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Porter Zachary Sadler IV Date

Effects of Supplemental Calcium and Vitamin D on Circulating Biomarkers of Inflammation in Colorectal Adenoma Patients: a Randomized Clinical Trial

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Master of Public Health

Epidemiology

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2016

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An abstract of

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Abstract

Effects of Supplemental Calcium and Vitamin D on Circulating Biomarkers of Inflammation in Colorectal Adenoma Patients: a Randomized Clinical Trial

By Porter Zachary Sadler IV

Vitamin D and calcium are known to affect several pathways involved in inflammation and immune surveillance relevant to carcinogenesis, and, in observational studies, were shown to be associated with lower risk for colorectal neoplasms. To investigate the effects of vitamin D and calcium, alone and in combination, on circulating biomarkers of inflammation in colorectal adenoma patients, we conducted a pilot biomarker study within a 2x2 factorial, placebo-controlled, double-blind, randomized controlled trial of 1.2 g/d calcium and/or 1000 UI/d vitamin D_3 supplementation over 3 or 5 years. Plasma concentrations of pro-inflammatory markers [tumor necrosis factor (TNF)-α and granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6, IL-1β, IL-2, IL-4, IL-8, IL-12p40, IL12p70, IL-17A, interferon-gamma (IFN-γ)] and an anti-inflammatory marker (IL-10) were measured using multiplex electrochemiluminescence assays. After 12 months, in the vitamin D_3 supplementation group, compared to the group that received no vitamin D_3, IL-2 and IL-10 increased 27% (p = 0.06) and 16% (p = 0.11), respectively, and IL-1β decreased 23% (p = 0.07). In the calcium supplementation group, compared to the group that received no calcium, IL-1β decreased 79% (P = 0.11); and, in the calcium plus vitamin D supplementation group, compared to the calcium group, IL-2 increased 30% (p = 0.13). A combined inflammatory markers z-score decreased by 51% (p = 0.03) in the calcium treatment group overall, and 73% (p = 0.09) among those with higher baseline total calcium, when compared to the group that received no calcium. These results suggest that supplemental vitamin D and calcium may influence colorectal neoplasms development through inflammatory pathways.

Effects of Calcium and Vitamin D Supplementation on Markers of Inflammation Among Colorectal Adenoma Patients in the PPS4B Randomized Clinical Trial

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Introduction

Colorectal Cancer

Colorectal cancer (CRC) is a malignant neoplasm of the large bowel or intestine. It is the third leading cause of cancer incidence among men and women combined, and the second leading cause of cancer mortality in the United States. The overall 5-year survival rate for CRC is 65%, differing by cancer stage. It is estimated that by the end of 2019 there will be 145,000 new cases of the disease and 51,000 CRC deaths. One in 22 men and 1 in 24 women are estimated to develop CRC in their lifetimes. Due to improvements in CRC screening, CRC incidence and mortality have been declining in populations ages 50 and older, however, between 2009 and 2013, the incidence of CRC in populations under the age of 50 has increased by 1.3% and mortality has increased by 3%. The majority of CRC are adenocarcinomas.(1) Most CRCs arise from precancerous polyps known as colorectal adenomas (CAs).(2) The prevalence of CA is between 35% and 60%, varying by region.(3) In three years following CA removal, 46% of patients experience CA recurrence.(4)

Vitamin D, Calcium, and Colorectal Carcinogenesis

A 4-year randomized controlled trial found the effect of 3000 mg calcium carbonate (1200 mg of calcium) on colorectal adenoma reoccurrence to yield a crude risk ratio of 0.77 (95% CI: 0.62, 0.96). Another trial of calcium and fiber supplementation's effects on CA reoccurrence yielded an odds ratio of 0.66 (95% CI:

0.38, 1.17) when treatment of 2000 mg calcium daily was compared to placebo over 3 years.(5) A pooled analysis of 10 cohort studies yielded an estimated 22% lower CRC risk when comparing the highest quintile of dietary calcium intake to the lowest. Daily calcium intake above 1000 mg/day was shown to have little further reduction on CRC risk, suggesting a plateau of effect at the level of calcium deficiency.(6) A prior study suggested that the plateau to be at 700 mg/day.(7) A randomized controlled trial (RTC) found that compared to placebo, those receiving 1200 milligrams (mg) of calcium carbonate had a 14% reduced risk of all colon polyps over the 4 year follow-up, with a more pronounced effect on advanced adenomas.(8) Vitamin D and calcium are closely interrelated and could potentially synergize in their effect on colorectal neoplasms.(9,10) Vitamin D is involved in promoting the absorption of calcium in the intestines and the most bioactive form of vitamin D, 1,25 α-dihydroxyvitamin $D_{3'}$ inhibits cell proliferation and promotes differentiation and apoptosis in targeted tissues including the colon.(11) Clinical trials on the effects of vitamin D and calcium on CRC and CA occurrence have shown inconsistent results. A 4-year randomized controlled trial found the effect of 3000 mg calcium carbonate (1200 mg of calcium) on colorectal adenoma reoccurrence to yield a crude risk ratio of 0.77 (95% CI: 0.62, 0.96). Another trial of calcium and fiber supplementation's effects on CA reoccurrence yielded an odds ratio of 0.66 (95% CI: 0.38, 1.17) when treatment of 2000 mg calcium daily was compared to placebo over 3 years.(5) A combination of 1000 IU vitamin D and 1200 mg calcium (as calcium carbonate) daily yielded an adjusted risk ratio of 0.93 (95% CI: 0.80, 1.08) of CA reoccurrence when both vitamin D and calcium treatment were compared

with two placebos over 3 or 5 years. The effects of calcium and vitamin D separately compared to placebos yielded 0.95 (0.85, 1.06) and 0.99 (95% CI: 0.89, 1.09) respectively. It is worth noting that the outcome was exceedingly rare in the study.(12)

Vitamin D, Calcium and Inflammation

Vitamin D has also been shown to have an anti-inflammatory effect. Vitamin D deficiency in animal models and vitamin D receptor (VDR) gene polymorphisms in humans were shown to be associated with inflammatory bowel disease (IBD).(13,14) Vitamin D may also dampen immune response.(15) Vitamin D intake has been significantly associated with a reduction in the inflammatory biomarker interleukin (IL)-6 both via experimental studies on supplements(16) and observational studies on total intake(17) and vitamin D supplementation has been associated with an increase in the anti-inflammatory IL-10(18) but results are inconsistent.(19–23) IL-6 and C-reactive protein (CRP), a protein that is used as an indicator of acute and chronic inflammation, have notably been associated directly with CA occurrence, and CRC tumor progression.(24) Ionized calcium forms insoluble soaps in the colon which can bind to carcinogenic an inflammation-causing fatty acids and bile acids.(25,26) Contrary to this concept, however a randomized controlled trial in colorectal adenoma patients on supplementation of 1g or 2g of calcium per day over a 4-month trial period found that both treatments may have caused an increase in biomarkers of inflammation (analyzed as a summary z-score) with the average z-scores increasing by 0.26 (95% CI: -0.64,

1.17) for the 1g dosage and 0.13 (-0.77, 1.03) for the 2g dosage relative to the control. However, this study had a small sample size and this observation may be due to random error.(27)

Methods

Study Population and Data Collection

The Calcium/Vitamin D, Biomarkers & Colon Polyp Prevention Study 4 Biomarkers (PPS4B) (n=459) study is an adjunct biomarker study based on the 2813 participant 2x2 factorial double-blind randomized clinical trial, The Calcium/Vitamin D, Polyp Prevention study (NCT00153816). The study population of the adjunct study didn't differ from the all study participants by baseline characteristics. The study population were age 45-75 at baseline, with good general health, and who had a CA removed within 120 days prior to enrollment. Those with a narcotic or alcohol dependence within the past 5 years, osteoporosis, hyperparathyroidism, liver disease, past kidney stones, women who intended to become pregnant, those with a familial history of CRC and those with serum levels of 25(OH)D and/or calcium outside of normal ranges were ineligible. Study participants were randomized into four treatment groups: 600 milligrams (mg) of calcium (as calcium carbonate) and a placebo twice a day and a placebo, 500 international units (IU) of vitamin D and a placebo twice a day, both the calcium and vitamin D supplements twice a day, or two placebos. At request, women could choose to be randomized into either calcium and placebo, or calcium and vitamin D in a separate two-group randomization.

After enrollment he study period lasted until either a 3 or 5 year follow up colonoscopy. Demographic data, medical history, and data regarding medications, diet, and nutritional supplements was gathered at baseline. Dietary information was

collected through a Block Brief 2000 food frequency questionnaire (FFQ) and was combined with obtained nutritional supplement data to estimated daily nutritional intake. Participants were randomized to treatment groups based on clinical center, expected follow up (3 year or 5 year) and sex. Blood drawn at baseline and again during a 1 and 3 year (for the 5-year expected follow up group) and during the end of study colonoscopy.

Blood Collection

Peripheral venous blood samples were at baseline, one year, and at the end of study follow-up. Blood was drawn into pre-chilled vacutainer tubes for whole blood, plasma, and serum, and then immediately placed on ice and shielded from light. Blood fractions were aliquoted 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 high sensitivity, validated, multiplex, electroluminescence V-PLEX immunoassays (Meso Scale Discovery, Maryland, DC) was used to measure IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-17A, IFN-γ, TNF-α, and GM-CSF, in duplicate, according to the

manufacturer's protocol. The average intra-assay coefficient of variation (CV) for INF was 5.1%, for TNF-α 3.8%, for IL-1β 13.8%, for IL-2 9.2%, for IL-4 26.3% for IL-6 5.5%, for IL-8 3.7%, for IL-10 8.5%, for IL-12p70 14.9%, for IL-12p40 4.8%, for IL-17A 14.1%, and for GM-CSF 32.5%. Low plasma cytokine concentrations create very high variability, and the results for cytokines with CVs above 15% were considered too variable. Therefore, the main

Statistical Analysis

Baseline characteristics of participants were compared across treatment groups using χ^2 test for categorical variables and ANOVA for continuous variables. Treatment groups and comparison groups and corresponding comparison groups were, those who received calcium compared to those who did not ("calcium *versus* no calcium", excluding two-arm participants), those who received vitamin D compared with those who did not ("vitamin D *versus* no vitamin D") and those who received calcium and vitamin D compared to those who received only calcium ("vitamin D + calcium *versus* calcium"). Treatment effects on serum cytokine concentration was assessed using a generalized linear mixed model to compare treatment groups and corresponding comparison groups at baseline and follow up with age, sex (by arm), study center, number of adenomas at baseline, and time between randomization and final checkup were included in the models as covariates. When variables that had shown heterogeneity at baseline were included in the models, education status, % of regular

non-aspirin NSAID users, BMI, dietary fiber (grams per day), servings of fruits and vegetables per day, total (dietary plus any non-study-treatment supplement) vitamin D (IU/d) and baseline 25(OH)D serum concentration and total daily calcium were not found to significantly affect the association. The relative effect was calculated for each cytokine by concentrations for [(treatment group follow-up) / (treatment group baseline)] / [(placebo group follow-up) / (placebo group baseline)].

Z-scores were calculated for individual cytokines using $z=(x-\mu)/\delta$, where x equals the natural log-transformed concentration of each cytokine and μ sex-specific mean and standard deviation of the natural log-transformed concentrations of each cytokine at baseline. The scores from each cytokine (IL-6, IL-8, IL-10, TNF-α, IL-1β, IL-12p40, IFN-γ, IL-17A, IL-2, IL-12p70) were then added into an aggregate inflammatory z-score except for IL-10 which was instead subtracted due to its anti-inflammatory properties. GM-CSF and IL-4 were not included in the aggregate score due to the two biomarkers having over 20% of observations missing. A generalized linear mixed model was used to compare differences in aggregate cytokine z-score between treatment and corresponding comparison groups at baseline and follow up period (either 3 year or 5 year), age, sex (by arm), study center, number of adenomas at baseline, and time between randomization and final checkup were included in the model as covariates. Absolute effect of treatment on inflammatory z-score was assessed using the formula [(treatment group follow-up mean z-score)] – [(treatment group baseline mean z-score) / (comparison group follow-up mean z-score) - (comparison group baseline mean z-score)]. Relative effect was calculated using the formula [(exponentiated treatment

group geometric mean at follow up) / (exponentiated treatment group geometric mean at baseline)] / [(exponentiated placebo group geometric mean at follow up) / (exponentiated placebo group geometric mean at baseline)].

A secondary stratified analysis was performed using a generalized linear mixed model with to compare differences in aggregate cytokine z-score between treatment and corresponding comparison groups at baseline and follow up with data stratified by median total calcium intake (in mg/day) and at baseline to assess interaction.

Results

Participants

The study population consisted of 459 participants (Table 1). The average age of the study population was 58; just under 72% of the study population were men, over 86% of the population were white, and 57% were had a college or post-college education. The baseline serum 25(OH)D was 25.0 ng/ml (SD = 9.0). At baseline, the treatment groups in the 4-arm full factorial randomization differed by education status, % of regular non-aspirin NSAID users, BMI, dietary fiber (grams per day), servings of fruits and vegetables per day, total (dietary plus any non-study-treatment supplement) vitamin D (IU/d) and 25(OH)D serum concentration. The treatment groups for the 2-group randomization differed by physical activity, number of adenomas removed at colonoscopy, dietary fiber consumption, fruit and vegetable consumption, and total calcium.

Vitamin D, Calcium, and Inflammatory Markers

There were no statistically significant associations between supplementation of vitamin D or calcium, and changes in plasma concentrations of biomarkers of inflammation between baseline and final follow up at α=0.05 (Table 2.). When compared to no vitamin D, the vitamin D treatment increased IL-2 by 27% (*p*-value "*p*"-0.057) and decreased IL-1β 23% (*p*-0.066). Compared to no calcium supplementation, calcium supplementation decreased IL-1β by 21% (*p*-0.114) and increased IL-10 by 16%

(*p*-0.105). Calcium and vitamin D supplementation, when compared only calcium supplementation increased IL-2 by 30% (*p*-0.126).

Vitamin D, Calcium, and Inflammatory Aggregate Z-score

Table 3 shows changes in aggregate Z-score based on treatment categories over the study period. When compared to no vitamin D supplementation, vitamin D supplementation decreased the average biomarker z-score by 0.23 points (95% CI: -0.67, 1.14). When compared to no calcium supplementation, calcium supplementation decreased average z-score by 0.89 points (95% CI: -0.08, 1.86) and when compared with solely calcium supplementation, vitamin D and calcium supplementation increased the average z-score by 0.18 points (95% CI:-1.03, 1.38).

Stratified Analyses

When stratified by daily total calcium intake (≥716.4 IU/d and <716.4 IU/d) at baseline, in the group below the median, calcium supplementation, when compared to no calcium supplementation, decreased the average z-score by 0.56 (95% CI: -0.68, 1.81) and in the group with daily total calcium intake above the median, calcium supplementation, when compare to no calcium supplementation, decreased the average z-score by 1.30 (95% CI: -0.23, 2.84).

Discussion

The findings of this study suggest that vitamin D treatment, when compared to placebo may cause an increase in IL-2 and a decrease in IL-1β. Calcium when compared to placebo treatment may cause a decrease in IL-1β levels and an increase in IL-10. Vitamin D along with calcium, when compared to solely calcium, may increase IL-2. Supplemental calcium may to cause a decrease in average inflammation when compared to no supplemental calcium. When stratified by average total calcium intake per day, the group with the higher calcium intake at baseline saw a marginally, though not statistically significantly greater effect than the group with the lower baseline total calcium intake. Vitamin D supplementation, when compared to placebo and vitamin D and calcium supplementation, when compared to solely calcium supplementation, did not have a strong effect on inflammatory z-score.

This consistent with hypothesis that vitamin D and calcium, either supplemented separately or in combination, may decrease the inflammatory markers that are associated with colorectal neoplasia. The results from the z-score analysis are inconsistent with the findings of pilot study from the same population that found that vitamin D supplementation significantly decreased overall inflammation, however the sample size was small in the previous study.(20) The decrease in inflammatory z-score by calcium is counter to the effect seen in a prior study that found that 1g or 2g of calcium a day caused an increase in inflammatory z-score.(27) Other human clinical studies on the interaction between vitamin D and calcium have had inconsistent, with small sample sizes, narrow follow up periods, widely varying dosages, and/or have

investigated populations with highly specific study populations (eg. Patients with multiple sclerosis or congestive heart failure).(28–34) It's notable that no reported studies investigated these inflammation biomarkers in localized tissue, instead using blood samples. IL-1β is of specific importance, as it is intimately linked with colon cell proliferation, and thus CRC risk, through the Wnt cycle-pathway,(35) and IL-2 has been associated with increased survival time in colorectal cancer in animal models(36) and is being explored as an immunotherapeutic agent against some cancers.(37)

IL-6, an inflammatory marker that is associated with CRC survival and tumor progression(24), has been shown to be affected by calcium and vitamin D intake in previous literature,(16,17) but that effect was not observed in this study. The effect of calcium on lowering average aggregate inflammation is consistent with the hypothesized biological mechanism of calcium binding to pro-inflammatory bile acids.(25–27) As calcium has been shown to have a plateau of effect on CRC risk between 700 and 1000 mg/day,(6,7) it is counterintuitive that the group with the higher baseline calcium intake in the stratified analysis had a greater effect from further calcium supplementation. If the mechanism of action between calcium and CRC risk primary through anti-inflammatory pathways, then it would be expected that the dose response of calcium on each would be similar. It is possible that this interaction was a product of random sampling, however. More research should be conducted to further explore the relationship between vitamin D and calcium intake and inflammation as a mediating factor for CRC protection.

Strengths and Limitations

As a secondary analysis of an RCT, this study's strength was in its randomization. Analysis of the comparison groups revealed some heterogeneity, so it is a possibility of unmeasured confounding affecting the analysis. As with any study involving supplementation, it is possible that the effects of nutrient supplementation very different effects than what would be seen if vitamin D and calcium were increased via dietary changes. Plasma levels of biomarkers were used for the analysis and it is possible that these levels are meaningfully different than what is found in colorectal tissue. In addition, there is a possibility that the dose of 1000 IU vitamin D and 1200 mg calcium are insufficient to produce the full possible benefit. Finally, it is possible that the high-risk population of colorectal adenoma patients in this study would have a different reaction to the study treatment than the general population would. Finally, there were samples where serum levels of inflammatory biomarkers were below the levels of detection which could affect the results of the analysis.

Conclusions and Public Health Impact

CRC is the second leading cause of cancer mortality in the United States of America. More research needs to be conducted on the interactions between vitamin D and calcium supplementation, inflammation, and CRC, however it is possible that with further research and understanding of the mechanisms, vitamin D and calcium supplementation may prove to be a useful tool in the prevention of CRC and potentially provide insights into the effects of vitamin D and calcium on other inflammation-related diseases for future hypothesis generation.

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Table 1. Selected Baseline Characteristics of Clinical Trial Participants by Treatment Group Assigment (n=459)[a]

	Full Factorial Randomization (4-Arm)					Two-Group Randomization (2-Arm)		
Baseline Characteristics	Placebo (n=94)	Calcium (n=95)	Vitamin D (n=101)	Calcium + Vitamin D (n=106)	*P*[b]	Placebo (n=33)	Vitamin D (n=30)	*P*[c]
Demographics, medical history, habits								
Age, years	59.2 (6.7)	58.3 (7.8)	58.0 (7.2)	57.6 (6.9)	*0.078*	58.9 (5.8)	57.6(6.2)	*0.422*
Men, %	85.3	82.1	84.2	82.1	*0.913*	0.0	0.0	--
White, %[d]	83.9	82.8	87.6	93.2	*0.098*	75.8	86.7	*0.516*
College graduate[e], %	69.8	68.4	75.2	67.6	*0.025*	68.6	48.4	*0.296*
1° family history of CRC, %[f]	20.2	21.5	16.8	16.0	*0.690*	11.4	10.3	*0.864*
Regular[g] non-aspirin NSAID users, %[**]	57.3	65.3	52.5	73.2	*0.011*	71.4	67.7	*0.811*
Regular[g] aspirin users, %[h]	61.3	63.3	57.1	56.1	*0.810*	48.6	61.3	*0.248*
If woman (n=133), HRT users, %[***]	35.7	17.7	18.8	21.1	*0.570*	18.2	29.0	*0.345*
Current smoker, %	7.3	5.1	3.0	11.1	*0.149*	2.86	12.9	*0.278*
Multivitamin users, %	44.8	51.0	52.5	50.0	*0.852*	77.14	87.1	*0.411*
Physical activity, MET-min/wk[****]	2820 (2774)	3091 (3281)	2999 (3172)	3337 (2936)	*0.081*	2017 (1832)	2761 (2909)	*0.015*
BMI, kg/m^2	28.7 (4.7)	30.2 (5.2)	28.7 (4.0)	28.5 (4.4)	*<.0001*	29.1 (5.6)	27.4 (4.7)	*0.035*
Adenomas removed at colonoscopy[i]	1.45 (0.73)	1.62 (1.19)	1.58 (0.99)	1.58 (1.09)	*0.369*	1.16 (0.57)	1.59 (0.95)	*0.001*
Had advanced adenoma, %[***]	24.5	19.2	22.0	25.5	*0.723*	12.1	13.3	*0.885*

Table 1. Selected Baseline Characteristics of Clinical Trial Participants by Treatment Group Assigment (n=459)[a] Continued.

	Full Factorial Randomization (4-Arm) Continued					Two-Group Randomization (2-Arm)		
Baseline Characteristics	Placebo (n=94)	Calcium (n=95)	Vitamin D (n=101)	Calcium + Vitamin D (n=106)	*P*[b]	Placebo (n=33)	Vitamin D (n=30)	*P*[c]
Dietary Intakes								
Total energy intake, kcal/d[j]	1554 (514)	1721 (559)	1530 (565)	1521 (487)	*<.0001*	1359 (509)	1508 (562)	*0.072*
Dietary fiber, g/d[***]	14.9 (7.5)	16.4 (7.2)	13.7 (6.2)	15.4 (7.7)	*0.043*	14.2 (5.4)	16.9 (5.4)	*0.001*
Red and/or processed meat, servings/d[*]	1.14 (0.64)	1.12 (0.87)	0.98 (0.77)	1.02 (0.65)	*0.063*	0.76 (0.71)	0.73 (0.56)	*0.971*
Fruits and vegetables, servings/d[k]	6.5 (3.5)	7.6 (4.6)	7.0 (4.2)	6.3 (3.3)	*0.020*	7.4 (3.3)	9.3 (4.0)	*0.001*
Alcohol intake, drinks/d	0.92 (1.03)	0.77 (0.97)	0.79 (1.05)	0.87 (1.04)	*0.168*	0.47 (0.86)	0.41 (0.58)	*0.424*
Total vitamin D[l], IU/d[*]	161 (197)	183 (211)	206 (225)	212 (259)	*0.048*	367 (300)	420 (233)	*0.118*
Total calcium[m], mg/d[*]	90 (147)	112 (157)	116 (218)	103 (158)	*0.584*	478 (387)	593 (482)	*0.049*
Serum levels								
25-OH-vitamin D_3, ng/mL	23.5 (7.4)	23.3 (9.6)	25.2 (8.5)	26.6 (9.3)	*0.002*	27.0 (9.5)	26.6 (9.9)	*0.977*
Ca^{2+}, mg/dL	9.32 (0.33)	9.28 (0.32)	9.35 (0.36)	9.31 (0.35)	*0.322*	9.44 (0.33)	9.49 (0.34)	*0.830*

Table 1. Selected Baseline Characteristics of Clinical Trial Participants by Treatment Group Assigment (n=459)[a] Continued.

[a]Data are given as means (SD) unless otherwise specified

[b]Chi squared for categorical variables; ANOVA for continuous variables

[c]Chi squared for categorical variables; Student t tests for continuous variables

[d]Missing information for 11 patients

[e]Received a Bachelor's degree or higher

[f]Missing information for 25 patients

[g]At least four times a week

[h]Missing information for 7 patients

[i]Missing information for 3 placebo patients, 6 calcium patients, 4 vitamin D, 4 combined, one placebo (2-arm), and one vitamin D (2-arm)

[j]Missing information for 2 placebo patients, 5 calcium patients, 9 vitamin D, 5 combined, one placebo (2-arm), and 2 vitamin D (2-arm)

[k]Missing information for 4 placebo patients, 6 calcium patients, 9 vitamin D, 5 combined, one placebo (2-arm), and 2 vitamin D (2-arm)

[l]Dietary vitamin D plus supplemental vitamin D. Missing information for 3 placebo patients, 2 calcium, 2 vitamin D, one combined, 6 placebo (2-arm) and 5 vitamin D (2-arm)

[m]Dietary calcium plus supplemental calcium. Missing information for 2 placebo patients, one calcium, one vitamin D, one combined, 6 placebo (2-arm), and one vitamin D (2-arm)

[*]One patient missing per asterisk

Table 2. Changes in Plasma Concentrations of Biomarkers of Inflammation Between Baseline and Final Follow Up According to Categories of Treatment Group

	Baseline				Final follow-up				Treatment effect			
Biomarker	*n*	Mean[a]	95% CI	*p* [b]	*n*	Mean[a]	95% CI	*p* [b]	Relative Rx effect[c]	95% CI	*p* [d]	Absolute Rx effect[e]
IL-12p40, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	146.47	(133.71, 160.44)		221	146.88	(134.09, 160.90)					
Vitamin D	237	144.75	(132.07, 158.67)	0.786	234	146.54	(133.65, 160.66)	0.957	1.01	(0.90, 1.14)	0.877	1.38
Calcium vs. no calcium												
No calcium	195	147.36	(133.53, 162.63)		192	153.42	(138.99, 169.36)					
Calcium	201	142.51	(128.87, 157.59)	0.475	200	138.13	(124.90, 152.77)	0.026	0.93	(0.82, 1.06)	0.275	-10.44
Calcium + vitamin D vs. calcium alone												
Calcium	128	146.51	(130.19, 164.88)		128	140.99	(125.29, 158.67)					
Calcium + vitamin D	136	139.18	(123.41, 156.96)	0.356	135	139.77	(123.90, 157.67)	0.876	1.04	(0.90, 1.21)	0.579	6.11
IL-6, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.53	(0.46, 0.60)		221	0.57	(0.50, 0.64)					
Vitamin D	237	0.54	(0.48, 0.61)	0.617	234	0.59	(0.52, 0.67)	0.471	1.01	(0.86, 1.19)	0.873	0.01
Calcium vs. no calcium												
No calcium	195	0.54	(0.48, 0.62)		192	0.59	(0.52, 0.68)					
Calcium	201	0.55	(0.48, 0.64)	0.811	200	0.60	(0.52, 0.68)	0.889	0.99	(0.83, 1.19)	0.944	-0.003
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.53	(0.45, 0.62)		128	0.56	(0.48, 0.66)					
Calcium + vitamin D	136	0.53	(0.45, 0.63)	0.905	135	0.60	(0.51, 0.71)	0.438	1.05	(0.85, 1.29)	0.634	0.03

Table 2. Changes in Plasma Concentrations of Biomarkers of Inflammation Between Baseline and Final Follow Up According to Categories of Treatment Group Contiuned

	Baseline				Final follow-up				Treatment effect			
Biomarker	*n*	Mean[a]	95% CI	*p* [b]	*n*	Mean[a]	95% CI	*p* [b]	Relative Rx effect[c]	95% CI	*p* [d]	Absolute Rx effect[e]
IL-8, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	4.33	(3.96, 4.73)		221	4.76	(4.36, 5.21)					
Vitamin D	237	4.23	(3.87, 4.62)	0.583	234	4.55	(4.16, 4.98)	0.275	0.98	(0.87, 1.10)	0.696	-0.12
Calcium vs. no calcium												
No calcium	195	4.14	(3.76, 4.57)		192	4.64	(4.21, 5.12)					
Calcium	201	4.08	(3.69, 4.51)	0.746	200	4.37	(3.96, 4.83)	0.199	0.96	(0.84, 1.09)	0.490	-0.21
Calcium + vitamin D vs. calcium alone												
Calcium	128	4.28	(3.82, 4.80)		128	4.50	(4.01, 5.05)					
Calcium + vitamin D	136	4.43	(3.94, 4.98)	0.533	135	4.79	(4.26, 5.38)	0.250	1.03	(0.89, 1.19)	0.700	0.14
TNF-α, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	1.71	(1.60, 1.83)		221	1.74	(1.63, 1.86)					
Vitamin D	237	1.68	(1.57, 1.80)	0.653	234	1.72	(1.61, 1.84)	0.760	1.00	(0.92, 1.10)	0.918	0.01
Calcium vs. no calcium												
No calcium	195	1.70	(1.58, 1.82)		192	1.77	(1.65, 1.90)					
Calcium	201	1.63	(1.52, 1.76)	0.275	200	1.62	(1.51, 1.75)	0.010	0.95	(0.87, 1.04)	0.286	-0.09
Calcium + vitamin D vs. calcium alone												
Calcium	128	1.80	(1.65, 1.98)		128	1.79	(1.63, 1.96)					
Calcium + vitamin D	136	1.73	(1.58, 1.90)	0.342	135	1.76	(1.61, 1.94)	0.742	1.03	(0.91, 1.16)	0.653	0.05

Table 2. Changes in Plasma Concentrations of Biomarkers of Inflammation Between Baseline and Final Follow Up According to Categories of Treatment Group Continued

	Baseline				Final follow-up				Treatment effect			
Biomarker	*n*	Mean[a]	95% CI	*p* [b]	*n*	Mean[a]	95% CI	*p* [b]	Relative Rx effect[c]	95% CI	*p* [d]	Absolute Rx effect[e]
IFN-γ, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	4.58	(3.98, 5.26)		221	4.22	(3.67, 4.85)					
Vitamin D	237	4.38	(3.80, 5.03)	0.492	234	4.23	(3.67, 4.87)	0.973	1.05	(0.88, 1.26)	0.607	0.21
Calcium vs. no calcium												
No calcium	195	4.35	(3.77, 5.02)		192	4.30	(3.73, 4.96)					
Calcium	201	4.10	(3.55, 4.74)	0.385	200	3.74	(3.24, 4.33)	0.042	0.92	(0.77, 1.11)	0.401	-0.31
Calcium + vitamin D vs. calcium alone												
Calcium	128	4.43	(3.63, 5.40)		128	3.99	(3.27, 4.86)					
Calcium + vitamin D	136	4.17	(3.41, 5.10)	0.515	135	3.85	(3.15, 4.72)	0.711	1.03	(0.80, 1.32)	0.840	0.12
IL-10,[e] pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.22	(1.91, 0.25)		221	0.21	(0.18, 0.24)					
Vitamin D	237	0.19	(0.16, 0.22)	0.021	234	0.21	(0.18, 0.24)	0.963	1.16	(0.97, 1.38)	0.105	0.03
Calcium vs. no calcium												
No calcium	195	0.21	(0.19, 0.25)		192	0.23	(0.20, 0.26)					
Calcium	201	0.19	(0.17, 0.22)	0.085	200	0.20	(0.17, 0.23)	0.054	0.99	(0.82, 1.19)	0.880	-0.004
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.21	(0.17, 0.25)		128	0.19	(0.16, 0.23)					
Calcium + vitamin D	136	0.17	(0.14, 0.21)	0.043	135	0.18	(0.15, 0.22)	0.648	1.15	(0.91, 1.45)	0.254	0.03

Table 2. Changes in Plasma Concentrations of Biomarkers of Inflammation Between Baseline and Final Follow Up According to Categories of Treatment Group Continued

	Baseline				Final follow-up				Treatment effect			
Biomarker	*n*	Mean[a]	95% CI	*p* [b]	*n*	Mean[a]	95% CI	*p* [b]	Relative Rx effect[c]	95% CI	*p* [d]	Absolute Rx effect[e]
GM-CSF, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.08	(0.07, 0.09)		221	0.08	(0.07, 0.10)					
Vitamin D	237	0.08	(0.07, 0.10)	0.778	234	0.08	(0.07, 0.10)	0.940	0.97	(0.78, 1.22)	0.798	-0.002
Calcium vs. no calcium							()					
No calcium	195	0.08	(0.07, 0.10)		192	0.08	(0.07, 0.10)					
Calcium	201	0.09	(0.07, 0.11)	0.618	200	0.09	(0.08, 0.11)	0.338	1.04	(0.82, 1.32)	0.740	0.004
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.09	(0.07, 0.11)		128	0.09	(0.07, 0.11)					
Calcium + vitamin D	136	0.08	(0.06, 0.10)	0.553	135	0.08	(0.07, 0.11)	0.872	1.05	(0.78, 1.41)	0.755	0.004
IL-17A, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.78	(0.66, 0.93)		221	0.84	(0.71, 0.99)					
Vitamin D	237	0.72	(0.61, 0.85)	0.275	234	0.76	(0.64, 0.90)	0.218	0.99	(0.79, 1.23)	0.917	-0.01
Calcium vs. no calcium												
No calcium	195	0.72	(0.61, 0.87)		192	0.83	(0.69, 0.99)					
Calcium	201	0.81	(0.67, 0.97)	0.194	200	0.82	(0.68, 0.98)	0.882	0.89	(0.70, 1.12)	0.301	-0.09
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.79	(0.64, 0.98)		128	0.83	(0.67, 1.03)					
Calcium + vitamin D	136	0.81	(0.65, 1.01)	0.822	135	0.78	(0.63, 0.98)	0.553	0.92	(0.70, 1.21)	0.554	-0.07

Table 2. Changes in Plasma Concentrations of Biomarkers of Inflammation Between Baseline and Final Follow Up According to Categories of Treatment Group Continued

	Baseline				Final follow-up				Treatment effect			
Biomarker	*n*	Mean[a]	95% CI	*p* [b]	*n*	Mean[a]	95% CI	*p* [b]	Relative Rx effect[c]	95% CI	*p* [d]	Absolute Rx effect[e]
IL-2, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.09	(0.08, 0.11)		221	0.09	(0.07, 0.11)					
Vitamin D	237	0.09	(0.08, 0.11)	0.898	234	0.12	(0.10, 0.14)	0.006	1.27	(0.99, 1.63)	0.057	0.02
Calcium vs. no calcium												
No calcium	195	0.08	(0.07, 0.10)		192	0.10	(0.08, 0.12)					
Calcium	201	0.09	(0.08, 0.11)	0.406	200	0.10	(0.09, 0.13)	0.535	0.98	(0.76, 1.27)	0.882	-0.001
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.10	(0.08, 0.14)		128	0.10	(0.08, 0.13)					
Calcium + vitamin D	136	0.11	(0.08, 0.14)	0.795	135	0.13	(0.10, 0.17)	0.018	1.30	(0.93, 1.81)	0.126	0.03
IL-4, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.011	(0.009, 0.013)		221	0.010	(0.009, 0.013)					
Vitamin D	237	0.010	(0.008, 0.012)	0.705	234	0.011	(0.009, 0.013)	0.745	1.07	(0.83, 1.37)	0.614	0.001
Calcium vs. no calcium												
No calcium	195	0.011	(0.009, 0.013)		192	0.011	(0.009, 0.013)					
Calcium	201	0.011	(0.009, 0.013)	0.721	200	0.011	(0.009, 0.014)	0.618	1.01	(0.78, 1.33)	0.918	0.0002
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.013	(0.010, 0.016)		128	0.012	(0.010, 0.016)					
Calcium + vitamin D	136	0.011	(0.008, 0.014)	0.156	135	0.012	(0.009, 0.015)	0.726	1.13	(0.83, 1.55)	0.439	0.001

Table 2. Changes in Plasma Concentrations of Biomarkers of Inflammation Between Baseline and Final Follow Up According to Categories of Treatment Group Continued

	Baseline				Final follow-up				Treatment effect			
Biomarker	*n*	Mean[a]	95% CI	*p* [b]	*n*	Mean[a]	95% CI	*p* [b]	Relative Rx effect[c]	95% CI	*p* [d]	Absolute Rx effect[e]
IL-12p70, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.05	(0.04, 0.07)		221	0.06	(0.05, 0.07)					
Vitamin D	237	0.06	(0.05, 0.08)	0.144	234	0.06	(0.05, 0.07)	0.710	0.90	(0.70, 1.17)	0.438	-0.01
Calcium vs. no calcium												
No calcium	195	0.06	(0.05, 0.07)		192	0.06	(0.05, 0.08)					
Calcium	201	0.06	(0.05, 0.07)	0.778	200	0.05	(0.04, 0.07)	0.147	0.89	(0.67, 1.18)	0.401	-0.01
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.05	(0.04, 0.07)		128	0.05	(0.04, 0.07)					
Calcium + vitamin D	136	0.06	(0.04, 0.07)	0.640	135	0.05	(0.04, 0.07)	0.830	0.97	(0.70, 1.34)	0.855	-0.002
IL-1β, pg/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.03	(0.02, 0.03)		221	0.03	(0.02, 0.03)					
Vitamin D	237	0.03	(0.02, 0.04)	0.388	234	0.02	(0.02, 0.03)	0.089	0.77	(0.59, 1.02)	0.066	-0.01
Calcium vs. no calcium												
No calcium	195	0.02	(0.02, 0.03)		192	0.02	(0.02, 0.03)					
Calcium	201	0.03	(0.02, 0.04)	0.093	200	0.02	(0.02, 0.03)	0.595	0.79	(0.59, 1.06)	0.114	-0.01
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.03	(0.02, 0.04)		128	0.03	(0.02, 0.03)					
Calcium + vitamin D	136	0.03	(0.03, 0.05)	0.244	135	0.03	(0.02, 0.04)	0.630	0.92	(0.66, 1.29)	0.621	-0.003

Table 2. Changes in Plasma Concentrations of Biomarkers of Inflammation Between Baseline and Final Follow Up According to Categories of Treatment Group Continued

	Baseline				Final follow-up				Treatment effect			
Biomarker	*n*	Mean[a]	95% CI	*p* [b]	*n*	Mean[a]	95% CI	*p* [b]	Relative Rx effect[c]	95% CI	*p* [d]	Absolute Rx effect[e]
Serum 25(OH)D, ng/mL												
Vitamin D vs. no vitamin D												
No Vitamin D	222	22.34	(20.93, 23.84)		221	22.20	(20.80, 23.70)					
Vitamin D	237	24.29	(22.75, 25.94)	0.007	234	31.82	(29.79, 33.98)	< 0.001	1.32	(1.21, 1.44)	< 0.001	7.66
Calcium vs. no calcium												
No calcium	195	21.99	(20.42, 23.68)		192	25.00	(23.21, 26.92)					
Calcium	201	22.49	(20.85, 24.25)	0.525	200	25.99	(24.10, 28.03)	0.272	1.02	(0.92, 1.12)	0.738	0.49
Calcium + vitamin D vs. calcium alone												
Calcium	128	22.85	(20.87, 25.01)		128	22.79	(20.82, 24.95)					
Calcium + vitamin D	136	25.35	(23.12, 27.79)	0.015	135	33.45	(30.51, 36.69)	< 0.001	1.32	(1.18, 1.49)	< 0.001	8.16

[a]Geometric means with 95% confidence intervals are reported, calculated by exponentiating the mean of the log-transformed values.

[b]P values for difference between each treatment group and reference group from mixed model.

[c] Relative Rx effect = [(treatment group follow-up) / (treatment group baseline)] / [(placebo group follow-up) / (placebo group baseline)].

[d] P values for difference between follow-up visit and baseline visit from mixed model.

[e] Absolute Rx effect = [(treatment group follow-up) - (treatment group baseline] - [(placebo group follow-up) - (placebo group baseline)].

Table 3. Changes in Plasma Cytokine Summary Z-score[a] Among Colorectal Adenoma Patients in Response to Vitamin D and Calcium Supplementation According to Categories of Treatment Group

	Baseline				Final follow-up				Treatment effect			
Cytokine Z Score	*n*	Mean[b]	95% CI	*p*[c]	*n*	Mean[b]	95% CI	*p*[c]	Absolute effect[d]	95% CI	*p*	Relative effect[e]
Vitamin D vs. no vitamin D												
No Vitamin D	222	0.13	(-0.57, 0.82)		221	0.52	(-0.18,1.22)					
Vitamin D	237	0.30	(-0.40, 1.00)	0.606	234	0.58	(-0.11, 1.27)	0.852	-0.23	(-1.14, 0.67)	0.616	0.90
Calcium vs. no calcium												
No calcium	195	0.05	(-0.69, 0.79)		192	0.83	(0.09, 1.58)					
Calcium	201	0.24	(-0.52, 1.00)	0.592	200	0.13	(-0.63, 0.89)	0.048	-0.89	(-1.86, 0.08)	0.071	0.41
Calcium + vitamin D vs. calcium alone												
Calcium	128	0.55	(-0.39, 1.49)		128	0.46	(-0.48, 1.40)					
Calcium + vitamin D	136	0.79	(-0.79, 1.75)	0.589	135	0.87	(-0.09, 1.84)	0.349	0.18	(-1.03, 1.38)	0.773	1.19

[a]Calculated as the sum of the z-values for each cytokine [$z=(x-\mu)/\delta$, where x is the natural log-transformed values for each individual marker, and μ and δ are the sex-specific mean and standard deviation of the natural log-transformed biomarker value, at baseline]. Includes the following cytokines: IL-6, IL-8, IL-10, TNF-α, IL-1β, IL-12p40, IFN-γ, IL-17A, IL-2, IL-12p70, where IL-10 is included with a negative sign due to its anti-inflammatory properties.

[b]Arithmetic means

[c]P values for difference between each treatment group and reference group from mixed model.

[d]Absolute treatment effect = (treatment group arithmetic mean at follow up - treatment group arithmetic mean at baseline) - (placebo group arithmetic mean at follow-up - placebo group arithmetic mean at baseline); mean, 95% CI, and p-vlaue obtained from mixed linear model

[e]Relative treatment effect = [(exponentiated treatment group geometric mean at follow up) / (exponentiated treatment group geometric mean at baseline)] / [(exponentiated placebo group geometric mean at follow up) / (exponentiated placebo group geometric mean at baseline)]

Table 4. Changes in Plasma Cytokine Summary Z-score[a] Among Colorectal Adenoma Patients in Response to Vitamin D and Calcium Supplementation Stratified by Levels of Baseline Daily Total Calcium Intake[b]

	Baseline				Final follow-up				Treatment effect			
	n	Mean[c]	95% CI	*P*[d]	*n*	Mean[c]	95% CI	*P*[d]	Absolute effect[e]	95% CI	*P*	Relative Effect[f]
Calcium mg/d												
<716.4												
Vitamin D vs. no vitamin D												
No Vitamin D	122	-0.27	(-1.11, 0.57)		122	0.06	(-0.78, 0.91)					
Vitamin D	135	-0.02	(-0.88, 0.83)	0.5154	101	0.26	(-0.60, 1.12)	0.61	-0.05	(-1.07, 0.97)	0.920	0.95
Calcium vs. no calcium												
No calcium	121	-0.12	(-0.94, 0.69)		121	0.65	(-0.16, 1.46)					
Calcium	115	0.03	(-0.81, 0.87)	0.685	115	-0.06	(-0.90, 0.79)	0.071	-0.86	(-1.91, 0.19)	0.109	0.42
Calcium + vitamin D vs. calcium alone												
Calcium	73	-0.03	(-1.30, 123)		73	-0.35	(-1.62, 0.92)					
Calcium + vitamin D	63	0.21	(-1.08, 1.49)	0.653	63	0.39	(-0.90, 1.68)	0.172	0.50	(-0.93, 1.92)	0.492	1.65
≥716.4												
Vitamin D vs. no vitamin D												
No Vitamin D	100	0.65	(-0.87, 2.17)		99	1.57	(0.05, 3.10)					
Vitamin D	102	0.53	(-1.06. 2.13)	0.883	99	0.63	(-0.98, 2.24)	0.229	-0.83	(-2.70, 1.04)	0.380	0.44
Calcium vs. no calcium												
No calcium	74	0.86	(-1.17, 2.90)		71	1.87	(-1.17, 2.90)					
Calcium	86	1.23	(-0.68, 3.14)	0.731	85	1.02	(-0.89, 2.93)	0.429	-1.22	(-3.60, 1.16)	0.311	0.30
Calcium + vitamin D vs. calcium alone												
Calcium	65	0.73	(-0.94, 2.40)		65	1.30	(-0.37, 2.98)					
Calcium + vitamin D	63	0.70	(-1.16, 2.56)	0.973	63	0.56	(-1.29, 2.42)	0.433	-0.71	(-2.90, 1.49)	0.523	0.49

[a] Calculated as the sum of the z-values for each cytokine [$z=(x-\mu)/\delta$, where x is the natural log-transformed values for each individual marker, and μ and δ are the sex-specific mean and standard deviation of the natural log-transformed biomarker value, at baseline]. Includes the following cytokines: IL-6, IL-8, IL-10, TNF-α, IL-1β, IL-12p40, IFN-γ, IL-17A, IL-2, IL-12p70, where IL-10 is included with a negative sign due to its anti-inflammatory properties.

[b] Daily Total Calcium Intake = Daily Dietary Calcium Intake + Calcium from non-study-perscribed suppements in International units per day (mg/d)

[c] Arithmetic means

[d] P values for difference between each treatment group and reference group from mixed model.

[e] Absolute Treatment (Rx) effect = (treatment group arithmetic mean at follow up - treatment group arithmetic mean at baseline) - (placebo group arithmetic mean at follow-up - placebo group arithmetic mean at baseline); mean, 95% CI, and p-vlaue obtained from mixed linear model

[f] Relative treatment effect = [(exponentiated treatment group arithmatic mean at follow up) / (exponentiated treatment group arithmatic mean at baseline)] / [(exponentiated placebo group arithmatic mean at follow up) / (exponentiated placebo group arithmatic mean at baseline)]