Impact of Vitamin D Supplementation on Inflammatory Markers in African-Americans:

Results of a Four-Arm, Randomized, Placebo-Controlled Trial

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Key words: vitamin D, inflammation, African-Americans, biomarkers, chemoprevention

Running title: Vitamin D supplementation and inflammation in blacks

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Conflict of Interest:

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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 D3 (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 D3 orally for 3 months) in 328 African-Americans (median age, 51 years) of public housing communities in Boston, MA who were enrolled over 3 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, interleukin (IL)-10, and soluble tumor necrosis factor alpha 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 vitamin D supplementation period. Baseline CRP was significantly inversely associated with 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 BMI. Future studies of longer supplemental vitamin D3 duration are necessary to examine the complex influence of vitamin D3 on CRP and other chronic inflammatory cytokines for possible reduction of cancer health disparities in African-Americans.
Introduction
African-Americans have a disproportionate elevation in chronic inflammation(1) after accounting for differences in 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, IL-6, IL-10 and sTNFR-2 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) Thus, we examined
whether oral vitamin D supplementation reduces pro-inflammatory factors CRP, IL-6, and sTNFR-2 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 D3) 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 1000 international units (IU) of vitamin D3, 2000 IU of vitamin D3, and 4000 IU of vitamin D3 and placebo on plasma 25(OH)D levels. Participants were drawn from Open Doors to Health (ODH), a colorectal cancer (CRC) 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 Dana-Farber Cancer Institute. All procedures were followed in accordance with 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 (Figure 1). Exclusion criteria included pregnancy, renal disease, pre-existing parathyroid, thyroid, or calcium metabolism disorders, sarcoidosis, requirement for calcium channel blockers, type I diabetes, and active malignancies (other than non-melanoma skin cancer). Those taking vitamin D supplementation were enrolled if they agreed to discontinue these medications for 6 months prior to enrollment and during the study.

Treatment

Participants were randomly assigned to 4 treatment arms: placebo; 1,000 IU; 2,000 IU; or 4,000 IU/day (Pharmavite LLC, Mission Hill, CA) of vitamin D₃ 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 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).

End Points and Follow-up

The primary end points 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 also were 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 PCP was notified.

Plasma Vitamin D Levels

Blood samples collected at baseline, 3, and 6 months were separated and plasma was stored in liquid nitrogen in the Dana Farber Cancer Institute Clinical Research Laboratory (Boston, MA). Once 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) were 25(OH)D concentrations were measured using the Diasorin (DiaSorin, Inc., Stillwater, MN) radioimmunoassay. 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 percent. Levels of sTNF-R2, and IL-6 and IL-10 were measured by means of enzyme-linked immunosorbent assays (R&D Systems), which have a day-to-day variability of 3.5 to 9.0 percent. 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 sTNFR-2, 3.3%.

**Statistical Analysis**

The trial was designed with a statistical power of 80% to detect differences in plasma 25(OH)D level of 5.3 ng/mL between treatment groups. Based on 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 1000 IU/d of vitamin D3. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Baseline characteristics of the study population were compared between supplementation arms using the Chi-Square test for categorical variables and the Kruskal-Wallis test for continuous variables.

The primary end points 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 D3...
D3 (per 1000 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 markers, varied according to baseline 25(OH)D or baseline BMI, we tested for interaction between treatment group and baseline 25(OH)D level or baseline BMI.

Results

Subject Characteristics According to Supplementation Arm

Among the 328 eligible participants, baseline characteristics were relatively well balanced with an overall median age of 51.0 and a median BMI of 31.0 kg/m² (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 $> 30$ kg/m$^2$) had significantly higher proinflammatory marker levels IL-6, sTNF-R2, and CRP than non-obese 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 ($P < .0001$; 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 ($P < .0001$; Table 3).

**Response to Vitamin D3 Supplementation by Treatment Arm**

The primary end point of the study was change in 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 D₃ 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 vitamin D₃ 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 D3 supplementation did not reduce pro-inflammatory markers CRP, IL-6, and sTNFR-2 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 D3, we did not observe a significant association between supplemental vitamin D3 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 et al., who also found no overall change in inflammatory markers with vitamin D supplementation (D3 20,000 IU/week or D3 40,000 IU/week)(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 non-specific measurement of short-term changes in the tissue-specific inflammatory pathways relevant to inflammatory chronic diseases such as diabetes and CVD. Several researchers report significant favorable effects of vitamin D supplementation in randomized control trials on pro-inflammatory cytokines like 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. (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 baseline 25(OH)D level with a significantly lower CRP level noted in participants with 25(OH)D ≥ 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 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 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 appeared adequate with low coefficients of variation for each marker: CRP, 2.6%; IL-6, 3.8%; IL-10, 10.3%; and sTNFR-2, 3.3%.

Some limitations should also be considered. Given the 3-month duration of vitamin D supplementation, the end point 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 $\geq 33$ ng/mL based on prospective observational studies(30), but clinical trials assessing the effect of achieving this level are not available. Lastly, since 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 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 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 since 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 pro-inflammatory 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 impact of vitamin D3 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 D3 intervention are necessary to examine the influence of vitamin D3 on CRP and other inflammatory cytokines for reduction of cancer disparities in African-Americans.

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References

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


Legends:
Figure 1. Consort Diagram
Table 1. Subject characteristics by supplementation arm<sup>a</sup>

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>PLACEBO (n=81)</th>
<th>1,000 (n=81)</th>
<th>2,000 (n=83)</th>
<th>4,000 (n=83)</th>
<th>P VALUE &lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td><strong>Median age, years (IQR)</strong></td>
<td>50.7 (44.1-58.0)</td>
<td>51.1 (43.4-60.1)</td>
<td>50.3 (43.5-58.3)</td>
<td>51.3 (44.1-59.7)</td>
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<td><strong>Sex, No. (%)</strong></td>
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<td></td>
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<tr>
<td>Male</td>
<td>27 (33.3)</td>
<td>22 (27.2)</td>
<td>28 (33.7)</td>
<td>29 (34.9)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>54 (66.7)</td>
<td>59 (72.8)</td>
<td>55 (66.3)</td>
<td>54 (65.1)</td>
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<td><strong>Median body-mass index, kg/m² (IQR)</strong></td>
<td>31.2 (26.5-35.9)</td>
<td>30.5 (27.0-37.5)</td>
<td>31.9 (26.2-36.9)</td>
<td>31.4 (27.4-35.7)</td>
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<td><strong>Inflammatory Markers</strong></td>
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</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>2.21(1.32-3.42)</td>
<td>2.38(1.53-4.02)</td>
<td>2.30(1.19-4.17)</td>
<td>2.24(1.09-3.84)</td>
<td>0.71</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>0.55(0.46-0.68)</td>
<td>0.51(0.47-0.58)</td>
<td>0.53(0.48-0.69)</td>
<td>0.52(0.47-0.61)</td>
<td>0.31</td>
</tr>
<tr>
<td>sTNF-R2 (pg/ml)</td>
<td>2047.3(1631.0-2670.5)</td>
<td>1984.8(1727.2-2342.9)</td>
<td>2143.2(1685.4-2652.5)</td>
<td>2011.5(1713.1-2513.7)</td>
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</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.74(1.14-4.56)</td>
<td>1.95(0.79-5.02)</td>
<td>2.18(0.60-6.74)</td>
<td>2.23(0.66-5.35)</td>
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<tr>
<td>25 (OHD) ng/ml</td>
<td>15.1 (10.4-23.6)</td>
<td>16.2 (11.0-22.7)</td>
<td>13.9 (9.5-22.3)</td>
<td>15.7 (11.0-23.3)</td>
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<td><strong>Smoking status, No. (%)</strong></td>
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<td>Never</td>
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<td>36 (44.4)</td>
<td>33 (39.8)</td>
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<tr>
<td>Past</td>
<td>20 (24.7)</td>
<td>16 (19.8)</td>
<td>27 (32.5)</td>
<td>20 (24.1)</td>
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<tr>
<td>Current</td>
<td>28 (34.6)</td>
<td>29 (35.8)</td>
<td>23 (27.7)</td>
<td>19 (22.9)</td>
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<tr>
<td><strong>Frequency of exercise, days per week,^c Median (IQR)</strong></td>
<td>3.0 (0.5-5.0)</td>
<td>3.0 (1.0-5.0)</td>
<td>3.0 (0-5.0)</td>
<td>3.0 (0-5.0)</td>
<td>0.99</td>
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<tr>
<td><strong>Dietary vitamin D intake, Median (IQR)</strong></td>
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<tr>
<td>Baseline (n=328)</td>
<td>147.3 (71.4-262.8)</td>
<td>162.5 (92.6-295.5)</td>
<td>144.0 (58.0-265.1)</td>
<td>198.1(83.2-306.4)</td>
<td>0.41</td>
</tr>
<tr>
<td>Variable</td>
<td>No. (%)</td>
<td>Median household income, %</td>
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<tr>
<td>----------------------------------------------</td>
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<td>-----------------------------</td>
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<tr>
<td>Regular multivitamin use, No. (%)</td>
<td>10 (12)</td>
<td>33 (40.8)</td>
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<tr>
<td>Regular vitamin D supplement use, No. (%)</td>
<td>8 (10)</td>
<td>15 (18)</td>
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<tr>
<td>Post-menopausal hormone use, No. (%)</td>
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<td>1 (0.5)</td>
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<td>Regular calcium supplement use, No. (%)</td>
<td>7 (8.7)</td>
<td>27 (32.5)</td>
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<td>Regular aspirin use, No. (%)</td>
<td>4 (4.9)</td>
<td>10 (13.6)</td>
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<tr>
<td>Regular NSAID use, No. (%)</td>
<td>6 (7.4)</td>
<td>11 (13.6)</td>
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<tr>
<td>Regular acetaminophen use, No. (%)</td>
<td>6 (7.4)</td>
<td>11 (13.6)</td>
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<tr>
<td>Marital status, Married, No. (%)</td>
<td>23 (28.4)</td>
<td>15 (4.6)</td>
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<td>History of cancer¹, No. (%)</td>
<td>6 (7.4)</td>
<td>35 (43.2)</td>
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<td>History of hypertension, No. (%)</td>
<td>35 (43.2)</td>
<td>36 (43.3)</td>
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</tr>
</tbody>
</table>

* IU = international units; IQR = interquartile range; No. = number; NSAID = non-steroidal anti-inflammatory drug

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**Legend**

- Data are No.(%) unless otherwise indicated. The numbers do not always sum to group totals due to missing information for some variables.
- The Kruskal-Wallis test was used to calculate P values for continuous variables. All statistical tests were two-sided.
- 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.
- Refers to intake during preceding month.
- Percentages calculated from a total of 222 females.
- Defined as supplement use for 7 days per week during preceding month.
- Defined as 3 or more pills per week during the past week.
- Defined as 3 or more pills per week during the past week. Types of NSAIDs included salicylate, diflupasal, ibuprofen, ketoprofen, nabumetone, piroxicam, naproxen, diclofenac, indomethacin, sulindac, tolmel, etodolac, ketorolac, and oxaprozin.
- Reported cancers include breast cancer, cervical cancer, uterine cancer, lung cancer, prostate cancer, and sarcoma.
Table 2. Baseline inflammatory markers, median(IQR), stratified by potential predictors

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>CRP</th>
<th>IL-6</th>
<th>sTNFR-2</th>
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<tbody>
<tr>
<td>25(OH)D&lt;20 ng/ml</td>
<td>215</td>
<td>2.61(0.89-4.49)</td>
<td>2.38(1.32-3.83)</td>
<td>2031.4(1687.7-2460.8)</td>
<td>0.53(0.46-0.67)</td>
</tr>
<tr>
<td>25(OH)D≥20 ng/ml</td>
<td>113</td>
<td>1.68(0.48-4.19)</td>
<td>2.10(1.16-3.88)</td>
<td>2139.0(1732.8-2610.0)</td>
<td>0.52(0.46-0.63)</td>
</tr>
<tr>
<td>BMI&lt;30 kg/m²</td>
<td>146</td>
<td>1.19(0.38-2.74)</td>
<td>1.68(0.99-2.73)</td>
<td>1882.8(1585.1-2316.5)</td>
<td>0.52(0.46-0.65)</td>
</tr>
<tr>
<td>25(OH)D&lt;20 ng/ml</td>
<td>113</td>
<td>2.10(1.16-3.88)</td>
<td>2.10(1.16-3.88)</td>
<td>2139.0(1732.8-2610.0)</td>
<td>0.52(0.46-0.63)</td>
</tr>
<tr>
<td>BMI≥30 kg/m²</td>
<td>182</td>
<td>3.84(1.51-7.21)</td>
<td>3.00(1.78-4.62)</td>
<td>2186.7(1815.7-2721.7)</td>
<td>0.53(0.46-0.65)</td>
</tr>
<tr>
<td>Smoker</td>
<td>99</td>
<td>2.76(0.87-5.51)</td>
<td>2.30(1.60-4.17)</td>
<td>2047.3(1763.1-2728.6)</td>
<td>0.52(0.45-0.64)</td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>223</td>
<td>2.07(0.67-4.88)</td>
<td>2.30(1.17-3.81)</td>
<td>2045.9(1658.5-2481.3)</td>
<td>0.53(0.46-0.65)</td>
</tr>
</tbody>
</table>

using Kruskal-Wallis test

*P value calculated using Kruskal-Wallis test
Table 3. Baseline mean unadjusted and unadjusted CRP levels stratified by baseline 25(OH)D level and baseline BMI level, mean (95% Confidence Interval)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted Mean (95% Confidence Interval)</th>
<th>Adjusted Mean (95% Confidence Interval)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>CRP</td>
<td>P value</td>
</tr>
<tr>
<td>25(OH)D&lt;20 ng/ml</td>
<td>212</td>
<td>2.15(1.77,2.60)</td>
<td>0.03</td>
</tr>
<tr>
<td>25(OH)D&gt;20 ng/ml</td>
<td>107</td>
<td>1.49(1.14,1.96)</td>
<td></td>
</tr>
<tr>
<td>BMI&lt;30 kg/m²</td>
<td>140</td>
<td>0.99(0.79,1.23)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI&gt;30 kg/m²</td>
<td>179</td>
<td>3.17(2.61,3.84)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>99</td>
<td>1.83(1.51,2.21)</td>
<td>0.33</td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>223</td>
<td>2.16(1.63,2.87)</td>
<td></td>
</tr>
</tbody>
</table>

b Adjusted for age, bmi (as continuous variable), sex, smoking status; c Adjusted for age, sex, smoking status; d P value was computed by linear regression model, proc glm; unadjusted and adjusted means are geometric mean of log(CRP)

bp<.0001
cp>0.05
dBMI missing for 3 patients
<table>
<thead>
<tr>
<th>TIME POINT / VARIABLE</th>
<th>VITAMIN D₃ DOSE ASSIGNMENT (IU)</th>
<th>P VALUE¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLACEBO 1,000 2,000 4,000</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. participants</td>
<td>71 67 76 78</td>
<td></td>
</tr>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>13.7 (7.2-18.6) 29.7 (25.6-32.9) 34.7 (28.8-41.0) 45.9 (39.4-55.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>2.25(1.30-3.46) 2.53(1.57-4.16) 2.24(1.29-4.28) 1.97(1.09-3.78)</td>
<td>0.42</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>0.51(0.47-0.63) 0.49(0.45-0.57) 0.52(0.45-0.59) 0.52(0.45-0.61)</td>
<td>0.63</td>
</tr>
<tr>
<td>sTNF-R2 (pg/ml)</td>
<td>2033.4(1595.4-) 2061.4(1670.5-) 2198.6(1851.0-) 1946.0(1674.5-)</td>
<td>0.32</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.46(1.27-4.06) 2.21(0.92-4.70) 2.39(0.75-5.29) 1.98(0.49-5.96)</td>
<td>0.98</td>
</tr>
<tr>
<td>∆ 3 month – baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. participants</td>
<td>71 67 76 78</td>
<td></td>
</tr>
<tr>
<td>25(OH)D ng/ml</td>
<td>-2.3(-5.4-1.7) 10.8(2.5-18.9) 19.2(11.5-26.2) 30.2(21.5-37.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>-0.03(-0.96-0.88) -0.07(-1.07-0.93) 0.01(-0.57-0.89) 0.08(-0.61-0.71)</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>IL-10 (pg/ml)</td>
<td>sTNF-R2 (pg/ml)</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td></td>
<td>0(-0.06-0.07)</td>
<td>-0.01(-0.08-0.02)</td>
</tr>
<tr>
<td></td>
<td>-0.89(-187.75-160.10)</td>
<td>-9.88(-130.28-149.19)</td>
</tr>
<tr>
<td></td>
<td>59.16(-132.66-214.59)</td>
<td>13.29(-137.03-144.32)</td>
</tr>
<tr>
<td></td>
<td>-0.05(-1.13-0.98)</td>
<td>0.07(-0.58-0.86)</td>
</tr>
<tr>
<td></td>
<td>0.03(-0.68-0.79)</td>
<td>0.03(-0.68-0.79)</td>
</tr>
</tbody>
</table>

IU = international units; No. = number; IQR = interquartile range; 

\(^a\)P value calculated using Kruskal-Wallis test
Figure 1.

Participants consented (n=576)

- Ineligible by primary care physician (n=126)
- Eligible, but primary care physician refused (n=84)

Eligible by primary care physician (n=366)

- Lost to follow-up (n=7)
- Refused (n=31)

Participants randomized (n=328)

Placebo (n=81)
- Vitamin D₃ 1,000 IU/day (n=81)
- Vitamin D₃ 2,000 IU/day (n=83)
- Vitamin D₃ 4,000 IU/day (n=83)

Lost to follow-up (n=11)
- Withdrawal (n=20)
- Adverse event (n=4)

Participants who completed 3-month assessment (n=293) (89%)
- Plasma 25(OH)D unavailable (n=1)

Placebo (n=71)
- Vitamin D₃ 1,000 IU/day (n=67)

Vitamin D₃ 2,000 IU/day (n=76)
- Vitamin D₃ 4,000 IU/day (n=78)

Lost to follow-up (n=15)
- Withdrawal (n=21)

Participants who completed 6-month assessment (n=292) (89%)

Placebo (n=75)
- Vitamin D₃ 1,000 IU/day (n=68)
- Vitamin D₃ 2,000 IU/day (n=72)
- Vitamin D₃ 4,000 IU/day (n=77)
Cancer Prevention Research

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

Paulette D. Chandler, Jamil B. Scott, Bettina F. Drake, et al.