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Research Article

Cancer Prevention Research

Effects of Calcium Supplementation on Biomarkers of Inflammation and Oxidative Stress in Colorectal Adenoma Patients: A Randomized Controlled Trial

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Abstract

Inflammation and oxidative stress play important roles in colorectal carcinogenesis. There is strong evidence that calcium reduces risk for colorectal neoplasms, possibly through its ability to bind bile acids and prevent their colonic toxicity (which occurs via an oxidative mechanism and results in an inflammatory response). In a previously reported pilot, randomized, controlled trial among sporadic colorectal adenoma patients we found that those on 2.0 g/day of calcium, relative to those on placebo, had an estimated drop in a combined cytokine *z*-score of 48% ($P = 0.18$) over 6 months. To follow-up these promising preliminary findings, we tested the efficacy of two doses of supplemental calcium (1.0 or 2.0 g/day) relative to placebo on modulating circulating biomarkers of inflammation [C-reactive protein (CRP) and 10 cytokines] and oxidative stress (F_2-isoprostanes) over a 4-month treatment period among 193 patients with previous sporadic, colorectal adenoma in a randomized, double-blinded, placebo-controlled clinical trial. The inflammation markers were measured in plasma using electrochemiluminescence detection-based immunoassays, and F_2-isoprostanes were measured in plasma using gas chromatography–mass spectrometry. Over a 4-month treatment period, we found no appreciable effects of calcium on CRP, cytokines, or F_2-isoprostanes ($P > 0.4$), overall or within strata of several major risk factors for colorectal carcinogenesis, such as body mass index and regular use of nonsteroidal anti-inflammatory drugs. Overall, our results provide no evidence that calcium supplementation favorably modulates concentrations of circulating biomarkers of inflammation or oxidative stress over 4 months among patients with a previous colorectal adenoma. *Cancer Prev Res; 8(11); 1069–75.* ©*2015 AACR.*

Introduction

Colorectal cancer, a disease highly correlated with Western lifestyles (1, 2), is the second leading cause of cancer deaths in the United States (3). Calcium is a plausible and evidentially well-supported dietary chemopreventive agent against colorectal neoplasms (4). A recent meta-analysis of 15 prospective observational studies reported that every 300 mg/day increase of total calcium intake was associated with a statistically significant 8% lower risk of colorectal cancer (5). In addition, a major randomized controlled trial found statistically significantly reduced recurrence of colorectal adenoma (a well-accepted precursor of colorectal cancer) with calcium supplementation (6).

Multiple mechanisms have been proposed for calcium's chemopreventive properties against colorectal carcinogenesis (7). The earliest and probably the most prominent hypothesis was that calcium can bind bile and fatty acids in the colon lumen by forming insoluble soaps and thus prevent their colonic toxicity, which occurs via an oxidative mechanism and results in an inflammatory response and increased proliferation (8–10). It is well accepted that inflammation is causally linked to colorectal carcinogenesis, and reducing inflammation reduces risk for colorectal neoplasms (11–15). Evidence that oxidative stress (which is intimately linked with inflammation; ref. 16) is modifiable and associated with risk for colorectal neoplasms is growing (17–20). We hypothesized that calcium may reduce oxidative damage and inflammation in the colon, which could be reflected in the circulation and unlikely be due to systemic actions of calcium, because circulating levels of calcium are maintained in a very narrow range. Our group previously conducted a pilot clinical trial among colorectal adenoma patients and found that calcium supplementation over 6 months reduced plasma levels of several proinflammatory biomarkers (individually and combined as an inflammation *z*-score; ref. 21), and colon tissue 8-hydroxy-2′-deoxyguanosine (8-OH-dG) as a biomarker of oxidative DNA damage (22). Other than our pilot study, only a few clinical trials previously reported on effects of calcium or dairy (a rich source of calcium) on inflammation or oxidative stress markers among humans (23–28), but some of these studies had relatively small sample sizes (23–26), or restricted the study population to generally healthy adults (26–28) for whom the levels of inflammation and oxidative stress markers may be relatively low and perhaps not amenable to subsequent change.

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To address these gaps in the literature, we tested the effects of two doses of calcium supplementation on panels of circulating biomarkers of inflammation [C-reactive protein (CRP), tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, IL-17, vascular endothelial growth factor (VEGF), and interferon γ (IFN-γ)] and oxidative stress (F_2-isoprostanes) in a randomized, clinical trial among 193 patients with previous sporadic colorectal adenomas. The biomarkers in the inflammation panel were chosen to represent different aspects of the inflammatory response/immunomodulation in order to provide a more complete summary of the overall effect of calcium on inflammation. Categories of markers represented included mediators of natural and adaptive immunity (e.g., TNF-α and IL-4, respectively); inflammation promotion and inhibition (e.g., IL-6 and IL-10, respectively); cytokines originating from different cell sources, such as T, B, NK, Th1, and Th2 cells, macrophages, fibroblasts, epithelial cells, and others; cytokines with different cell targets; and cytokines with different primary effects.

Materials and Methods

This study was an adjunct investigation using data and blood samples from a chemoprevention trial conducted from 1990 to 1994 in the Minneapolis, Minnesota, metropolitan area (29). The parent trial was approved by the Committee on Use of Human Subjects in Research of the University of Minnesota. Each study participant provided written informed consent.

Participant population

Details on the eligibility criteria and recruitment protocol of the parent trial were described previously (29). Briefly, adults ages 30 to 74 years and in general good health were eligible if they had a history of pathology-confirmed adenomatous polyps within the previous 5 years. Subjects were recruited from the patient population of a major private-practice gastroenterology group in Minneapolis-St. Paul, Minnesota. Subjects were excluded if any of the following criteria were met: having contraindications to calcium supplementation or rectal biopsies; having clinical conditions, dietary habits, or medication that would otherwise affect safety, adherence, or interpretation of the study results; or failure to take >80% of their pills in a 4-week placebo run-in trial.

Clinical trial protocol

As previously described (29), individuals who passed the initial eligibility screening were invited for an eligibility visit, during which they were interviewed and their medical/pathology records were reviewed. Those eligible then entered a 4-week placebo run-in trial. Only individuals without substantial perceived side effects and who had taken >80% of their pills in the run-in trial were ultimately considered eligible ($n = 193$). Eligible participants then underwent a baseline visit and were randomly assigned (stratified by sex) to one of three groups: a placebo control group ($n = 66$) and 1.0 g ($n = 64$) and 2.0 g ($n = 63$) elemental calcium supplementation groups. Randomization was blinded to all participants and all study personnel and laboratory staff. The calcium carbonate tablets (prepared by SmithKline Beecham, Pittsburgh, PA) were taken twice daily with meals. The placebo pills contained no calcium, magnesium, vitamin D, or chelating agents; they were otherwise identical to calcium tablets in size, appearance, and taste.

At the baseline visit, we collected information on demographic and lifestyle factors as well as medical history and medication use for each participant via a self-administered questionnaire, and additionally collected dietary data using a Willett semiquantitative food-frequency questionnaire. The treatment period for the parent trial was 6 months, and participants were instructed to maintain their usual diets during the study. After random assignment, all participants attended follow-up visits at 1, 2, 4, and 6 months. Pill-taking adherence was evaluated at each follow-up visit by questionnaire, interview, and pill count. Blood samples were collected only at the baseline and 4-month follow-up visits. Participants sat comfortably in a chair for 5 minutes with both of their feet on the floor before venipuncture. Blood was drawn into prechilled vacutainer tubes, and immediately placed on ice and shielded from light. Tubes were immediately processed, plasma and serum were aliquotted into separate cryopreservation tubes, the air was displaced with nitrogen, and the aliquots were immediately taken to the laboratory for storage in a −80°C freezer.

Laboratory protocol

Concentrations of inflammation biomarkers were measured at the Emory Multiplexed Immunoassay Core (EMIC) from July to November 2013 using electrochemiluminescence detection-based immunoassays based on the Meso-Scale Discovery Sector 2400 instrument. For each assay, the baseline and follow-up samples from each given study participant were included in the same analysis batch. We conducted an individual assay for CRP, and a 10-plex assay for IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, IL-17, TNF-α, and VEGF. All biomarkers were measured in duplicate, according to the manufacturer's protocol, and technicians were blinded to treatment assignment. The average intra-assay coefficient of variation (CV) for CRP was 4.59%, for IFN-γ 16.71%, for IL-10 5.66%, for IL-12p40 6.89%, for IL-17 21.26%, for IL-1β 13.01%, for IL-4 17.61%, for IL-6 6.99%, for IL-8 3.48%, for TNF-α 4.29%, and for VEGF 4.49%. The results for biomarkers with CVs ≥15% (IFN-γ, IL-17, and IL-4) were excluded from further analyses because they were considered insufficiently reliable.

F_2-isoprostanes were measured at the University of Minnesota Molecular Epidemiology Biomarker Research Laboratory by gas chromatography–mass spectrometry (GC-MS) using an Agilent 6890 Series GC and an Agilent 5973N Mass Selective Detector. For quality control, we included two control samples, measured in duplicate, for each batch; the average intra-assay CV was 11.5% and 12.5%, respectively, for these two control samples.

Statistical analysis

Treatment groups were assessed for comparability of baseline characteristics using analysis of covariance (ANCOVA) for continuous variables, and the χ^2 or Fisher exact test for categorical variables, adjusting for sex as appropriate. Among the 193 participants, blood samples were available for measuring inflammation biomarkers on 190 participants and for F_2-isoprostanes on 188 participants at baseline; blood samples were available for all biomarkers at follow-up among 176 participants. For one biomarker (IL-1β), the biomarker levels for 4% ($n = 13$) of the samples were below the detection limit, and were assigned a value equal to half of the detection limit. Measurements of duplicate samples were averaged before statistical analysis.

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Primary analysis was based on the original treatment group assignment at randomization regardless of adherence (intent-to-treat). Because the biomarker values were not normally distributed, they were log-transformed before statistical testing. Treatment effects on the biomarkers from baseline to 4 months follow-up for the 1 and 2 g/day calcium groups relative to the placebo group were estimated using a mixed linear models procedure for repeated measures data as implemented in SAS Institute's Mixed Procedure (SAS version 9.4; SAS Institute, Cary, NC). Predictors in the model included visit, treatment groups, and a treatment by visit interaction term. Because it was necessary to apply natural log transformation to the biomarker values, the main effect for each individual biomarker was estimated on a multiplicative scale based on geometric means. Accordingly, a relative effect, defined as [(treatment group follow-up mean)/(treatment group baseline mean)]/[(placebo follow-up mean)/(placebo baseline mean)], was obtained from the Mixed model. Its interpretation is somewhat analogous to that of an odds ratio (e.g., a relative effect of 1.10 means that the proportional change in the treatment group is 10% higher than that in the placebo group). In addition, we also manually calculated an absolute treatment effect defined as [(treatment group follow-up mean) − (treatment group baseline mean)] − [(placebo follow-up mean) − (placebo baseline mean)], directly using the geometric means for each group at baseline and follow-up.

We considered that no single marker of inflammation could represent all of the complex aspects of inflammation/immunomodulation, and thus calculated a cytokine summary *z*-score. Briefly, an individual *z*-score was calculated for each cytokine as $z = (x - \mu)/\delta$, where x is the natural log-transformed values for an individual marker at a given visit, and μ and δ are the sex-specific mean and standard deviation of the log-transformed biomarker value at baseline, respectively. Each individual *z*-score at baseline fits a standard normal distribution with a mean of 0 and a standard deviation of 1. Then, a combined *z*-score was calculated by summing the individual *z*-scores (we included the *z*-score for IL-10 with a negative sign considering its anti-inflammatory properties; ref. 30). Because the *z*-score was normally distributed, it was not log-transformed in the modeling process, and its main effect was estimated using the mixed model on an additive scale as an absolute treatment effect (defined above), based on arithmetic means.

We also conducted cross-sectional analyses of the baseline data using analysis of covariance (ANCOVA), to investigate whether baseline levels of CRP, the cytokine summary *z*-score, or F_2-isoprostanes were associated with sex, body measurements, smoking status, regular use of nonsteroidal anti-inflammatory drugs (NSAID), and a comprehensive oxidative balance score (OBS, which reflects combined contributions of anti-oxidant and pro-oxidant diet and lifestyle exposures, with a higher score indicating lower oxidative stress; refs. 31, 32), with adjustment for sex and BMI as appropriate.

Results

The mean age of the study subjects was 59 years, 63% were men, 99% were White, and 28% had a family history of colorectal cancer in a first-degree relative. The treatment groups were balanced on major demographic, diet, and lifestyle factors at baseline (Table 1).

Adherence to visit attendance averaged 95.3%, and did not differ among the three groups. In each group, the mean percentage of pills taken was 97%, and >98% of all participants took >80% of their pills. Table 2 shows the geometric mean concentrations of each biomarker at baseline and follow-up, as well as the estimated relative and absolute calcium supplementation treatment effects.

Table 1. Selected baseline characteristics of the study participants ($n = 193$)[a]

	Treatment group			
Characteristics	**Placebo ($n = 66$)**	**Calcium 1 g ($n = 64$)**	**Calcium 2 g ($n = 63$)**	***P*[b]**
Age, y	60 (9)	60 (9)	58 (10)	0.37
Men, %	64	63	62	0.98
White, %	98	100	100	> 0.99
College graduate, %	35	19	33	0.08
Employed, %	52	45	56	0.48
Family history, %	26	25	30	0.78
Take aspirin[c], %	21	27	16	0.34
Take non-aspirin NSAID[c], %	9	11	10	0.92
Currently smoke, %	20	16	24	0.53
Alcohol intake, g/d	11 (19)	13 (20)	8 (13)	0.20
Body mass index, kg/m^2				
Men	28.0 (3.8)	29.0 (3.1)	28.8 (4.5)	0.47
Women	30.1 (5.2)	28.1 (8.4)	26.3 (4.4)	0.12
Vigorous/moderate physical activity, MET-hours/day	33 (21)	30 (22)	28 (21)	0.47
Dietary intakes				
Total energy, kcal/day	2,097 (753)	2,000 (627)	2,102 (633)	0.63
Total fat, g/day	64 (27)	62 (24)	70 (24)	0.19
Dietary fiber, g/day	24 (10)	22 (7)	22 (9)	0.33
Total vitamin D, IU/day	345 (251)	294 (268)	314 (207)	0.48
Total calcium, mg/day	884 (339)	787 (364)	855 (416)	0.33
Phosphorous, mg/day	1,359 (435)	1,248 (441)	1,327 (418)	0.34
Omega-3 fatty acids, g/day	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.41
Take any vitamin supplement(s), %	38	38	33	0.82

Abbreviation: MET, metabolic equivalents of task.

[a]Unless otherwise specified, values presented are mean (standard deviation).

[b]*P* values calculated from analysis of covariance for continuous variables, and χ^2 or Fisher's exact test for categorical variables. Sex was included as a covariate when appropriate.

[c]Regularly take once or more a week.

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Table 2. Changes in plasma concentrations of biomarkers of inflammation and oxidative stress among colorectal adenoma patients in response to calcium supplementation

	Baseline		Four-month follow-up		Relative treatment effect[b]		
Biomarker	***n***	**Mean[a] (95% CI)**	***n***	**Mean[a] (95% CI)**	**Mean (95% CI)**	***P***	**Absolute effect[c]**
Inflammation							
CRP, μg/mL							
Placebo	65	1.62 (1.20–2.18)	60	1.68 (1.24–2.28)	—	—	—
1 g calcium	62	2.81 (2.08–3.82)	58	2.65 (1.94–3.61)	0.91 (0.66–1.25)	0.55	−0.22
2 g calcium	63	1.66 (1.23–2.26)	58	1.96 (1.44–2.68)	1.14 (0.83–1.56)	0.43	0.24
IL-6, pg/mL							
Placebo	65	2.05 (1.77–2.38)	60	2.10 (1.80–2.44)	—	—	—
1 g calcium	62	2.75 (2.36–3.20)	58	2.52 (2.16–2.94)	0.89 (0.72–1.10)	0.30	−0.28
2 g calcium	63	1.95 (1.67–2.26)	58	2.32 (1.98–2.71)	1.16 (0.94–1.43)	0.16	0.32
IL-8, pg/mL							
Placebo	65	5.59 (5.03–6.21)	60	5.39 (4.84–6.00)	—	—	—
1 g calcium	62	5.72 (5.14–6.37)	58	5.58 (5.00–6.22)	1.01 (0.91–1.13)	0.84	0.06
2 g calcium	63	5.70 (5.12–6.35)	58	5.20 (4.66–5.81)	0.95 (0.85–1.05)	0.32	−0.30
IL-10, pg/mL							
Placebo	65	1.86 (1.40–2.49)	60	2.07 (1.55–2.76)	—	—	—
1 g calcium	62	2.17 (1.62–2.90)	58	2.29 (1.71–3.07)	0.95 (0.81–1.12)	0.55	−0.09
2 g calcium	63	2.21 (1.65–2.96)	58	2.51 (1.87–3.36)	1.02 (0.87–1.20)	0.77	0.09
IL-12p40, pg/mL							
Placebo	65	18.73 (16.05–21.86)	60	18.71 (16.02–21.86)	—	—	—
1 g calcium	62	18.75 (16.05–21.92)	58	20.94 (17.90–24.50)	1.12 (1.01–1.23)	0.03	2.21
2 g calcium	63	17.42 (14.89–20.38)	58	18.23 (15.56–21.35)	1.05 (0.95–1.16)	0.36	0.83
TNF-α, pg/mL							
Placebo	65	1.41 (1.28–1.55)	60	1.41 (1.28–1.55)	—	—	—
1 g calcium	62	1.32 (1.20–1.45)	58	1.42 (1.29–1.56)	1.08 (1.01–1.15)	0.04	0.10
2 g calcium	63	1.42 (1.29–1.56)	58	1.45 (1.32–1.60)	1.02 (0.96–1.10)	0.48	0.03
VEGF, pg/mL							
Placebo	65	76.39 (63.95–91.26)	60	76.17 (63.62–91.20)	—	—	—
1 g calcium	62	85.02 (71.01–101.8)	58	79.56 (66.32–95.44)	0.94 (0.81–1.09)	0.42	−5.24
2 g calcium	63	80.10 (66.87–95.96)	58	73.00 (60.80–87.66)	0.91 (0.79–1.06)	0.25	−6.88
IL-1β, pg/mL							
Placebo	65	0.16 (0.13–0.20)	60	0.13 (0.10–0.16)	—	—	—
1 g calcium	62	0.17 (0.14–0.22)	58	0.16 (0.12–0.20)	1.12 (0.72–1.72)	0.62	0.02
2 g calcium	63	0.16 (0.13–0.21)	58	0.14 (0.11–0.17)	1.03 (0.67–1.59)	0.90	0.01
Oxidative stress							
F_2-isoprostane, pg/mL							
Placebo	64	86.82 (77.01–97.87)	60	85.94 (76.02–97.15)	—	—	—
1 g calcium	61	80.15 (70.93–90.57)	58	81.81 (72.24–92.65)	1.03 (0.87–1.22)	0.72	2.54
2 g calcium	63	84.61 (74.96–95.49)	58	85.38 (75.37–96.72)	1.02 (0.86–1.21)	0.82	1.65

[a]Geometric means.
[b]Calculated as (treatment group geometric mean at follow-up/treatment group geometric mean at baseline)/(placebo group geometric mean at follow-up/placebo group geometric mean at baseline); mean, 95% CI, and *P*-value obtained from the repeated measures mixed linear model.
[c]Calculated as (treatment group geometric mean at follow-up − treatment group geometric mean at baseline) − (placebo group geometric mean at follow-up − placebo group geometric mean at baseline).

Overall, we did not observe an effect of calcium supplementation (1 or 2 g/d) on individual biomarkers of inflammation and oxidative stress. Opposite to our hypothesis, we noted statistically significant increases of 12% and 8% in the concentrations of IL-12p40 and TNF-α, respectively, for those treated with 1 g/d but not 2 g/d of calcium. The estimated effect of calcium on the cytokine summary *z*-score is presented in Table 3. From baseline to follow-up, the *z*-score decreased by 0.39, 0.13, and 0.26 in the placebo group and the 1 and 2 g/d calcium groups, respectively, suggesting relative increases of the cytokine levels in both treatment groups compared with the placebo; however, none of these estimates were statistically significant. The results were also null within strata of age at enrollment, sex, smoking status, family history of colorectal cancer in a first degree relative, body mass index, regular use of aspirin and/or non-aspirin NSAIDs, total fat intake, dietary fiber intake, and the OBS, or limiting the analysis to those with good adherence (data not shown).

To provide possible insight into whether the null results for the calcium intervention were valid or possibly due to the age of the blood samples, we analyzed baseline associations of CRP, the cytokine summary *z*-score, and F_2-isoprostanes with selected participant characteristics previously reported to be associated with inflammation and oxidative stress (Table 4). Overall, mean concentrations of these biomarkers were higher among women, those with a larger BMI or waist–hip ratio, current smokers, or those with higher oxidative stress as indicated by a lower OBS (overall, diet-specific, or lifestyle-specific); aspirin regular users, compared with nonusers, had lower levels of F_2-isoprostanes but higher levels of inflammatory cytokines.

Discussion

The results from this first full-scale, dose–response trial of calcium and biomarkers of inflammation and oxidative stress indicate that supplementation with 1 or 2 g/d of elemental calcium has no effects on circulating biomarkers of inflammation and oxidative stress in sporadic colorectal adenoma patients over a 4-month treatment period.

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Table 3. Changes in plasma cytokine summary *z*-score[a] among colorectal adenoma patients in response to calcium supplementation

	Baseline		Four-month follow-up			Absolute treatment effect[c]	
	n	Mean[b] (95% CI)	*n*	Mean[b] (95% CI)	Change from baseline to follow-up	Mean (95% CI)	*P*
Placebo	65	−0.11 (−0.92 to 0.70)	60	−0.50 (−1.33 to 0.32)	−0.39	—	—
1 g calcium	62	0.40 (−0.42 to 1.22)	58	0.27 (−0.57 to 1.10)	−0.13	0.26 (−0.64 to 1.17)	0.57
2 g calcium	63	−0.25 (−1.07 to 0.57)	58	−0.51 (−1.35 to 0.33)	−0.26	0.13 (−0.77 to 1.03)	0.78

[a]Summary *z*-score of pro- and anti-inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-8, IL-12p40, VEGF, and IL-10) 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, respectively, at baseline]. The *z*-value for IL-10 was included with a negative sign.
[b]Arithmetic means.
[c]Calculated as (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*-value obtained from the repeated measures mixed linear model.

Chronic inflammation is an important hallmark of cancer (33), including colorectal cancer (11). Several biomarkers of inflammation have been previously linked to colorectal cancer risk in population studies. For example, in a meta-analysis of prospective studies (including 1,159 colorectal cancer cases and 37,986 controls), CRP was statistically significantly associated with higher risk for colorectal cancer [RR per unit increase of log-transformed CRP 1.12; 95% confidence interval (CI), 1.01–1.25; ref. 34]. Also, serum levels of several proinflammatory cytokines, including VEGF, TNF-α, IL-6, and IL-8, were found to be higher in colorectal cancer cases than in controls (35). Oxidative stress, intimately linked with inflammation (16), primarily acts through reactive oxygen and nitrogen species (RONS); RONS can induce damage in almost all cellular components, including oxidizing cellular lipids (lipid peroxidation; ref. 36), which is believed to be a major determinant of oxidative stress-related colorectal carcinogenesis (37, 38), and F_2-isoprostanes has been recognized as the most reliable marker of lipid peroxidation *in vivo* (36, 39). Therefore, the selection of CRP, cytokines, and F_2-isoprostanes as the endpoints in our calcium intervention trial is well supported, and modulation of these biomarkers by calcium could have implications for further modulation of risk for colorectal neoplasms.

In mice, calcium with or without vitamin D reduced inflammation (IL-1β, TNF-α, IL-6; refs. 23, 40, 41) or oxidative stress (ROS production, NADPH oxidase mRNA, and plasma malondialdehyde; ref. 23). The results from our previous pilot clinical trial suggested that calcium may reduce plasma IL-6, IL-1β, and an inflammation *z*-score (21) as well as oxidative DNA damage in the normal colorectal mucosa of patients with colorectal adenoma (22). Apart from our pilot study, three other human clinical trials tested the effect of calcium on inflammation biomarkers. Gannagé-Yared and colleagues reported no effect of 1.0 g/d of calcium and 800 IU/d of vitamin D_3 on serum CRP, IL-6, and TNF-α among 47 healthy postmenopausal women over 12 weeks (26). Similarly, Grey and colleagues reported no effect of 1 g/day of calcium on serum CRP level among 116 healthy postmenopausal women over 12 months (28), and Pittas and colleagues reported no effect of 500 mg/day calcium and 700 IU/day vitamin D_3 supplementation on CRP and IL-6 among nondiabetic adults over 3 years (27). However, the null results in the above three studies may be partially explained by the relatively low levels of cytokines in healthy participants, as opposed to those likely to have higher levels of gut or systemic inflammation, such as patients with colorectal adenoma (42). In addition, three studies reported that diets high in dairy reduced the levels of CRP, TNF-α, IL-6, monocyte chemoattractant protein-1, and oxidative stress biomarkers in overweight or obese adults (23–25) who may have higher levels of systemic inflammation than do normal weight individuals (43), but whether the effects were due to calcium or other dairy components could not be ascertained.

In this study, we observed no effect of calcium on circulating biomarkers of inflammation and oxidative stress, which is inconsistent with the preliminary findings from our pilot trial. This discrepancy could be due to several reasons. Two important possibilities are that calcium truly has no effect on systemic inflammation and oxidative stress—whether or not it has effects on inflammation in the colorectal mucosa—and that our previously reported preliminary findings were due to chance. Our null results are consistent with recent findings from a multicenter randomized clinical trial ($n = 2{,}259$) that calcium and/or vitamin D did not reduce risk of metachronous colorectal adenomas over 3 to 5 years (44), despite previous clinical trial evidence that calcium supplementation did so (6). The original blood samples for this study were collected in the early 1990s, which raised concerns that some analytes in the samples could have deteriorated over the years. However, the blood samples were immediately processed and appropriately stored in a −80°C freezer since the original collection, and no additional freeze–thaw cycles were introduced before we aliquotted the samples for this study. Concentrations of inflammation markers were comparable to those in the pilot trial, which had a much shorter gap between sample collection and laboratory measurement (21). Most importantly, associations of these biomarkers at baseline with sex, body measurements, and an oxidative balance score were consistent with previous findings from our group (42, 45, 46) and other groups (e.g., refs. 47–49), supporting the validity of our biomarker measurements. The baseline association of regular aspirin use with the cytokine *z*-score was opposite to what one might expect; however, this may have been the result of confounding by indication (i.e., subjects who took aspirin regularly likely were doing so for medical conditions associated with higher systemic inflammation), and thus should be interpreted with caution.

A major strength of the study is that it is the first full-scale dose–response trial to test the effect of calcium on systemic indicators of inflammation and oxidative stress. For the inflammation biomarkers, we chose a panel of markers to represent different aspects of the inflammatory response/immunomodulation in order to provide a more complete summary of the overall effect of calcium on inflammation. There are also several limitations of this study, including the above-mentioned long storage period of blood samples. Also, because this study was based on a clinical trial population, the findings may not be generalizable to a general population; however, the population may reflect a typical clinic population with adenoma removal. Although not all adenomas become cancerous, most sporadic cancers form from adenomas,

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Table 4. Mean levels of inflammation and oxidative stress biomarkers by demographic and lifestyle factors

	CRP, μg/mL			Cytokine *z*-score			F_2-isoprostanes, pg/mL		
	n	Mean[a] (95% CI[a])	*P*[a]	*n*	Mean[a] (95% CI[a])	*P*[a]	*n*	Mean[a] (95% CI[a])	*P*[a]
Sex									
Male	119	1.91 (1.54–2.36)		119	−0.02 (−0.61 to 0.57)		118	74.99 (68.06–82.63)	
Female	71	2.05 (1.56–2.70)	0.69	71	0.03 (−0.73 to 0.80)	0.91	70	101.72 (89.67–115.38)	<0.01
BMI, kg/m^2									
<25	49	1.31 (0.94–1.82)		49	−1.07 (−1.99 to −0.14)		49	82.52 (70.95–95.98)	
25–27.49	34	1.24 (0.83–1.87)		34	−0.68 (−1.82 to 0.47)		32	73.97 (60.95–89.76)	
27.50–29.99	41	2.30 (1.59–3.33)		41	0.75 (−0.29 to 1.78)		41	92.64 (78.23–109.71)	
30–34.99	51	3.29 (2.37–4.56)		51	0.86 (−0.05 to 1.78)		51	90.81 (78.14–105.53)	
≥35	15	2.70 (1.49–4.88)	<0.01	15	0.42 (−1.25 to 2.08)	<0.01	15	108.22 (82.42–142.09)	0.05
Waist–hip ratio[b]									
Tertile 1	63	1.34 (1.00–1.81)		63	−1.06 (−1.87 to −0.24)		62	74.43 (65.11–85.09)	
Tertile 2	64	2.00 (1.48–2.69)		64	−0.06 (−0.87 to 0.75)		63	95.70 (83.72–109.39)	
Tertile 3	63	2.86 (2.12–3.86)	<0.01	63	1.12 (0.31–1.93)	<0.01	63	93.28 (81.67–106.53)	0.02
Current cigarette smoker									
Yes	38	3.46 (2.37–5.05)		38	1.01 (−0.05 to 2.07)		38	97.55 (81.91–116.18)	
No	149	1.70 (1.40–2.06)	<0.01	149	−0.28 (−0.82 to 0.26)	0.03	147	85.45 (78.13–93.46)	0.19
OBS[b]									
Tertile 1	70	2.48 (1.88–3.28)		70	0.09 (−0.70 to 0.88)		69	94.13 (82.71–107.14)	
Tertile 2	57	2.29 (1.69–3.12)		57	0.28 (−0.59 to 1.16)		57	88.62 (76.88–102.16)	
Tertile 3	60	1.39 (1.04–1.87)	0.01	60	−0.24 (−1.08 to 0.60)	0.59	59	80.34 (69.97–92.23)	0.10
OBS-diet[b]									
Tertile 1	61	2.32 (1.72–3.13)		61	0.60 (−0.23 to 1.43)		60	103.12 (90.11–118.02)	
Tertile 2	66	1.95 (1.45–2.61)		66	−0.52 (−1.33 to 0.29)		66	84.95 (74.57–96.77)	
Tertile 3	60	1.78 (1.31–2.40)	0.22	60	0.06 (−0.77 to 0.9)	0.37	59	77.19 (67.40–88.40)	<0.01
OBS-lifestyle[b]									
Tertile 1	45	2.79 (1.97–3.94)		45	0.65 (−0.33 to 1.64)		45	95.79 (81.55–112.53)	
Tertile 2	78	2.31 (1.78–2.99)		78	−0.04 (−0.78 to 0.69)		77	84.80 (75.09–95.77)	
Tertile 3	67	1.31 (0.98–1.74)	<0.01	67	−0.37 (−1.19 to 0.45)	0.13	66	84.91 (74.24–97.13)	0.30
Regular use of aspirin									
Yes	40	2.17 (1.49–3.15)		40	1.31 (0.30–2.32)		39	72.06 (60.88–85.30)	
No	150	1.93 (1.58–2.35)	0.58	150	−0.35 (−0.88 to 0.19)	<0.01	149	91.90 (84.18–100.33)	0.01
Regular use of non-aspirin NSAIDs									
Yes	19	1.82 (1.06–3.13)		19	−0.75 (−2.23 to 0.74)		19	88.50 (69.25–113.11)	
No	167	1.99 (1.65–2.14)	0.76	167	0.09 (−0.43 to 0.61)	0.30	165	87.59 (80.33–95.50)	0.94

Abbreviation: BMI, body mass index.

[a] Mean, standard error, and *P* value were calculated using analysis of covariance. Models for all variables except sex were adjusted for sex (men/women). Models for all variables except BMI and waist–hip ratio also adjusted for BMI (continuous). *P* value is for trend if explanatory variable has more than two categories. Geometric means presented for CRP and F_2-isoprostane because of the nonnormality of the original observations.

[b] OBS reflects combined contributions of antioxidant and pro-oxidant diet and/or lifestyle exposures, with a higher score indicating lower oxidative stress. Tertiles are sex specific.

and it is important to understand mechanisms and intervene preventively at earlier points in the carcinogenic process.

In summary, taken together with previous literature, the results from this study do not support the hypothesis that calcium supplementation favorably modulates circulating biomarkers of inflammation and oxidative stress among patients with previous colorectal adenoma over a 4-month treatment period. Future full-scale studies with tissue-based preneoplastic biomarkers of risk for colorectal cancer are needed to provide additional insights into the effects of calcium and further clarify its potential role as a chemopreventive agent against colorectal neoplasia.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: B. Yang, M.D. Gross, R.M. Bostick
Development of methodology: B. Yang, R.M. Bostick
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.D. Gross, R.M. Bostick
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B. Yang, M.D. Gross, V. Fedirko, R.M. Bostick
Writing, review, and/or revision of the manuscript: B. Yang, V. Fedirko, M.L. McCullough, R.M. Bostick
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B. Yang, R.M. Bostick
Study supervision: R.M. Bostick

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Cancer Prevention Research

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