Published in final edited form as:
*Cancer Prev Res (Phila)*. 2011 October ; 4(10): 1645–1654. doi:10.1158/1940-6207.CAPR-11-0105.

# Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: A randomized, controlled clinical trial

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## Abstract

Vitamin D and calcium affect several pathways involved in inflammation, tumor growth, and immune surveillance relevant to carcinogenesis. Also, epidemiologic evidence indicates that calcium and vitamin D may reduce risk for colorectal adenomas and cancer. To investigate the effects of calcium and vitamin D on biomarkers of inflammation in colorectal adenoma patients, we conducted a pilot, randomized, double-blind, placebo-controlled, 2×2 factorial clinical trial (n=92), of 2 g/day calcium and/or 800 IU/day vitamin $D_3$ supplementation vs. placebo over six months. Plasma concentrations of pro-inflammatory markers (CRP, TNF-α, IL-6, IL-1β, and IL-8) and an anti-inflammatory marker (IL-10) were measured using enzyme-linked immunoassays. After six months of treatment, in the vitamin $D_3$ supplementation group, CRP decreased 32% overall (p=0.11), 37% in men (p=0.05), and 41% among non-NSAID users (p=0.05) relative to placebo. In the vitamin $D_3$ supplementation group, TNF-α decreased 13%, IL-6 32%, IL-1β 50%, and IL-8 15%; in the calcium supplementation group, IL-6 decreased 37%, IL-8 11%, and IL-1β 27%. Although these changes were not statistically significant, a combined inflammatory markers z-score decreased 77% (p=0.003) in the vitamin $D_3$ treatment group overall, 83% (p=0.01) among men, and 48% among non-NSAID users (p=0.01). There was no evidence of synergy between vitamin $D_3$ and calcium or effects on IL-10. These preliminary results are consistent with a pattern of reduction in tumor-promoting inflammation biomarkers with vitamin $D_3$ or calcium supplementation alone, and support further investigation of vitamin $D_3$ as a chemopreventive agent against inflammation and colorectal neoplasms.

### Keywords

Vitamin D; calcium; colonic neoplasms; inflammation; biomarkers; chemoprevention trial

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## Introduction

Colorectal cancer (CRC) is the second leading cause of cancer mortality in the United States and is consistently inversely associated with calcium intake and serum vitamin D levels (1–9). Inflammation is intricately linked to the etiology of colorectal cancer, and may also be a key in understanding the mechanisms linking calcium and vitamin D to colorectal cancer risk reduction. Inflammatory conditions such as Crohn's disease and ulcerative colitis are established risk factors for colorectal cancer, nonsteroidal anti-inflammatory drug (NSAID) use reduced both polyposis in FAP patients and sporadic colorectal adenoma recurrence in clinical trials, and specific pro-inflammatory markers, such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), are elevated in inflammatory bowel disease patients (10–14). These inflammatory markers are also associated with neoplastic growth, higher tumor grade, and increased risk of mortality in colorectal cancer patients (11, 12, 15–19). In addition, in a case-control study, risk factors for colorectal adenomas, such as old age, smoking, and adiposity, were found to be associated with higher levels of these inflammatory markers (10).

The mechanisms by which calcium is proposed to reduce risk for colorectal cancer are closely related to inflammation. Calcium binds to free fatty acids and bile acids, precipitating them from solution in the colon, which is hypothesized to reduce oxidative stress and inflammation in the colon (20). Calcium also activates the calcium sensing receptor, which is involved in cell-cycle events and differentiation, and promotes cell-cell and cell-matrix adhesion (21, 22). Vitamin D, along with increasing the absorption of calcium and regulating calcium homeostasis, also regulates more than 200 genes through the vitamin D receptor (VDR). Activation of the VDR is involved in bile acid degradation, direct transcriptional regulation of several inflammatory cytokines, cell cycle regulation, DNA repair, differentiation, and apoptosis (22, 23).

Despite the basic science evidence, there are no published trials of the effects of vitamin D and/or calcium supplementation on blood markers of inflammation in patients at risk for developing colorectal cancer. To address this, we conducted a pilot, randomized, double-blind, placebo-controlled 2×2 factorial chemoprevention trial of calcium and vitamin $D_3$ supplementation, alone or in combination, versus placebo over six months to estimate their effects on a panel of circulating pro- and anti-inflammatory markers in patients with a history of sporadic colorectal adenoma. We hypothesized that vitamin $D_3$ and calcium, alone or in combination, would decrease tumor-promoting pro-inflammatory markers, and increase tumor-inhibiting, anti-inflammatory markers.

## Materials and Methods

This study was approved by the Emory University Institutional Review Board. Written informed consent was obtained from each study participant.

### Study population

The detailed protocol of study recruitment and procedures was published previously (24). Briefly, study participants were recruited from the patient population attending the Digestive Diseases Clinic of Emory University. Eligibility included age 30 to 75 years, in good general health, capable of informed consent, and at least one pathology-confirmed sporadic colon or rectal adenoma in the past 36 months. Exclusions included contraindications to calcium or vitamin $D_3$ supplementation or rectal biopsy procedures, and medical conditions, habits, or medication usage that would otherwise interfere with the study (24).

## Clinical trial protocol

Between April 2005 and January 2006, potential participants attended an eligibility visit during which they were interviewed, signed a consent form, completed questionnaires, provided a blood sample, and were entered into a 30-day placebo run-in trial (24). Diet was assessed with a semi-quantitative food frequency questionnaire (25). After the 30-day placebo run-in trial, 92 participants without significant perceived side effects who had taken at least 80% of their capsules during the run-in trial were eligible for randomization. Eligible participants then underwent a baseline blood draw and rectal biopsy and were randomly assigned (stratified by sex and nonsteroidal anti-inflammatory drug use) to the following four treatment groups: a placebo control group (n = 23), a 2.0 g elemental calcium (1 g twice daily as calcium carbonate) group (n = 23), an 800 IU vitamin $D_3$ (400 IU twice daily) group (n = 23), and a 2.0 g elemental calcium plus 800 IU vitamin $D_3$ group (n = 23).

Study tablets were custom manufactured by Tishcon Corp. (Westbury, New York). The corresponding supplement and placebo pills were identical in size, appearance, and taste. The placebo was free of calcium, magnesium, vitamin D, and chelating agents. Additional details and rationale for the doses and forms of calcium and vitamin D supplementation were described previously (24).

Over the 6-month treatment period, participants attended follow-up visits at 2 and 6 months after randomization and were contacted by telephone at monthly intervals between the second and final follow-up visits. At follow-up visits, pill-taking adherence was assessed by questionnaire, interview, and pill count. Adverse events were monitored by interview at each study visit, interim telephone call, and questionnaires and graded according to NIH Common Toxicity Criteria 2.0 and the likelihood that they were study related. Participants were instructed to remain on their usual diet and not take any nutritional supplements not in use on entry into the study. At each follow-up visit, participants were interviewed and completed questionnaires. At the first and last visits, all participants underwent venipuncture and a rectal biopsy procedure. Participants were asked to abstain from aspirin (but not non-aspirin NSAIDs) use for 7 days before each biopsy/blood draw visit. All visits for a given participant were scheduled at the same time of day to control for possible circadian variability in the outcome measures. Factors hypothesized to be related to inflammatory cytokines (e.g., diet and NSAID use) were assessed at baseline, several were reassessed at the first follow-up visit, and all were reassessed at the final follow-up visit.

Peripheral venous blood samples were taken after the subject sat upright with their legs uncrossed for five minutes. Blood was drawn into red-coated, pre-chilled vacutainer tubes for whole blood, plasma, and serum, and then immediately placed on ice and shielded from light. Blood fractions were aliquotted into amber-colored cryopreservation tubes, the air was displaced with argon gas, and then the aliquots were immediately placed in a −80° C freezer until analysis.

## Inflammation biomarker analyses

All samples were blinded to treatment group and treated identically. A single enzyme linked immunoassay (ELISA) (R&D systems, Minneapolis, MN) was used to measure CRP, in duplicate, according to the manufacturer's protocol. The average intra-assay coefficient of variation (CV) for CRP was 6.6%. A High Sensitivity Multiplex enzyme linked immunoassay (R&D systems, Minneapolis, MN) was used to measure TNF-α, IL-6, IL-1β, IL-8, IL-5, IL-4, VEGF, IL-2, IL-10, IL-12, GM-CSF, and IFN-γ, in duplicate, according to the manufacturer's protocol. The average intra-assay coefficient of variation (CV) for TNF-α was 11.5%, for IL-6 11.7%, for IL-1β 10.6%, for IL-8 7.9%, for IL-5 34.1%, for IL-4 39.4%, for VEGF 21.0%, for IL-2 45.0%, for IL-10 11.5%, for IL-12 24.5%, and for GM-

CSF 38.5%. Low plasma cytokine concentrations create very high variability, and the results for cytokines with CVs above 15% were considered too variable and inaccurate to be reported.

### Statistical analysis

Treatment groups were assessed for comparability of characteristics at baseline and final follow-up by the Fisher's exact test for categorical variables and ANOVA for continuous variables. ELISA reliability was assessed using coefficients of variation.

Primary analyses were based on assigned treatment at the time of randomization regardless of adherence (intent-to-treat analysis). Biomarker levels below the limits of detection were assigned a value equal to the lower limit of detection for that biomarker. Variables not normally distributed were transformed, as appropriate, before statistical testing. Mean biomarker concentrations were calculated for each treatment group for the baseline and six-month follow-up visits. Treatment effects were evaluated by assessing the differences in biomarker concentrations from baseline to 6-months follow-up between each active treatment group and the placebo group by a repeated-measures linear mixed effects model, as implemented using the Proc MIXED procedure of the Statistical Analysis System (SAS, version 9.2 Copyright© 2002–2008 by SAS Institute Inc., Cary, NC, USA). The model included the intercept, indicators for treatment group and visit (baseline and follow-up), and a treatment by visit interaction term. Study participant was treated as a random effect, and absolute treatment effects were calculated and reported. A cutoff level of $P \leq 0.05$ (two-sided) was used for assessing statistical significance. Since concentrations of the measured biomarkers in plasma are not widely familiar, to provide perspective on the magnitude of treatment effects, relative effects were also calculated, defined as (treatment group follow-up/treatment group baseline)/(placebo follow-up/placebo baseline) (24, 26). The relative effect provides a conservative estimate of the average proportional change in the treatment group relative to that in the placebo group. The interpretation of the relative effect is somewhat analogous to that of an odds ratio (e.g., a relative effect of 2.0 means that the relative proportional change in the treatment group was twice as great as that in the placebo group). Stratified analyses were conducted to investigate potential differential treatment effects by sex, age, BMI, and NSAID use.

To assess the effects of vitamin $D_3$ and/or calcium supplementation on a summary score of all the pro- and anti-inflammatory markers combined, a summary inflammation z-score was calculated. This score was calculated as follows: first, a normalized z-score for each individual biomarker value, with a mean of zero and standard deviation of 1.0, was calculated as $z = (x - \mu)/\sigma$, where x is a participant's biomarker value at a given visit, and $\mu$ and $\sigma$ are the study population mean and standard deviation, respectively, at baseline; and then the combined inflammation z-score for each participant at each trial visit was created by summing the z-scores of each inflammatory marker (IL-10 was included with a negative sign, because it has been shown to protect against colonic inflammation (27)). This inflammation z-score was then analyzed as for the individual biomarkers.

## Results

### Study participants

Treatment groups were quite similar on characteristics measured at baseline (Table 1) or at final follow-up (data not shown; in particular, there was no change in NSAID use by treatment group over the course of the trial). The mean age of participants was 61 years, 70% were men, 71% were white, and 20% had a family history of colorectal cancer in a first-degree relative. Adherence to visit attendance averaged 92% and did not differ

significantly among the four treatment groups. On average, at least 80% of pills were taken by 93% of participants at the first follow-up visit and 84% at the final follow-up visit. There were no complications attributed to study procedures or treatments. Seven participants (8%) were lost to follow-up due to perceived drug intolerance (n = 2), unwillingness to continue participation (n = 3), physician's advice (n = 1), and death attributed to cardiovascular disease (n = 1). Participant dropouts from the trial included one person from the vitamin $D_3$ supplementation group and two persons from each of other three groups.

At baseline, there were no significant differences between the four study groups in serum 25-OH-vitamin D. By study end, serum 25-OH-vitamin D levels statistically significantly ($p<0.0001$) increased by 60% to 29.5 ng/ml in the vitamin $D_3$ group and by 56% to 28.5 ng/ml in the calcium plus vitamin $D_3$ group relative to placebo (24).

Changes in CRP, TNF-α, IL-6, IL-1β, IL-8, and IL-10 plasma concentrations relative to placebo in the calcium, vitamin $D_3$, or combined supplementation groups are shown in Table 2. After six months of treatment, in the vitamin $D_3$ supplementation group, CRP decreased by 32%, TNF-α by 13%, IL-6 by 32%, IL-1β by 50%, and IL-8 by 15%, relative to placebo, although these changes were not statistically significant. In the calcium supplementation group, relative to placebo, CRP decreased 8%, IL-6 decreased 37%, IL-8 by 11%, and IL-1β by 27%, although these changes were also not statistically significant. In the vitamin $D_3$ plus calcium supplementation group, IL-6 decreased by 8%, IL-8 by 13%, and IL-1β by 35%, relative to placebo, although these changes were not statistically significant. IL-10 decreased a minor non-significant amount in all active treatment groups.

The effects of vitamin $D_3$ and/or calcium on the combined "inflammation z-score" of all reported inflammatory markers (CRP, TNF-α, IL-6, IL-8, IL-1β, and IL-10) are summarized in Table 3. An individual's inflammation z-score allows for the calculation of an aggregate score of all biomarkers by converting them to a comparable score, a z-score, and then totaling the values for each individual. The overall inflammation z-score significantly dropped 77% ($p=0.003$) in the vitamin $D_3$ treatment group, 48% ($p=0.18$) in the calcium treatment group, and 33% ($p=0.40$) in the combined treatment group relative to placebo.

Men and women have differences in their biochemical makeup (such as estrogen levels) that could lead to differences in response to vitamin D and calcium supplementation; therefore, we investigated potential differences in response by sex (Table 4). In men, CRP decreased 37% ($p=0.05$) in the vitamin $D_3$ treatment group relative to placebo, but did not change substantially in women. Similar to the results for CRP, the inflammation z-score statistically significantly dropped in men (83%, $p=0.01$) but not in women in the vitamin $D_3$ treatment group relative to placebo. Changes in TNF-α, IL-6, IL-8, IL-1β, and IL-10 did not differ substantially by sex (data not shown).

Since NSAID use may overwhelmingly affect inflammation pathways, we investigated the effects of vitamin $D_3$ and calcium among study participants who were not currently taking NSAIDs (Table 4). In non-NSAID users, the decrease in CRP (41%; $p=0.05$) was slightly stronger than in all participants combined (32%; $p=0.11$) in the vitamin $D_3$ treatment group relative to placebo. The inflammation z-score also decreased significantly by 58% ($p=0.01$) among non-NSAID users in the vitamin $D_3$ treatment group. Changes in TNF-α, IL-6, IL-8, IL-1β, and IL-10 among non-NSAID users did not differ substantially from changes among all participants combined (data not shown).

## Discussion

The results from this pilot, randomized, controlled clinical trial suggest that supplementation with vitamin $D_3$ or calcium alone may decrease tumor-promoting pro-inflammatory markers

in the plasma of sporadic colorectal adenoma patients. These findings are consistent with the hypothesis that vitamin $D_3$ or calcium may decrease inflammation in the colon, and thus reduce risk for colorectal neoplasms. Consistent with previous findings in this same study on oxidative DNA damage in the normal colorectal mucosa (28), our findings also suggest that vitamin $D_3$ combined with calcium may have a lesser treatment effect on pro-inflammatory markers than do vitamin $D_3$ or calcium alone.

Inflammation is intricately linked to the etiology of CRC, as evidenced by inflammatory conditions of the colon, such as Crohn's disease and ulcerative colitis, which are established risk factors for the disease (29). Several inflammatory molecules, including CRP, TNF-α, IL-6, and IL-8, were found to be higher in the blood of CRC patients than in controls (11, 12, 19), and have been associated with other risk factors for CRC, such as age, smoking, and high BMI (10). In addition, CRP, TNF-α, and IL-6 are associated with higher tumor grade and poorer prognosis (15, 19), and higher levels of CRP and IL-6 are associated with increased mortality among colorectal cancer patients (11). In a case-control study, polymorphisms in the genes for IL-6, TNF-α, IL-1β, and IL-8 that are linked to increased expression of their corresponding cytokines were associated with increased adenoma risk (18, 30). IL-1β is involved in COX-2 activation and activates the Wnt cell cycle activation pathway, the primary pathway of colon cell proliferation (31). Vitamin $D_3$ inhibited this pathway *in vitro* by decreasing IL-1β production by macrophages, thus decreasing colon carcinoma cell proliferation (31).

Calcium and vitamin D have several mechanisms of action relevant to our hypothesis that they may decrease inflammatory markers and risk for CRC. Only about 30% of calcium is absorbed in the GI tract, with the other 70% free to bind with and precipitate bile acids, which have been shown to cause damage to epithelial cell membranes and produce an inflammatory response in these cells (32, 33). This inflammatory response, in turn, may represent a large source of circulating cytokines. Vitamin D, acting through the vitamin D receptor, also reduces bile acids in the colon by increasing the bile acid catabolizing enzyme CYP3A4 (21, 34). 1-25-$(OH)_2$-vitamin D binding of the vitamin D receptor acts as a transcriptional regulator to enhance IL-10 transcription, and represses several pro-inflammatory cytokines, including IL-6, IL-8, and TNF-α (26, 35). In addition, the vitamin D receptor, when activated by vitamin D, suppresses the transcription of RelB, a component of the global transcriptional regulator NF-κB (36), a key regulator of inflammation and response to oxidative stress and a downstream target of TNF-α (37). NF-κB induces the transcription of inflammatory cytokines and anti-apoptotic proteins that together promote cellular transformation and tumor formation (38). Mice lacking IL-10 quickly develop inflammatory bowel disease, but supplementation with vitamin $D_3$ ameliorated symptoms and blocked the progression of the disease (27). Combined with this biological evidence, the results of our study support vitamin $D_3$ and calcium as possible inflammation-reducing agents in humans.

Contrary to our original hypothesis, and the findings of some epidemiological and clinical studies, we found no evidence for a greater than additive effect of combined supplementation of calcium and vitamin $D_3$ (28, 39–42). Our estimated treatment effects in the calcium plus vitamin $D_3$ group tended to be less than those for the individual agents. In this same population, we previously reported that combined calcium and vitamin $D_3$ supplementation may have lesser effects on colorectal epithelial apoptosis, differentiation, and oxidative DNA damage than do calcium or vitamin $D_3$ alone (24, 28, 43). These statistically non-significant findings of a smaller treatment effect in the combined treatment group may simply be due to chance because of our small sample size. However, given the consistency of this pattern, it is possible that calcium and vitamin D negatively regulate one another. 1,25-$(OH)_2$-vitamin $D_3$ regulates calcium absorption, and calcium suppresses 1,25-

$(OH)_2$-vitamin $D_3$ synthesis by 1α-hydroxylase (44). One animal study found that high calcium supplementation led to lower circulating levels of 25-OH-vitamin D (34); however, in humans, risk of adenoma recurrence was only decreased by calcium supplementation in individuals with higher serum 25-OH-vitamin D levels (40). In human colon carcinoma cells, calcium and vitamin D synergistically enhanced the expression of E-cadherin; however, the enhanced expression of p21 and p27 by calcium and vitamin D separately was not changed with a combined treatment (45). Another possible explanation for the less than additive effects of calcium plus vitamin $D_3$ is that too little vitamin $D_3$ was given. Although 800 IU daily vitamin $D_3$ supplementation in this population statistically significantly raised serum 25-OH-vitamin D levels, the mean in all treatment and placebo groups was below 32 ng/ml, the suggested level to be considered sufficient for this vitamin (24, 46). Taken together, the combined effect of calcium and vitamin $D_3$ on biomarkers of colon carcinogenesis and inflammation in humans is unclear, and requires further clarification through larger studies.

Calcium and vitamin D have several known and likely unknown downstream targets involved in inflammation regulation as discussed above, and, therefore, biological effects of these agents may be best measured using a combined detection method. We developed an inflammation z-score to assess the inflammation status of an individual more comprehensively, and then analyzed the effects of calcium and/or vitamin $D_3$ on this inflammation z-score. We hypothesized that vitamin $D_3$ and/or calcium would affect this inflammation z-score more substantially than any single measure of inflammation. Vitamin $D_3$, but not calcium or the two combined, significantly reduced the inflammation z-score in this study population by 77% ($p=0.003$) relative to placebo. This finding suggests that vitamin $D_3$ may reduce inflammation in multi-factorial ways. Inflammatory markers, including CRP, IL-6, and TNF-α, were found to be significantly higher in colorectal cancer patients than in controls (11, 12, 19); however, it is not known whether these individual markers are also elevated in colorectal adenoma patients. We propose the use of this inflammation z-score to measure sub-clinical inflammation or to detect small changes in multiple cytokines that combined may produce clinically important changes in inflammation and risk for disease. Further investigation is needed, however, and this score should be explored in cohort and case-control studies to investigate whether it is associated with risk for colorectal adenomas or cancer, as well as in larger chemoprevention trials to investigate its usefulness as an intervention response marker.

In our analysis stratified by sex, there was a significant reduction in CRP and the inflammation z-score with vitamin $D_3$ supplementation in men but not in women. There are several possible explanations for this, the most obvious one being chance related to the small sample size, especially in women. Another possible explanation is that most women in this study were post-menopausal and not taking hormone replacement therapy, and, therefore, likely had low estrogen levels. Estrogen supplementation was found to increase 1-25-$(OH)_2$-vitamin D signaling and down-regulate inflammation pathways in the rectal epithelium of postmenopausal women (47). The findings of our study support the hypothesis that low estrogen levels may interfere with response to vitamin D supplementation, VDR signaling, and inflammatory pathways; however, larger studies are needed to investigate these issues more definitively.

When only non-NSAID users were considered, CRP and the inflammation z-score were found to be statistically significantly reduced with vitamin $D_3$ supplementation. Other than chance due to the small sample size, a possible explanation is that NSAIDs have powerful effects on inflammation pathways that could mask effects of vitamin $D_3$ or calcium. NSAIDs largely reduce risk for colorectal cancer by blocking a major colon carcinogenesis and inflammatory pathway enzyme, COX-2 (48, 49). Vitamin D supplementation effects on

inflammation may only be detectable and important in individuals not already using NSAIDs, although more investigation is needed to clarify this issue.

Our pilot study has several limitations and strengths. First, the sample size was small, limiting the statistical power for detecting treatment effects. A second potential limitation to the study is that all of the blood biomarker analyses except for CRP were done using a high-sensitivity multiplex ELISA. While the low limit of detection allowed for a higher number of samples with detectable analytes, the measurements may have been less reliable and accurate than would have been found with non-multiplex ELISA. We accounted for this lower accuracy of the ELISA by calculating coefficients of variation (CV), and did not report data for those cytokines with a CV > 20%. Third, with the relatively low dose of vitamin $D_3$ supplementation, although serum 25-OH-vitamin D levels increased significantly, the average serum levels did not reach the “sufficiency” range of above 32 ng/ml (24, 46). Therefore, higher doses of supplemental vitamin $D_3$ may produce more definitive changes in pro-inflammatory markers.

Strengths of this study included the randomized, double-blind, placebo-controlled clinical trial design; the high protocol adherence by the study participants; investigation of both the individual and combined effects of calcium and vitamin $D_3$; and the balance in the treatment groups on many potential confounding risk factors for colorectal cancer and inflammation.

In summary, our preliminary findings suggest vitamin $D_3$ or calcium alone may decrease tumor-promoting pro-inflammatory markers in the plasma of sporadic colorectal adenoma patients. Also, taken together with previous literature, this study supports further investigation of a) vitamin $D_3$ or calcium supplementation for reducing inflammatory biomarkers in sporadic colorectal adenoma patients, b) our investigated biomarkers of inflammation or a combined inflammation z-score as potential treatable biomarkers of risk for colorectal cancer, and c) a larger trial with higher doses of vitamin $D_3$ on biomarkers of inflammation and risk for colorectal neoplasms.

## Acknowledgments

This study was funded in part by grants from the NIH, the Franklin Foundation, and a Georgia Cancer Coalition Distinguished Scholar Award (to RMB). We thank Bill Liang for equipment management and advice, Jill Woodward for managing the trial, and all study participants for their time and dedication to the study.

**Grant support:** R01 CA104637/CA/NCI NIH HHS/United States; R03 CA136113/CA/NCI NIH HHS/United States; The Franklin Foundation; a Georgia Cancer Coalition Distinguished Scholar Award (to R.M.B.). The NIH, the Georgia Cancer Coalition, and the Franklin Foundation had no influence on the design of the study; the collection, analysis, and interpretation of the data; the decision to submit the manuscript for publication; or the writing of the manuscript.

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**Table 1**

Selected baseline characteristics of the clinical trial participants

| Characteristics | Treatment group | | | | P* |
|---|---|---|---|---|---|
| | Placebo (n = 23) | Calcium (n = 23) | Vitamin D (n = 23) | Calcium + Vit. D (n = 23) | |
| Demographics | | | | | |
| Age (y) | 58.5 (7.9) | 61.9 (8.0) | 60.2 (8.1) | 61.7 (7.4) | 0.39 |
| Men (%) | 70 | 70 | 70 | 70 | 1.00 |
| White (%) | 74 | 83 | 65 | 61 | 0.39 |
| College graduate (%) | 65 | 61 | 57 | 44 | 0.53 |
| Medical history | | | | | |
| History of colorectal cancer in 1° relative (%) | 17 | 30 | 17 | 13 | 0.60 |
| Take NSAID regularly† (%) | 17 | 13 | 13 | 30 | 0.26 |
| If woman (n = 5), taking estrogens (%) | 4 | 9 | 4 | 4 | 1.00 |
| Habits | | | | | |
| Current smoker (%) | 9 | 4 | 0 | 0 | 0.61 |
| Take multivitamin (%) | 30 | 30 | 26 | 39 | 0.86 |
| Mean dietary intakes | | | | | |
| Total energy intake (kcal/d) | 1,596 (528) | 1,788 (691) | 1,848 (821) | 1,845 (752) | 0.59 |
| Total calcium‡ (mg/d) | 618 (308) | 746 (335) | 843 (526) | 824 (714) | 0.41 |
| Total vitamin D‡ (IU/d) | 277 (230) | 336 (202) | 360 (317) | 415 (316) | 0.40 |
| Total fat (g/d) | 67 (32) | 72 (35) | 70 (32) | 74 (28) | 0.59 |
| Dietary fiber (g/d) | 15 (7) | 17 (9) | 18 (9) | 17 (11) | 0.97 |
| Alcohol (g/d) | 9 (14) | 11 (15) | 14 (18) | 10 (20) | 0.84 |
| Anthropometrics | | | | | |
| Body mass index (kg/m$^2$) | 30.6 (7.2) | 29.4 (5.5) | 29.0 (5.56) | 31.6 (6.0) | 0.44 |
| Waist-to-hip ratio | 0.9 (0.1) | 0.9 (0.1) | 0.9 (0.1) | 1.0 (0.1) | 0.17 |
| 25-OH-vitamin D (ng/mL) | 20.4 (7.6) | 25.7 (7.6) | 21.0 (8.3) | 20.9 (9.7) | 0.12 |

NOTE: Data are given as means (SD) unless otherwise specified.

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug.

*By Fisher's exact test for categorical variables and by ANOVA for continuous variables.

† At least once a week.

‡ Diet plus supplements.

**Table 2**

Changes in biomarkers of inflammation in plasma of colorectal adenoma patients

| Biomarkers | Baseline | | | | 6-month follow-up | | | | Absolute treatment effect† | | | Relative treatment effect‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | P* | n | Mean | SD | P§ | Mean | 95% CI | P* | |
| **Pro-inflammatory** | | | | | | | | | | | | |
| CRP# (μg/ml) | | | | | | | | | | | | |
| Placebo | 22 | 1.77 | 3.80 | N/A | 21 | 1.88 | 4.16 | 0.77 | N/A | N/A | N/A | 1.00 |
| Calcium | 21 | 1.13 | 3.50 | 0.21 | 21 | 1.09 | 4.32 | 0.85 | −0.09 | (−0.40, 0.56) | 0.71 | 0.92 |
| Vitamin D | 22 | 1.39 | 2.79 | 0.49 | 22 | 0.99 | 1.97 | 0.05 | −0.39 | (−0.91, 0.86) | 0.11 | 0.68 |
| Calcium + Vit D | 21 | 1.93 | 2.94 | 0.82 | 21 | 2.21 | 3.06 | 0.42 | 0.08 | (−0.40, 0.57) | 0.74 | 1.09 |
| TNF-α# (pg/ml) | | | | | | | | | | | | |
| Placebo | 23 | 4.13 | 1.92 | N/A | 21 | 4.57 | 2.05 | 0.09 | N/A | N/A | N/A | 1.00 |
| Calcium | 23 | 3.04 | 1.94 | 0.28 | 21 | 3.35 | 1.88 | 0.67 | 0.06 | (−0.21, 0.33) | 0.66 | 1.06 |
| Vitamin D | 22 | 2.92 | 1.78 | 0.09 | 22 | 2.73 | 2.52 | 0.46 | −0.14 | (−0.40, 0.13) | 0.30 | 0.87 |
| Calcium + Vit D | 23 | 3.62 | 1.75 | 0.50 | 21 | 4.00 | 1.62 | 0.28 | 0.03 | (−0.23, 0.30) | 0.81 | 1.03 |
| IL-6# (pg/ml) | | | | | | | | | | | | |
| Placebo | 23 | 1.13 | 4.54 | N/A | 21 | 1.41 | 2.67 | 0.30 | N/A | N/A | N/A | 1.00 |
| Calcium | 23 | 1.09 | 3.65 | 0.45 | 21 | 0.85 | 3.29 | 0.27 | −0.46 | (−1.07, 0.14) | 0.13 | 0.63 |
| Vitamin D | 22 | 0.78 | 4.68 | 0.42 | 22 | 0.67 | 3.76 | 0.47 | −0.38 | (−0.98, 0.23) | 0.22 | 0.68 |
| Calcium + Vit D | 23 | 1.39 | 4.49 | 0.63 | 21 | 1.62 | 3.25 | 0.50 | −0.08 | (−0.69, 0.53) | 0.80 | 0.92 |
| IL-8# (pg/ml) | | | | | | | | | | | | |
| Placebo | 23 | 4.74 | 1.59 | N/A | 21 | 5.01 | 1.78 | 0.10 | N/A | N/A | N/A | 1.00 |
| Calcium | 23 | 5.97 | 1.72 | 0.51 | 21 | 5.34 | 1.52 | 0.56 | −0.12 | (−0.49, 0.22) | 0.45 | 0.89 |
| Vitamin D | 22 | 5.60 | 1.52 | 0.30 | 22 | 5.03 | 1.79 | 0.62 | −0.15 | (−0.62, 0.08) | 0.13 | 0.85 |
| Calcium + Vit D | 23 | 5.61 | 1.68 | 0.63 | 21 | 5.09 | 1.54 | 0.60 | −0.14 | (−0.50, 0.21) | 0.42 | 0.87 |
| IL-1β# (pg/ml) | | | | | | | | | | | | |
| Placebo | 23 | 0.22 | 2.03 | N/A | 21 | 0.23 | 2.64 | 0.56 | N/A | N/A | N/A | 1.00 |
| Calcium | 23 | 0.27 | 3.82 | 0.56 | 21 | 0.22 | 3.06 | 0.62 | −0.32 | (−1.16, 0.51) | 0.44 | 0.73 |
| Vitamin D | 22 | 0.16 | 2.04 | 0.30 | 22 | 0.13 | 2.28 | 0.07 | −0.70 | (−1.53, 0.12) | 0.09 | 0.50 |
| Calcium + Vit D | 23 | 0.27 | 2.62 | 0.59 | 21 | 0.23 | 2.24 | 0.40 | −0.43 | (−1.27, 0.41) | 0.31 | 0.65 |

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| Biomarkers | Baseline | | | | 6-month follow-up | | | | Absolute treatment effect† | | | Relative treatment effect‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | P* | n | Mean | SD | P§ | Mean | 95% CI | P* | |
| **Anti-inflammatory** | | | | | | | | | | | | |
| IL-10# (pg/ml) | | | | | | | | | | | | |
| Placebo | 23 | 0.54 | 1.57 | N/A | 21 | 0.53 | 1.96 | 0.63 | N/A | N/A | N/A | 1.00 |
| Calcium | 23 | 0.58 | 1.68 | 0.63 | 21 | 0.50 | 1.56 | 0.16 | −0.07 | (−0.29, 0.15) | 0.52 | 0.93 |
| Vitamin D | 22 | 0.48 | 1.53 | 0.37 | 22 | 0.43 | 1.38 | 0.23 | −0.05 | (−0.27, 0.16) | 0.62 | 0.95 |
| Calcium + Vit D | 23 | 0.26 | 1.49 | 0.66 | 21 | 0.55 | 1.55 | 0.35 | −0.04 | (−0.26, 0.19) | 0.75 | 0.96 |

Abbreviations: C-reactive protein, CRP; Tumor necrosis factor alpha, TNF-α; Interleukin-6, IL-6; Interleukin-8, Il-8; Interleukin-1 beta, IL-1β; Interleukin-10, IL-10.

† Absolute treatment effect is the absolute change from baseline to follow-up in the treatment group minus the absolute change from baseline to follow-up in the placebo group from mixed model.

‡ Relative treatment effect is defined as: (treatment group follow-up /treatment group baseline)/(placebo follow-up /placebo baseline). The interpretation of the relative effect is similar to that of an odds ratio (e.g., a relative effect of 2.0 would mean that the relative proportional change in the treatment group was twice as great as that in the placebo group).

* P values for difference between each treatment group and placebo group from mixed model.

§ P values for difference between follow-up visit and baseline visit from mixed model.

# Geometric means with standard errors are reported, calculated by exponentiating the mean of the log transformed values.

**Table 3**

Changes in inflammation Z-score in plasma of colorectal adenoma patients

| | | Baseline | | 6-month follow-up | | Absolute treatment effect† | | | Relative treatment effect‡ |
|---|---|---|---|---|---|---|---|---|---|
| **Inflammation z-score§** | **n** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **95% CI** | **P*** | |
| Placebo | 23 | 0.20 | 2.96 | 0.69 | 3.48 | N/A | N/A | N/A | 1.00 |
| Calcium | 23 | −0.35 | 2.79 | −0.50 | 2.94 | −0.65 | (−1.62, 0.31) | 0.18 | 0.52 |
| Vitamin D | 22 | −0.81 | 2.66 | −1.78 | 2.74 | −1.47 | (−2.42, −0.51) | 0.003 | 0.23 |
| Calcium + Vit D | 23 | 0.60 | 3.05 | 0.69 | 2.99 | −0.41 | (−1.37, 0.55) | 0.40 | 0.67 |

† Absolute treatment effect is the absolute change from baseline to follow-up in the treatment group minus the absolute change from baseline to follow-up in the placebo group from mixed model.

‡ Relative treatment effect is defined as: (treatment group follow-up /treatment group baseline)/(placebo follow-up /placebo baseline).

§ Inflammation z-score: Z-score of pro- and anti-inflammatory markers (CRP, IL6, IL-1β, TNF-α, IL-8 and IL-10) calculated by 1) subtracting the mean and dividing by the standard deviation (thus creating a mean of zero and standard deviation of 1.0) for each participants individual biomarker value at each visit, and then 2) summing the biomarker z-score values for each participant at each visit (IL-10 was included with a negative sign).

* P values for difference between each treatment group and the placebo group from mixed model.

**Table 4**

Changes in plasma CRP and inflammation z-score levels stratified by sex and NSAID use in colorectal adenoma patients

| | | Baseline | | 6-month follow-up | | Absolute treatment effect† | | | Relative treatment effect‡ |
|---|---|---|---|---|---|---|---|---|---|
| **CRP (μg/ml)** | **n** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **95% CI** | **P*** | |
| **Women** | | | | | | | | | |
| Placebo | 7 | 3.55 | 3.50 | 2.99 | 2.85 | N/A | N/A | N/A | 1.00 |
| Calcium | 7 | 1.78 | 4.51 | 2.07 | 5.66 | 0.32 | (−0.91, 1.55) | 0.60 | 1.38 |
| Vitamin D | 6 | 1.52 | 3.32 | 0.98 | 1.89 | −0.27 | (−1.55, 1.00) | 0.66 | 0.76 |
| Calcium + Vit D | 7 | 2.65 | 4.13 | 2.37 | 4.06 | −0.06 | (−1.17, 1.28) | 0.92 | 0.94 |
| **Men** | | | | | | | | | |
| Placebo | 16 | 1.28 | 3.61 | 1.48 | 4.79 | N/A | N/A | N/A | 1.00 |
| Calcium | 16 | 0.90 | 2.98 | 0.79 | 3.51 | −0.28 | (−0.74, 0.19) | 0.24 | 0.76 |
| Vitamin D | 16 | 1.34 | 2.70 | 0.99 | 2.04 | −0.45 | (−0.90, −0.01) | 0.05 | 0.63 |
| Calcium + Vit D | 16 | 1.65 | 2.42 | 2.14 | 2.74 | 0.11 | (−0.36, 0.57) | 0.64 | 1.12 |
| **Non-NSAID users#** | | | | | | | | | |
| Placebo | 19 | 1.37 | 3.59 | 1.51 | 4.13 | N/A | N/A | N/A | 1.00 |
| Calcium | 20 | 1.13 | 3.80 | 1.07 | 4.47 | 0.22 | (−0.70, 0.37) | 0.43 | 1.25 |
| Vitamin D | 20 | 1.55 | 2.64 | 1.02 | 1.90 | −0.53 | (−1.05, −0.01) | 0.05 | 0.59 |
| Calcium + Vit D | 16 | 2.19 | 2.83 | 3.05 | 2.82 | −0.17 | (−0.34, 0.78) | 0.53 | 0.84 |
| **Inflammation Z-Score§** | | | | | | | | | |
| **Women** | | | | | | | | | |
| Placebo | 7 | 2.15 | 3.91 | 2.32 | 3.94 | N/A | N/A | N/A | 1.00 |
| Calcium | 7 | −1.39 | 2.78 | −1.45 | 3.39 | −0.23 | (−1.91, 1.44) | 0.77 | 0.79 |
| Vitamin D | 6 | −1.07 | 2.50 | −1.68 | 3.70 | −0.78 | (−2.52, 0.96) | 0.36 | 0.46 |
| Calcium + Vit D | 7 | −0.06 | 3.74 | 0.37 | 3.12 | −0.14 | (−1.54, 1.81) | 0.87 | 0.87 |
| **Men** | | | | | | | | | |
| Placebo | 16 | −0.72 | 1.94 | −0.12 | 3.05 | N/A | N/A | N/A | 1.00 |
| Calcium | 16 | 0.17 | 2.75 | −0.03 | 2.69 | −0.85 | (−2.09, 0.39) | 0.18 | 0.42 |
| Vitamin D | 16 | −0.71 | 2.79 | −1.82 | 2.44 | −1.75 | (−2.95, −0.55) | 0.01 | 0.17 |
| Calcium + Vit D | 16 | 0.88 | 2.77 | 0.86 | 3.03 | −0.67 | (−1.91, 0.57) | 0.29 | 0.51 |

| | | Baseline | | 6-month follow-up | | Absolute treatment effect† | | | Relative treatment effect‡ |
|---|---|---|---|---|---|---|---|---|---|
| **CRP (μg/ml)** | **n** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **95% CI** | **P*** | |
| **Non-NSAID users#** | | | | | | | | | |
| Placebo | 19 | −0.10 | 2.84 | 0.20 | 3.19 | N/A | N/A | N/A | 1.00 |
| Calcium | 20 | −0.60 | 3.57 | −0.84 | 2.88 | −0.56 | (−1.57, 0.46) | 0.28 | 0.57 |
| Vitamin D | 20 | −0.80 | 2.51 | −1.89 | 2.67 | −1.42 | (−2.41, −0.45) | 0.01 | 0.42 |
| Calcium + Vit D | 16 | 0.02 | 2.42 | 1.08 | 3.01 | −0.08 | (−1.14, 0.98) | 0.89 | 0.92 |

Abbreviations: C-reactive protein, CRP; nonsteroidal anti-inflammatory drug, NSAID.

†Absolute treatment effect is the absolute change from baseline to follow-up in the treatment group minus the absolute change from baseline to follow-up in the placebo group from mixed model.

‡Relative treatment effect is defined as: (treatment group follow-up /treatment group baseline)/(placebo follow-up /placebo baseline).

*P values for difference between each treatment group and the placebo group from mixed model.

§Inflammation z-score: Z-score of pro- and anti-inflammatory markers (CRP, IL6, IL-1β, TNF-α, IL-8 and IL-10) calculated by 1) subtracting the mean and dividing by the standard deviation (thus creating a mean of zero and standard deviation of 1.0) for each participants individual biomarker value at each visit, and then 2) summing the biomarker z-score values for each participant at each visit (IL-10 was included with a negative sign).

#NSAID user status at baseline (NSAID use by treatment group did not change during the course of the trial).

*Cancer Prev Res (Phila)*. Author manuscript; available in PMC 2012 October 1.