran M, Sari M, Uysal OK, et al. Serum vitamin D levels are independently associated with severity of coronary artery disease. J Investig Med 2012;60:869–73.
5. Anuurad E, Tracy RP, Pearson TA, Kim K, Berglund L. Synergistic role of inflammation and insulin resistance as coronary artery disease risk factors in African Americans and Caucasians. Atherosclerosis 2009;205:290–5.
6. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.
7. Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. J Hepatol 2012;56:952–64.
8. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 1994;43:1271–8.
9. Thorand B, Lowel H, Schneider A, Kolb H, Meisinger C, Frohlich M, et al. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984–1998. Arch Intern Med 2003;163:93–9.
10. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol 2012; 188:2127–35.
11. Gepner AD, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH. A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. PLoS ONE 2012;7: e36617.
12. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med 2004;351:2599–610.
13. Liu KD, Glidden DV, Eisner MD, Parsons PE, Ware LB, Wheeler A, et al. Predictive and pathogenetic value of plasma biomarkers for acute kidney injury in patients with acute lung injury. Crit Care Med 2007; 35:2755–61.
14. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest 2006;116:2062–72.
15. Grant WB, Peiris AN. Differences in vitamin D status may account for unexplained disparities in cancer survival rates between African and white Americans. Dermatoendocrinol 2012;4:85–94.
16. McNeill LH, Coeling M, Puleo E, Suarez EG, Bennett GG, Emmons KM. Colorectal cancer prevention for low-income, sociodemographically-diverse adults in public housing: baseline findings of a randomized controlled trial. BMC Public Health 2009;9:353.
17. Forman JP, Scott JB, Ng K, Drake BF, Suarez EG, Hayden DL, et al. Effect of vitamin D supplementation on blood pressure in blacks. Hypertension 2013;61:779–85.
18. U.S. Bureau of the Census. Overview of race and Hispanic origin. Census 2000 Brief. Washington, DC: U.S. Bureau of the Census; 2001.
19. McKenney NR, Bennett CE. Issues regarding data on race and ethnicity: the Census Bureau experience. Public Health Rep 1994;109: 16–25.
20. Williams DR. Race/ethnicity and socioeconomic status: measurement and methodological issues. Int J Health Serv 1996;26: 483–505.
21. Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. Methods Enzymol 1997;282:174–86.
22. Jorde R, Sneve M, Torjesen PA, Figenschau Y, Gøransson LG, Omdal R. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. Cytokine 2010;50:175–80.
23. Shab-Bidar S, Neyestani TR, Djazayery A, Eshraghian MR, Houshiarrad A, Kalayi A, et al. Improvement of vitamin D status resulted in amelioration of biomarkers of systemic inflammation in the subjects with type 2 diabetes. Diabetes Metab Res Rev 2012;28: 424–30.
24. Hopkins MH, Owen J, Ahearn T, Fedirko V, Flanders WD, Jones DP, et al. Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: a randomized, controlled clinical trial. Cancer Prev Res 2011;4:1645–54.
25. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Intern Emerg Med 2011;8:33–40.
26. Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RB, Dawson-Hughes B, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. Am J Epidemiol 2008;167:313–20.
27. Oh J, Weng S, Felton SK, Bhandare S, Riek A, Butler B, et al. 1,25(OH)2 vitamin d inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. Circulation 2009;120:687–98.
28. Agrawal T, Gupta GK, Agrawal DK. Calcitriol decreases expression of importin alpha3 and attenuates RelA translocation in human bronchial smooth muscle cells. J Clin Immunol 2012;32:1093–103.
29. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107:499–511.
30. Giovannucci E. Epidemiology of vitamin D and colorectal cancer. Anticancer Agents Med Chem 2013;13:11–9.
31. Manson JE, Bassuk SS, Lee IM, Cook NR, Albert MA, Gordon D, et al. The VITamin D and OmegA-3 TriaL (VITAL): rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. Contemp Clin Trials 2012;33:159–71.
32. Manson JE, Mayne ST, Clinton SK. Vitamin D and prevention of cancer–ready for prime time? N Engl J Med 2011;364:1385–7.
33. Bouillon R, Eelen G, Verlinden L, Mathieu C, Carmeliet G, Verstuyf A. Vitamin D and cancer. J Steroid Biochem Mol Biol 2006;102: 156–62.
34. Verstuyf A, Carmeliet G, Bouillon R, Mathieu C. Vitamin D: a pleiotropic hormone. Kidney Int 2010;78:140–5.
35. Amer M, Qayyum R. Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). Am J Cardiol 2012;109:226–30.
36. Kahn SE, Zinman B, Haffner SM, O'Neill MC, Kravitz BG, Yu D, et al. Obesity is a major determinant of the association of C-reactive protein levels and the metabolic syndrome in type 2 diabetes. Diabetes 2006;55:2357–64.

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