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Effects of Calcium and/or Vitamin D Supplementation on Biomarkers of Gut Barrier Function in Colorectal Adenoma Patients: a Randomized Clinical Trial

By

Kelly Vermandere

Degree to be awarded: MPH

Epidemiology

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By

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B.S.

University of Michigan

2018

Faculty Thesis Advisor: Dr. Veronika Fedirko, PhD, MPH

An abstract of

a thesis submitted to the Faculty of the
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2020

Abstract

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By Kelly Vermandere

Background: Gut barrier dysfunction may lead to chronic inflammation and contribute to several gastrointestinal diseases, including colorectal cancer. Preliminary evidence suggests that vitamin D and calcium could prevent colorectal carcinogenesis in part by influencing gut barrier function, however, human data are scarce.

Methods: We tested the effects of supplemental calcium (1,200 mg/day) and/or vitamin D_3 (1,000 IU/day) on circulating biomarkers of gut permeability [anti-flagellin (FLIC) and anti-lipopolysaccharide (LPS) immunoglobulins (Igs), measured *via* ELISA] at year 1 and following 3 or 5 years of treatment after baseline examination among colorectal adenoma patients in a randomized, double-blinded, placebo-controlled clinical trial (n = 175), and assessed factors associated with baseline levels of these biomarkers.

Results: We found that vitamin D_3 and/or calcium supplementation has no substantial effects on individual or aggregate biomarkers of gut permeability. Subgroup analyses by baseline BMI, aspirin use, calcium intake, and blood 25(OH)-vitamin D concentrations yielded similar results. At baseline, a combined permeability score (the summed concentrations of all four biomarkers) was 14% higher among women (P= 0.01) and 10% higher among those who had >1 serving/day of red or processed meat compared to those having 0 servings/day (P_{trend}= 0.03).

Conclusions: Our results suggest that daily supplementation with vitamin D_3 and/or calcium may not modify levels of gut permeability biomarkers, and support

continued investigation of modifiable factors such as diet that could affect gut permeability.

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Chapter I: Background/ Literature Review

Descriptive Epidemiology of Colorectal Cancer

Colorectal cancer is the third most common cancer and the fourth most common cause of cancer-related death. By gender, it is the second most common cancer in women and the third in men. The probability of having colorectal cancer is about 4%–5% (1). However, the incidence of colorectal cancer has risen by ~ 200,000 cases per year from 1990 to 2012. Although 55% amount of cases are found in Western countries, research has shown that only 33% of related deaths in the world occur there. This may be due to improvements in health systems and the implementation of screening programs (2).

Colorectal cancer is caused by mutations in tumor suppressor genes, oncogenes, and genes related to DNA repair mechanisms. These mutations can be considered sporadic (70%); inherited (5%), or familial (25%) (3). Thus, the onset of colorectal cancer is caused by both genetic and environmental risk factors. One major risk factor for colorectal cancer is age. The risk of developing colorectal cancer increases past the age of 50, while onset below 50 is rare. Additionally, there are other unmodifiable risk factors, such as a personal history of colorectal cancer, diabetes, or inflammatory bowel disease. Another risk factor is the presence of a family history of colorectal cancer, especially in relatives that were diagnosed under the age of 50 (4).

Some other risk factors, which are related to lifestyle, can be reduced by changing one's dietary and physical activity habits. A sedentary lifestyle is also related to obesity, another important risk factor for colorectal cancer. The increased risk is linked to both food intake and increased levels of visceral adipose tissue, the hormonally active

component of total body fat. Visceral adipose tissue can promote the development of colorectal cancer through the secretion of proinflammatory cytokines, which leads to inflammatory changes in the colon and rectum. In terms of diet, a high red meat-intake, high-fat diet, and inadequate intake of fiber all increase risk (5). Additionally, smoking and alcohol consumption increase risk of colorectal cancer (6). Since inflammation plays a role in the development of colorectal cancer, many anti-inflammatory drugs have become important in the prevention and treatment of colorectal cancer. Most of the anti-inflammatory agents used are non-steroidal anti-inflammatory drugs (NSAIDs). For instance, aspirin has had good results in the prevention of colorectal cancer, reducing risk by up to 50% (7).

In the past decade, there has been substantial progress made towards understanding colorectal cancer. Screening has improved outcomes and there is more knowledge on the genetic basis of inherited colorectal cancer and identification of at-risk patients. Improvements have been made in surgery procedures for patients with localized forms of colorectal cancer and active targeted drugs for treatments have been made, yet cure rates remain low. Another important step towards understanding colorectal cancer may be using biomarkers to aid selection of patients that will best respond to therapy (8). Hopefully research in the future can progress to improve the prevention and treatment of colorectal cancer.

Anti-carcinogenic Effects of Calcium and Vitamin D in Colorectal Cancer

The primary hypotheses that describe how calcium may reduce risk of colorectal cancer are the bile acid binding hypothesis and the cell cycle regulation hypothesis. The bile acid binding hypothesis states that if an excess amount is consumed then calcium can

bind to toxic bile acids and fatty acids that were formed as a result of fat digestion. This would prevent their mutagenic, mitogenic, and injury-induced inflammatory effects. The cell cycle regulation hypothesis states that calcium also has direct effects in reducing proliferation and increase differentiation (8).

Vitamin D was originally considered due to its relationship to calcium homeostasis. However, the vitamin D receptor is also expressed in the colon and many other tissues. Additionally, vitamin D modulates more than 200 genes involved in activities relevant to colorectal carcinogenesis. This includes cell cycle regulation, growth factor signaling, protection against oxidative stress, bile acid and xenobiotic metabolism, cell adhesion, DNA repair, angiogenesis, and inflammation and immune function (9).

Gut Barrier Biomarkers and Colorectal Cancer

Research has pointed to roles of gut microbial communities in understanding the link between obesity, chronic inflammation, and the development of colorectal cancer. For my thesis project, I focused on four gut barrier biomarkers: lipopolysaccharide (LPS), flagellin (FLIC), immunoglobulin G (IgG), and immunoglobulin A (IgA). LPS is an endotoxin and cell wall component of gram-negative bacteria and an underlying factor of obesity-driven low-grade inflammation. High fat, high caloric, or high carbohydrate diets have been shown to increase serum LPS concentrations. LPS increases inflammatory response signaling, alters gut barrier function, and may play a role in the pathogenesis of several adverse outcomes, such as colorectal cancer. LPS and lipopolysaccharide-binding protein (LBP), a marker of LPS exposure, have been shown to be associated with reduced apoptosis and increased proliferation in metastatic tumor cells (10).

FLIC is a bacterial product that is related to LPS and is also associated with gram-negative bacteria. It is a subunit protein of the flagellum, a whip-like appendage that enables bacterial motility. Currently, research has defined FLIC as an immune activator that shapes both the innate and adaptive arms of immunity during microbial infections. FLIC has also been examined for anti-tumor and radioprotective activities and has shown potential in combating tumor growth and radiation-associated tissue damage (11).

Chronic inflammation has been associated with increased risk of colorectal cancer. It has been hypothesized that the colorectal cancer development can be due to long term exposure to the localized inflammatory responses. A recently published nested case-control study investigated the association between serum LPS- and FLIC-specific immunoglobulin levels and risk of colorectal cancer (12). Some dietary and lifestyle exposures as well as physiological factors were found to potentially exacerbate intestinal permeability leading to increased exposure of the colonic epithelium to endotoxins. This would lead to a greater leakage of endotoxins into the systemic circulation. However, So Yeon Kong et al. found no overall association between bacterial exposure levels and risk of colorectal cancer. In the sub-group analysis by sex, some biomarker levels were positively associated with colorectal cancer risk among men (fully-adjusted OR for highest vs. lowest quartile for total anti-LPS + flagellin, 1.66; 95% CI, 1.10-2.51; P_{trend}, 0.049), but inversely associated with the risk among women (fully-adjusted OR, 0.70; 95% CI, 0.47-1.02; P_{trend}, 0.18).(12).

Additional studies on these biomarkers have shown that flagellin- and LPS-specific serum immunoglobulin levels (IgA and IgG) were increased in patients with short bowel syndrome (SBS) compared with healthy controls (13). Furthermore, IgA and

IgG antibodies (specific for flagellin monomers) were shown to be a target of the elevated adaptive immune response associated with Crohn's disease (14).

Observational Studies on Calcium and Vitamin D and Colorectal Cancer

There have been many observational studies that support the hypothesis that higher intakes of calcium reduce the risk of colorectal cancer. Bostick et al. found that of 20 cohort studies, 18 (90%) found inverse associations between calcium and colorectal cancer. Among these studies, eight were statistically significant. The other two studies found direct associations, but neither of them were statistically significant (15). Cho et al. designed a pooled analysis of 10 cohort studies from five countries and found a 22% lower risk of colorectal cancer among those consuming the highest vs. the lowest levels of calcium, which was statistically significant (16).

For vitamin D exposure, 25-OH-vitamim D blood levels are used as the most accurate indicator of this exposure. Circulating 25-OH-vitamin D concentrations often come from sunlight exposure, which provides 90 – 95% of vitamin D in most people, but we also need to consider dietary and supplemental intakes. Unlike the results from studies on calcium and colorectal cancer risk, the results from studies of vitamin D are consistent with there being an inverse association between vitamin D exposure and colorectal neoplasms (9).

Fedirko et al. conducted a pooled analysis of three case-control studies on colorectal adenomas and found that those in the highest quartile of circulating 25-OH-vitamin D_3 concentrations were at a statistically significant, ~40% lower risk (17). Wei et

al. looked at seven other observational studies of 25-OH-vitamin D and colorectal adenoma. Among these studies, six found inverse associations, but only three were statistically significant (18). Furthermore, Lee et al. found that of nine prospective cohort studies that investigated associations of circulating 25-OH-vitamin D and incident colorectal cancer, seven had inverse associations, but only two were statistically significant. When they created a meta-analysis with these studies, the estimated relative risk for those in the upper relative to the lower quantiles of 25-OH-vitamin D was 0.66 (95% CI 0.54 – 0.81) (19). This amount of consistency for vitamin D studies is interesting given the low 25-OH-vitamin D blood levels in the studies. However, there were a small number of studies that assessed 25-OH-vitamin D blood levels, so these results are just suggestive (9).

Clinical Trials on Calcium and Vitamin D and Colorectal Neoplasms

The "parent study" of my thesis project was designed by Baron and co-workers. They designed a Vitamin D/Calcium Polyp Prevention Study, which considered the effects of daily supplementation with vitamin D_3 (1,000 IU), calcium (1,200 mg), or both after removal of colorectal adenomas. This was a randomized, multi-center, double-blind, placebo-controlled trial that took place at 11 genetically diverse centers in the United States. The results showed that none of these treatments significantly altered the risk of recurrent colorectal adenomas over a period of 3 to 5 years. Thus, the results did not necessarily justify vitamin D or calcium supplementation (20).

When the paper by Bostick et al. was published in 2015, there was mention of seven clinical trials of calcium and adenoma recurrence, two of which had large sample sizes, and one major trial of colorectal cancer prevention (9). The Calcium Polyp

Prevention Study (a precursor to the previous Baron study) was noteworthy as a multi-center, randomized, double-blind, placebo-controlled clinical trial of calcium supplementation (1,200 mg of elemental calcium daily) and adenoma recurrence. Baron et al. found that the relative risk for any recurrence of adenoma was 0.85 (95% CI 0.74–0.98) and for advanced adenomas, 0.46 (95% CI 0.26–0.83) (21). Another noteworthy study was the European Cancer Prevention Organization Intervention Study, which found a non-significant reduction in adenoma recurrence (RR 0.66, 95% CI 0.38–1.17) among those randomized to 2,000 mg of elemental calcium daily compared to placebo (22). The meta-analysis that included these two studies and five other clinical trials by Shaukat et al. found an overall RR of 0.80 (95% CI 0.68–0.93) (23).

A combination of both calcium and vitamin D was studied in the Women's Health Initiative, which was a randomized, double-blind, placebo- controlled clinical trial. In this study, 36,282 postmenopausal women were randomized to 1,000 mg of elemental calcium plus 400 IU (10 μg) of vitamin D *vs.* placebo over an average of seven years. The authors Wactawski-Wende et al. found no evidence for a reduction in the incidence of invasive colorectal cancers (RR 1.08, 95% CI 0.86–1.34). However, this study had major limitations because there was a low adherence in the active treatment group (only 60% took 80% or more of their pills). On the other hand, there was a high rate of subjects in the placebo group taking supplements (69% took calcium and vitamin D supplements, so intakes were twice that of the national averages). Additional limitations include the low doses administered, the short length of follow-up for the downstream endpoint, and the interpretation of the results was considered problematic (24).

Conclusion

The development of modifiable biomarkers of risk for colorectal cancer would be useful for assessing and managing risk for colorectal cancer (9). Based on the bile acid binding and the cell cycle regulation hypothesis mentioned previously, there is strong biological rationale for vitamin D3 and calcium in reducing risk for colorectal cancer. The observational literature for calcium in reducing risk for colorectal neoplasms is consistent in cohort studies and large randomized controlled trials. The literature for circulating 25-OH-vitamin D concentrations and colorectal neoplasms is also consistent, but still sparse (9). The studies mentioned in this literature review and the research on gut barrier biomarkers demonstrated the need for further investigation of vitamin D and calcium as potential chemopreventative agents against colorectal neoplasms. In addition, there is a need for the development of modifiable biomarkers of risk for colorectal neoplasms that can eventually be used in clinical applications.

Chapter II: Manuscript

Effects of Calcium and/or Vitamin D Supplementation on Biomarkers of Gut Barrier Function in Colorectal Adenoma Patients: a Randomized Clinical Trial

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Abstract

Background: Gut barrier dysfunction may lead to chronic inflammation and contribute to several gastrointestinal diseases, including colorectal cancer. Preliminary evidence suggests that vitamin D and calcium could prevent colorectal carcinogenesis in part by influencing gut barrier function, however, human data are scarce.

Methods: We tested the effects of supplemental calcium (1,200 mg/day) and/or vitamin D_3 (1,000 IU/day) on circulating biomarkers of gut permeability [anti-flagellin (FLIC) and anti-lipopolysaccharide (LPS) immunoglobulins (Igs), measured *via* ELISA] at year 1 and following 3 or 5 years of treatment after baseline examination among colorectal adenoma patients in a randomized, double-blinded, placebo-controlled clinical trial (n = 175), and assessed factors associated with baseline levels of these biomarkers.

Results: We found that vitamin D_3 and/or calcium supplementation has no substantial effects on individual or aggregate biomarkers of gut permeability. Subgroup analyses by baseline BMI, aspirin use, calcium intake, and blood 25(OH)-vitamin D concentrations yielded similar results. At baseline, a combined permeability score (the summed concentrations of all four biomarkers) was 14% higher among women (P= 0.01) and 10% higher among those who had >1 serving/day of red or processed meat compared to those having 0 servings/day (P_{trend}= 0.03).

Conclusions: Our results suggest that daily supplementation with vitamin D_3 and/or calcium may not modify levels of gut permeability biomarkers, and support continued investigation of modifiable factors such as diet that could affect gut permeability.

Introduction

The largest mucosal surface in the body is located in the gastrointestinal tract, which has a selectively permeable barrier and adapts in response to extracellular stimuli, such as nutrients and harmful wastes (25). The gut barrier plays an important role in defending against microbes and foreign antigens and can affect pro-inflammatory and immunoregulatory responses (25). Abnormal gut barrier function contributes to multiple gastrointestinal disorders, such as inflammatory bowel disease and colorectal neoplasms (26, 27). There is also emerging evidence that elevated bacterial endotoxin concentrations may be associated with colorectal adenomas (28). Thus, identifying the role and mechanism of gut barrier and its interplay with inflammatory response can lead to further understanding of colorectal cancer pathogenesis.

LPS increases inflammatory response signaling, alters gut barrier function, and may play a role in the pathogenesis of several adverse outcomes, such as colorectal cancer (10, 29). Flagellin is a bacterial product related to LPS, which has also been examined for anti-tumor activities and has shown potential in combating tumor growth (11, 30). Circulating levels of flagellin- and LPS-specific IgA and IgG may contribute to gut barrier dysfunction and may indicate altered adaptive immune responses (31,32). Additional factors affect gut permeability, such as toxins, gut bacteria, and lifestyle factors, but have not been well characterized (33). There is strong evidence for the association between diet, lifestyle, and the development of colorectal cancer (34).

There are strong observational and experimental studies that support the rationale for protection against colorectal cancer by calcium and vitamin D (9, 15-19, 35-36). However, due to the limited human data, more studies are needed to further understand

the biological pathways that lead to a decreased risk in colorectal cancer. Calcium and vitamin D may be involved in maintaining the gut barrier against dysfunction that leads to endotoxemia, inflammation, and colorectal carcinogenesis (36, 37, 38). Calcium can bind bile and fatty acids in the colon lumen, which prevents them from causing colonic cytotoxicity and producing inflammation(23, 38, 39). Calcium also directly affects the cell cycle, reducing proliferation and increasing differentiation (40). Furthermore, there is evidence that vitamin D levels are inversely associated with colorectal adenomas and colorectal cancer incidence (18-19, 41). Vitamin D is related to calcium homeostasis and is involved in activities related to colorectal carcinogenesis. This includes cell cycle regulation, growth factor signaling, protection against oxidative stress, and inflammation and immune function (9, 42).

To further investigate the chemopreventative potential of vitamin D and calcium, we measured circulating levels of flagellin- and LPS-specific IgA and IgG among patients with previous colorectal adenomas in a full-scale, randomized, double-blinded, placebo-controlled clinical trial (n= 175). We also evaluated factors associated with these circulating biomarkers of gut permeability at baseline and tested whether biomarker levels were affected by calcium supplementation over 1 and 3-5 years of treatment.

Methods

Clinical Trial Protocol

The participants in this adjunct biomarker study were recruited from a larger 11-center, randomized, placebo-controlled, partial 2 x 2 factorial chemoprevention clinical trial (“parent study”; Vitamin D and Calcium Polyp Prevention Study) testing the

efficacy of supplemental calcium and vitamin D_3, alone or in combination, over 3 to 5 years on colorectal adenoma recurrence in colorectal adenoma patients in the United States. The parent study protocol, eligibility, and exclusion criteria were previously published (20).

Briefly, participants were 45 to 75 years of age, had at least one colorectal adenoma removed within 120 days of enrollment with no remaining polyps after a complete colonoscopy, and anticipated to undergo a 3-year or 5-year colonoscopic follow-up examination. For participation in the adjunct biomarker study, additional exclusion criteria included being in two participating study centers in GA and SC. Patients for this biomarker study were recruited at 2 of the 11 clinical centers (Georgia and South Carolina). Of 2,259 patients randomized in the parent study, 175 patients met the additional eligibility criteria and agreed to provide blood samples at baseline, after 1 year of supplementation, and EOT with study agents and were consented and recruited July 2004 through July 2008 into the adjunct biomarker study.

Eligible patients were in good general health and did not have familial colorectal cancer syndromes or serious intestinal disease. We did not include patients who had conditions that indicated that the study agents would pose a health risk (e.g., a history of kidney stones or hyperparathyroidism) or who had conditions that would indicate a need for either agent (e.g., osteoporosis). We also did not include patients who had a serum calcium level that was outside the normal range, a creatinine level that was more than 20% above the upper limit of the normal range, or a 25-hydroxyvitamin D level that was lower than 12 ng per milliliter or higher than 90 ng per milliliter.

We evaluated four regimens, all of which involved two identical tablets taken daily: 1000 IU of vitamin D_3, 1200 mg of calcium as carbonate, both agents, or placebo. Women could elect to be randomly assigned to receive either calcium or calcium plus vitamin D (two-arm randomization); all other patients were randomly assigned to receive one of the four regimens (full factorial randomization). The doses of study agents were chosen to increase the total intake substantially, with a margin of safety below the highest mean daily intake level believed unlikely to cause adverse effects in most people at the time that the trial began (2000 IU of vitamin D and 2.5 g of calcium). In accordance with the protocol, study treatment was to continue until the anticipated 3-year or 5-year colonoscopic examination.

At enrollment, participants provided information regarding demographic data, medical history, medications, nutritional supplements, behavioral factors, and diet (using the NutritionQuest Block Brief 2000 food frequency questionnaire). Enrollment was followed by a placebo run-in period of 56 to 84 days to identify and exclude participants who were considered unlikely to follow study procedures. Subsequent randomization by the coordinating center was performed with the use of computer-generated random numbers with permuted blocks and stratification according to clinical center, sex, anticipated colonoscopic examination at 3 years or 5 years, and full factorial or two-group randomization. All study staff were unaware of the treatment assignments, with the exception of the data analyst and statistician, some of the programmers, and pharmacy personnel.

Participants agreed to avoid taking study agents outside the trial. However, because of increasing publicity regarding the possible benefits of these supplements,

daily personal use of up to 1000 IU of vitamin D, 400 mg of elemental calcium, or both were permitted, although discouraged, from April 2008 onward.

Participants were contacted by telephone every 6 months and questioned regarding adherence to study agents, illnesses, medication and supplement use, dietary calcium intake, and colorectal procedures. Records were collected that included data on major medical events, colorectal surgical procedures, and endoscopic examinations. Two physicians who were unaware of the study group assignments adjudicated the diagnosis of adverse events. Bottles of study tablets were mailed to participants every 4 months. Patients who wanted to take a multivitamin were offered a special preparation that did not include calcium and vitamin D. The study intervention ended on August 31, 2013; the treatment-phase follow-up continued until November 30, 2013, to accommodate the final 5-year participants. Blood levels of 25-hydroxyvitamin D and calcium were measured at baseline and at year 1, as well as at year 3 for participants with 5-year surveillance cycles. The level of 25-hydroxyvitamin D was also measured shortly before the end-of-treatment examination. The net change in 25-hydroxyvitamin D levels was defined as the post-treatment level minus the pre-treatment level in participants who received vitamin D, minus that difference in participants who were given no vitamin D.

The study end points included all adenomas that were diagnosed in any colorectal endoscopic or surgical procedure at least 1 year after randomization and up to 6 months after the anticipated 3-year or 5-year colonoscopic examination. A single study pathologist who was unaware of the treatment assignments reviewed the slides for all excised colorectal lesions. We distinguished between lesions that were proximal to the splenic flexure and lesions that were more distal. Advanced adenomas were defined as

those with cancer, high-grade dysplasia, more than 25% villous features, or an estimated diameter of at least 1 cm. Study diagnoses were compared with the diagnoses made by the pathologists at the clinical centers.

All participants provided written informed consent; the research was approved by the institutional review board at each center. An independent data and safety monitoring committee oversaw the study.

Laboratory Measurements

Levels of flagellin and LPS-specific IgA and IgG were measured via a previously described custom-made ELISA at Georgia State University (13,43-44). ELISA plates (Costar) were coated overnight with laboratory-made flagellin (100 ng/well; prepared from Salmonella typhimurium, strain SL 3201 fljB$^{-/-}$ as previously described (45) or purified Escherichia coli LPS (2 mg/well; from E. coli 0128: B12, Sigma, Catalog No. 2887). Plasma samples diluted 1:200 were applied to wells coated with flagellin or LPS. After incubation and washing, the wells were incubated either with anti-IgG coupled to horseradish peroxidase (GE, Catalog No. 375112) or, in the case of IgA- specific antibodies, with horseradish peroxidase–conjugated anti-IgA (KPL, Catalog No. 14-10-01). Using the established platform, specificity of flagellin/LPS is observed when the signal is extremely low when using serum from germ-free mice (very low flagellin- or LPS-specific Igs) and completely abolished using serum from RAG-1 knockout mice and germ-free mice on an elemental diet (no flagellin- or LPS-specific Igs). The specificity of the anti-human IgA and anti-human IgG is in accordance to the manufacturer's specifications, KPL and GE Healthcare Life Sciences, respectively.

Quantitation of total Igs was performed using the colorimetric peroxidase substrate tetramethylbenzidine, and optical density (OD) was read at 450 nm and 540 nm (the difference was taken to compensate for optical interference from the plate), with an ELISA plate reader. Data are reported as OD corrected by subtracting background (determined by readings in blank samples) and are normalized to each plate's control sample, which was prepared in bulk, aliquoted, frozen, and thawed daily as used. Standardization was performed using preparations of known concentrations of IgA and IgG. The technician was blinded to treatment group and treated all samples identically.

Baseline, follow-up, and end of study samples from each participant were included in the same batch. The laboratory previously performed assays of these biomarkers in replicates with a very low coefficient of variation (CV < 5%); therefore, our samples were analyzed in singleton to minimize costs and time. The average within-batch CVs were 11%, 16%, 15%, and 18% for flagellin IgA, flagellin IgG, LPS IgA, and LPS IgG, respectively, on the basis of three quality control samples included in each batch. The corresponding between-batch CVs were 7%, 12%, 10%, and 5% for flagellin IgA, flagellin IgG, LPS IgA, and LPS IgG, respectively.

Plasma levels of the inflammation biomarkers (interleukin [IL-10], IL-6, tumor necrosis factor α [TNFa]) were measured using electrochemiluminescence detection-based immunoassays (Meso Scale Discovery; MSD) in the Emory Multiplexed Immunoassay Core (EMIC). All biomarkers were measured in duplicate, according to the manufacturer's protocol, and the technicians were blinded to the treatment group assignment. These three markers were chosen as cytokines related to inflammatory response/immunomodulation

that modify gut permeability to provide a more complete summary of systemic inflammation.

Statistical Analysis

We compared the baseline characteristics of the participants across treatment groups using the chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables. We assessed differences in LPS, FLIC, IgA, and IgG expression from baseline to year 1 follow-up or baseline to the colonoscopic examination 3-5 years later. Treatment effects were analyzed between participants in the treatment group of interest and those in the comparison group using multivariable general linear mixed models. The models included as predictors the intercept, visit (baseline, year 1 follow-up, years 3-5), treatment group, and a treatment-by-visit interaction term. We evaluated changes in biomarker levels over time for the treatment groups that were separated through full factorial, two-arm randomization, or vitamin D versus no vitamin D, calcium versus no calcium, and calcium plus vitamin D versus calcium alone.

We initially analyzed each biomarker for gut permeability individually. Then we created several combinations to better capture different aspects of gut barrier function, which included all four biomarkers combined as a permeability score (flagellin IgA + flagellin IgG + LPS IgA + LPS IgG), LPS (LPS IgA + LPS IgG), FLIC (flagellin IgA + flagellin IgG), IgG (flagellin IgG + LPS IgG), and IgA (flagellin IgA + LPS IgA). These biomarkers were directly summed up because the measurements were approximately on the same scale. Since biomarker values were normally distributed, they were not transformed before statistical testing.

In all analyses of randomized treatments, participants were retained in their originally assigned treatment group, regardless of adherence to study treatment and procedures. To assess potential confounders, identified by differed by categories of a priori– selected biologically plausible factors, two additional models were run. They were adjusted by age, sex, clinical center, number of baseline adenomas, and follow up-period. Adjustment for these potential confounders did not affect the estimated treatment effects; therefore, only unadjusted results are presented. To explore potential factors associated with differences in biomarker levels, we used additional categories of a priori-selected biologically plausible factors.

Treatment effects were calculated on the ratio scale according to the following: relative treatment effect [(treatment group follow-up)/(treatment group baseline)]/ [(control group follow-up)/(control group baseline)]. A relative effect of 1.2 would indicate a 20% increase in biomarker expression in the treatment group relative to the control group. We conducted secondary analyses for treatment effects for LPS, FLIC, and LPS-FLIC by stratifying by median baseline levels of BMI, calcium intake, vitamin D intake, and regular or irregular aspirin use.

Finally, we assessed whether the expression of biomarkers at baseline differed by categories of a priori-selected biologically plausible factors, including age, sex, body mass index (BMI), smoking status, alcohol intake, red/processed meat intake, regular aspirin use, regular NSAID use, number of adenomas and advanced adenomas, diabetes diagnosis, and levels of interleukin (IL-10), IL-6, and tumor necrosis factor α (TNFa). Means, 95% confidence intervals (CI), and P values were calculated using general linear models adjusted for age, sex, BMI, and, study center. P values for trend for categorical

variables with more than two levels were calculated by treating the ordered categories as a continuous variable in the same general linear model.

All statistical analyses were conducted using SAS 9.4 statistical software. A two-sided *P*-value < 0.05 was considered statistically significant.

Results

Baseline patient characteristics

Baseline demographic, lifestyle, and adenoma characteristics of the 175 study participants by treatment arm are shown in Table 1. The mean age of all participants was 58 years and mean BMI was 29 kg/m^2. The demographic distribution was 62% male and 81% white. The average baseline levels of serum calcium and serum 25-OH vitamin D were 9.34 mg/dL and 23.61 ng/mL, respectively. The overall adherence to study tablets at the end of treatment were 89% in the calcium group, 91% in the vitamin D group, and 92% in the calcium and vit. D groups. There were observed differences ($P = 0.002$) in physical activity levels across the 4-arm treatment groups (Table 1).

Treatment effects of calcium and/or vitamin D on gut permeability markers

Mean serum 25-OH-vitamin D concentrations increased by 45% and 32% (all $P < 0.0001$) in the groups taking vitamin D and calcium at year 1 and the end of treatment, respectively (Supplementary Table S7). The mean percentage of pills taken in each treatment combination was 87% and 80% of all participants in each group took > 80% of their pills.

Changes in the gut barrier function biomarkers, alone or in combination, for each treatment comparison, are shown in Table 2. None of the treatment combinations statistically significantly affected individual or aggregate biomarkers of gut permeability (more details in Supplementary Tables S1-S3). Similar null results were found when we performed a secondary analysis by stratifying participants by median values of BMI (28.5), aspirin use (no. > 4/week), total calcium intake (162 elemental mgs/day), and serum 25-OH vitamin D (21.6 ng/mL; Supplementary Table S6).

Associations between baseline levels of gut permeability markers and demographic and lifestyle factors

Proportional differences of mean concentrations of gut barrier biomarkers across categories of *a priori*-selected participant characteristics at baseline are presented in Table 3 (a full version of the table, with means and confidence intervals, is included as Supplementary Tables S4 and S5). Women, on average, relative to men, had lower levels of permeability score (-10.77%, P= 0.01), and lower levels of LPS (-11.44%, P= 0.03), FLIC (-10.80%, P= 0.001), and IgG (-7.48%, P= 0.00). Participants who had >1 serving/day relative to those who had 0 servings/day of red or processed meat had higher levels of permeability score (10.02%, P= 0.03), FLIC (8.58%, *P=0.01*), and IgA (12.18%, P= 0.01). Participants who had a BMI >35 kg/m^2, relative to those who had a BMI < 22.5 kg/m^2, had higher levels of permeability score (48.70%, P_{trend}=0.17), LPS (71.82%, P_{trend}=0.06), FLIC (33.69%, P_{trend}=0.54), IgG (34.55%, P_{trend}=0.72), and IgA (61.92%, P_{trend}=0.11).

Discussion

Our results suggest that calcium and/or vitamin D have no substantial effect on levels of biomarkers of gut barrier function over one year and 3 to 5 years among individuals with previously diagnosed colorectal adenoma. Subgroup analysis based on BMI, aspirin use, calcium intake, and blood 25-(OH)-vitamin D concentrations at baseline yielded similar results. However, our results suggest that men, participants with higher overall adiposity, and those who have high red and processed meat intake may have higher levels of gut barrier markers, indicating greater gut permeability.

Gut microbiota may initiate colorectal cancer development by inducing epithelial DNA damage. The microbiota could then be replaced by bacteria that promote or hinder carcinogenesis and have a growth advantage in the tumor microenvironment (46). Gut permeability and inflammation are closely related and may be associated with the incidence of metabolic diseases and several types of cancer, including colorectal cancer (47,48). The biomarkers we studied may not be the most direct measurements for measuring gut permeability. However, there is evidence that antibodies against LPS and FLIC are elevated in patients with conditions that involve gut barrier dysfunction, such as Crohn's disease (49).

Additionally, we investigated associations of *a priori*–selected patient characteristics with the levels of gut barrier biomarkers at baseline. Women had lower levels of gut barrier biomarkers relative to men. This is in line with studies that indicate that women have higher innate and adaptive immune responses than men (50). This indicates that sex should be considered as a potential confounder and/or effect modifier in

future studies for the association of gut permeability and colorectal cancer. However, due to limited knowledge, there is a need for the need for more detailed analysis of the effects of sex differences in immune responses (51).

We also found that at baseline, participants who had >1 serving/day of red or processed meat, relative to those who had 0 serving/day, had higher levels of biomarkers of inflammation. Epidemiologic studies suggest that red and processed meat intake is associated with high colorectal cancer risk (52). Hypothesis about this association include that cooking meat at high temperature forms mutagenic and carcinogenic compounds. The heme iron in red meat may also promote carcinogenesis because it increases cell proliferation in the colonic mucosa. Biologic mechanisms have not been demonstrated yet, but there are studies that indicate that processed meat intake is associated with a higher colorectal cancer risk than unprocessed meat (53).

Lastly, participants who had a BMI >35 kg/m^2, relative to those who had a BMI < 22.5 kg/m^2, had higher levels of biomarkers of gut barrier function. BMI is an established risk factor for many forms of cancer, including colorectal cancer (54,55). Obesity may also play a role in colorectal cancer recurrence, treatment outcomes and survival (56). Yang et al. found that BMI and waist circumference are positively associated with colonic permeability, which is consistent with previous literature (40). Several human cross-sectional studies support a positive association of obesity with measurements of intestinal permeability, such as IgG against bacterial antigens, and LPS-binding protein (LBP) (57-59). One possible explanation is that obese individuals may have different gut microbiota and/or gut microbiome patterns. Gram-negative bacteria often increase in growth due to a high-fat diet and may have a greater ability to translocate across the gut

mucosa into the circulation when compared to gram-positive microbes. LPS is a major component of the outer membrane of Gram-negative bacteria; thus, obese individuals may have higher levels of anti-LPS and anti-flagellin Igs. However, these cross-sectional studies have not been able to assess the chronological sequence the succession between gut barrier dysfunction and obesity (40).

Strengths of this study include that the adherence to study treatment was high, and participants largely avoided taking vitamin D and calcium in substantial amounts outside the study, and the inclusion of novel gut permeability biomarkers. We also collected detailed questionnaire information and were able to evaluate associations of baseline demographic, diet, and lifestyle factors with gut permeability biomarkers, which may provide insights for future epidemiologic studies.

The trial was conducted among patients with a recent history of colorectal adenomas; thus, the results might not apply to persons without such a history. The vitamin D dose was lower than the dose many experts now recommend, with our dose of vitamin D (1000 IU per day) exceeding the currently recommended intake for adults up to 70 years of age (600 IU per day) (20). Most of our study participants were white, limiting our ability to detect differences in biomarker expression by race.

In summary, contrary to our hypothesis, supplementation with vitamin D and/or calcium did not modify biomarkers of gut barrier function over the period of 1 or 3 to 5 years, at least in sporadic colorectal adenoma patients. Our results suggest also suggest that sex, red or processed meat consumption, and BMI may be associated with gut permeability. These findings support continued investigation of potential modifiable

factors that could alter gut barrier function to inform development of treatable biomarkers of risk for colorectal neoplasms.

Table

Table 1. Selected baseline characteristics of the adjunct biomarker study participants (n = 175), by treatment group assignment

	Randomization to Ca and to vit. D (4-arm)					Randomization to vit. D only (2-arm)		
Characteristics[a]	**Calcium (n = 33)**	**Vit. D (n = 33)**	**Vit. D + Calcium (n = 36)**	**Placebo (n = 33)**	***P*[b]**	**Vit. D (n = 19)**	**Placebo (n = 21)**	***P*[c]**
Age, yrs	58.45 (6.91)	58.15 (7.50)	57.86 (6.30)	59.58 (5.98)	*0.74*	58.84 (6.59)	57.95 (5.34)	*0.64*
Women	9 (27.27)	7 (21.21)	4 (11.11)	6 (18.18)		19 (100)	21 (100)	
Regular aspirin use, n (%)[d]	19 (57.58)	10 (30.30)	11 (30.56)	15 (45.45)	*0.07*	5 (26.32)	5 (23.81)	*0.86*
Regular NSAID use, n (%)[e]	26 (78.79)	21 (63.64)	26 (72.22)	22 (66.67)	*0.56*	11 (57.89)	15 (71.43)	*0.38*
Diabetes, n (%)	6 (18.18)	3 (9.09)	4 (11.11)	3 (9.09)	*0.63*	3 (15.79)	3 (14.29)	*0.90*
Current smoker, n (%)	2 (6.06)	0	4 (11.11)	3 (9.09)	*0.29*	3 (15.79)	0	*0.06*
Former smoker, n (%)	8 (24.24)	14 (42.42)	14 (38.89)	14 (42.42)	*0.37*	6 (31.58)	7 (33.33)	*0.91*
White, n (%)	24 (72.73)	27 (81.82)	34 (97.14)	25 (75.76)	*0.22*	16 (84.21)	15 (71.43)	*0.49*
BMI, kg/m^2	31.45 (6.17)	28.99 (4.99)	29.39 (3.95)	28.60 (4.24)	*0.09*	27.43 (4.81)	29.26 (4.69)	*0.23*
IPAQ, n (%)[f]	1.82 (0.68)	2.30 (0.81)	2.50 (0.65)	2.12 (0.82)	*0.002*	2.11 (0.90)	1.90 (0.83)	*0.46*
Study Center,n (%)								*0.22*
GA	21 (63.64)	19 (57.58)	19 (52.78)	19 (57.58)	*0.84*	10 (52.63)	15 (71.43)	*0.22*
SC	12 (36.36)	14 (42.42)	17 (47.22)	14 (42.42)		9 (47.37)	6 (28.57)	
Highest Education Level, n (%)								
Some college or less	15 (45.45)	6 (18.18)	12 (33.33)	14 (42.42)	*0.09*	8 (42.11)	9 (42.86)	*0.96*
Associate's or other degrees	18 (54.55)	27 (81.82)	24 (66.67)	19 (57.58)		11 (57.89)	12 (57.14)	
Adenoma characteristics, n (%)								
Any adenomas	1.48 (0.87)	1.39 (0.66)	1.28 (0.57)	1.39 (0.56)	*0.65*	1.58 (0.96)	1.19 (0.68)	*0.15*
Advanced adenomas	0.24 (0.50)	0.36 (0.60)	0.33 (0.59)	0.45 (0.71)	*0.56*	0.16 (0.37)	0.10 (0.30)	*0.56*
Serrated polyps	0.39 (0.75)	0.15 (0.36)	0.42 (0.69)	0.30 (0.59)	*0.28*	0.32 (0.67)	0.29 (0.46)	*0.87*
Multivitamin use, n (%)	17 (51.52)	16 (48.48)	19 (52.78)	15 (45.45)	*0.94*	17 (89.47)	15 (71.43)	*0.16*
Dietary Intake								
Total calories, kcal/d[h]	1695.92 (559.98)	1493.70 (509.73)	1558.73 (561.54)	1462.88 (427.93)	*0.31*	1433.94 (589.85)	1351.11 (554.89)	*0.65*
Calcium, IU/d[g]	1114.88 (145.91)	99.06 (119.88)	113.29 (184.22)	93.64 (109.20)	*0.91*	608.37 (467.62)	485.88 (369.38)	*0.40*
Red or procd meat, serv/d	0.87 (0.71)	0.87 (0.77)	1.02 (0.67)	1.10 (0.68)	*0.46*	0.66 (0.55)	0.70 (0.69)	*0.85*
Vitamin D, IU/d[g]	171.88 (195.49)	200.00 (236.64)	225.00 (241.29)	175.00 (201.61)	*0.74*	500.00 (260.18)	416.00 (328.88)	*0.45*
Alcohol, drinks/d	0.62 (0.96)	0.83 (0.97)	0.83 (0.83)	0.79 (0.72)	*0.71*	0.36 (0.51)	0.55 (1.02)	*0.46*

Abbreviations: BMI, body mass index; d, day; IPAQ, International Physical Activity Questionnaire; IU/day, International Units/day; kcal, kilocalorie, No. number;

NSAID, nonsteroidal anti-inflammatory drug; procd, processed; serv, servings; Vit. D, vitamin D; Yrs, years.
[a]Data presented as means (SD) unless otherwise specified
[b]X^2 for categorical variables; general linear model for continuous variables
[c]X^2 for categorical variables; Student t test for continuous variables
[d]Regular aspiring use= no. ≥ 4/week
[e]Regular NSAID use= no. ≥ 4/week
[f]Missing data on 1 patient
[g]Total intake represents multivitamin and extra supplements
[h]Missing data on 10 patients

Table 2: Effects of calcium and/or vitamin D supplementation on circulating concentrations of gut barrier biomarkers at year 1 and end of treatment in the adjunct biomarker study participants (n = 175)

	Baseline			EOT			Abs. Tx EOT[b]		Δ tx EOT[c]
	n	Mean (SE)	*P*	n	Mean (SE)	*P*	Mean (SE)	*P*	
Permeability Score									
Calcium	69	6.10 (0.20)	*0.25*	68	5.19 (0.20)	*0.18*	-0.06 (0.40)	*0.88*	0.98
No calcium	66	6.42 (0.20)		63	5.58 (0.21)				
Vitamin D	88	6.73 (0.17)	*0.11*	85	5.13 (0.18)	*0.33*	0.15 (0.35)	*0.66*	0.87
No Vitamin D	87	6.13 (0.17)		87	5.37 (0.17)				
Vit. D + Calcium	55	5.52 (0.21)	*0.46*	54	4.98 (0.21)	*0.58*	0.05 (0.42)	*0.90*	1.01
Calcium only	54	5.74(0.21)		55	5.15 (0.21)				
LPS									
Calcium	69	2.65 (0.11)	*0.35*	68	2.22 (0.11)	*0.23*	-0.04 (0.22)	*0.85*	0.97
No calcium	66	2.80 (0.11)		63	2.41 (0.12)				
Vitamin D	88	2.45 (0.09)	*0.07*	85	2.20 (0.10)	*0.35*	0.11 (0.19)	*0.55*	1.04
No Vitamin D	87	2.69 (0.09)		87	2.33 (0.09)				
Vit. D + Calcium	55	2.30 (0.11)	*0.10*	54	2.08 (0.11)	*0.19*	0.06 (0.23)	*0.79*	1.01
Calcium only	54	2.57 (0.11)		55	2.29 (0.11)				
FLIC									
Calcium	69	3.45 (0.10)	*0.23*	68	2.98 (0.10)	*0.19*	-0.02 (0.21)	*0.93*	0.99
No calcium	66	3.63 (0.11)		63	3.17 (0.11)				
Vitamin D	88	3.28 (0.09)	*0.25*	85	2.93 (0.10)	*0.40*	0.04 (0.19)	*0.84*	1.00
No Vitamin D	87	3.43 (0.09)		87	3.05 (0.09)				
Vit. D + Calcium	55	3.22 (0.11)	*0.76*	54	2.91 (0.11)	*0.78*	-0.01 (0.23)	*0.98*	1.00
Calcium only	54	3.17 (0.11)		55	2.86 (0.11)				
IgG									
Calcium	69	2.86 (0.09)	*0.28*	68	2.42 (0.09)	*0.06*	-0.10 (0.18)	*0.57*	0.96
No calcium	66	3.00 (0.09)		63	2.65 (0.09)				
Vitamin D	88	2.70 (0.08)	*0.15*	85	2.43 (0.08)	*0.30*	0.04 (0.15)	*0.78*	1.01
No Vitamin D	87	2.86 (0.08)		87	2.54 (0.08)				
Vit. D + Calcium	55	2.59 (0.09)	*0.38*	54	2.36 (0.09)	*0.62*	0.05 (0.18)	*0.78*	1.02
Calcium only	54	2.71 (0.09)		55	2.43 (0.09)				
IgA									

Calcium	69	3.24 (0.15)	*0.36*	68	2.78 (0.15)	*0.47*	0.04 (0.30)	*0.90*	1.00
No calcium	66	3.43 (0.15)		63	2.93 (0.15)				
Vitamin D	88	3.03 (0.13)	*0.19*	85	2.70 (0.13)	*0.48*	0.11 (0.26)	*0.67*	1.03
No vitamin D	87	3.27 (0.13)		87	2.83 (0.13)				
Vit. D + Calcium	55	2.93 (0.16)	*0.65*	54	2.62 (0.16)	*0.65*	0.003 (0.31)	*0.99*	1.00
Calcium only	54	3.03 (0.16)		55	2.72 (0.16)				

Abbreviations: Abs., absolute; EOT, end of treatment; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; LPS, lipopolysaccharide; Tx, treatment; Vit. D, vitamin D.

[a]The effect of treatment agent on biomarker level was modeled using mixed linear models.
[b]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) – ([placebo group EOT − placebo group baseline]).
[c]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/ [treatment group baseline]) /([placebo group EOT) /(placebo group baseline]) .

Table 3: Mean baseline plasma levels of gut permeability biomarkers by demographic and lifestyle factors[a]

		Permeability Score		LPS		FLIC		IgG		IgA	
Characteristics	**N**	**% Diff[b]**	***P*[c]**	**% Diff**	***P***	**% Diff**	***P***	**% Diff**	***P***	**% Diff**	***P***
Age, years											
≤55	57	Ref.		Ref.		Ref.		Ref.		Ref.	
55.1-60	44	-0.67		0.78		-1.18		-0.37		-0.95	
60.1-65	38	-5.37		-5.84		-5.03		-6.45		-4.42	
>65	36	0.50	*0.84*	2.33	*0.74*	-0.30	*0.97*	-2.15	*0.64*	3.15	*0.59*
Sex											
Men	109	Ref.		Ref.		Ref.		Ref.		Ref.	
Women	66	14.35	*0.01*	-11.44	*0.03*	-10.80	*0.001*	-7.48	*0.001*	-8.51	*0.13*
Study center											
GA	103	Ref.		Ref.		Ref.		Ref.		Ref.	
SC	72	8.32	*0.07*	10.29	*0.07*	-6.83	*0.11*	1.85	*0.63*	14.29	*0.03*
BMI, kg/m^2											
<22.5	8	Ref.		Ref.		Ref.		Ref.		Ref.	
22.5-25	24	26.96		34.25		22.22		25.91		28.45	
25-27.5	36	36.30		51.93		26.16		33.18		39.75	
27.5-30	45	28.04		44.75		17.20		25.45		30.96	
30-35	38	13.91		21.55		8.96		12.73		15.48	
>35	24	48.70	*0.17*	71.82	*0.06*	33.69	*0.54*	34.55	*0.72*	61.92	*0.11*
Intake of red or processed meat, serv/d											
0	14	Ref.		Ref.		Ref.		Ref.		Ref.	
0.1-0.5	50	8.51		13.22		4.95		4.65		12.18	
0.51-1.0	48	18.53		16.74		19.80		6.98		29.89	
>1	63	10.02	*0.03*	11.89	*0.18*	8.58	*0.01*	8.14	*0.31*	12.18	*0.01*

Abbreviations: BMI, body mass index; d, day; FLIC, flagellin; No., number, LPS, lipopolysaccharide; IgA, immunoglobulin A; IgG, immunoglobulin G; immunoglobulin A; IgG, immunoglobulin G; procd, processed; serv, servings.

[a]All means, SEs, and P-values were calculated using ANCOVA. Models for all variables were adjusted for by age, sex, center, and BMI.

[b][b]% difference= [(comparison mean – reference mean)/reference mean] x 100%

[c]P-value is for trend if the explanatory variable has more than two categories.

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Supplementary Tables

Supplementary Table 1: Mean levels of gut barrier biomarkers at baseline, year 1, and end of treatment according to treatment assignment[a]

	Baseline			Year 1			EOT			Abs. Tx Year 1[b]		Abs. Tx EOT[c]		Δ tx Year 1[d]	Δ tx EOT[e]
	n	Mean (SE)	*P*	n	Mean (SE)	*P*	n	Mean (SE)	*P*	Mean (SE)	*P*	Mean (SE)	*P*		
Permeability Score															
Calcium	69	6.10 (0.20)	*0.25*	69	5.94 (0.20)	*0.10*	68	5.19 (0.20)	*0.18*	-0.14 (0.40)	*0.72*	-0.06 (0.40)	*0.88*	0.98	0.98
No calcium	66	6.42 (0.20)		66	6.41 (0.20)		63	5.58 (0.21)							
Vitamin D	88	6.73 (0.17)	*0.11*	87	5.80 (0.17)	*0.52*	85	5.13 (0.18)	*0.33*	0.23 (0.35)	*0.50*	0.15 (0.35)	*0.66*	0.89	0.87
No Vitamin D	87	6.13 (0.17)		87	5.96 (0.17)		87	5.37 (0.17)							
Vit. D + Calcium	55	5.52 (0.21)	*0.46*	54	5.52 (0.21)	*0.78*	54	4.98 (0.21)	*0.58*	0.14 (0.42)	*0.75*	0.05 (0.42)	*0.90*	1.03	1.01
Calcium only	54	5.74(0.21)		54	5.60 (0.21)		55	5.15 (0.21)							
LPS															
Calcium	69	2.65 (0.11)	*0.35*	69	2.58 (0.11)	*0.17*	68	2.22 (0.11)	*0.23*	-0.07 (0.22)	*0.76*	-0.04 (0.22)	*0.85*	0.97	0.97
No calcium	66	2.80 (0.11)		66	2.80 (0.11)		63	2.41 (0.12)							
Vitamin D	88	2.45 (0.09)	*0.07*	87	2.50 (0.09)	*0.47*	85	2.20 (0.10)	*0.35*	0.14 (0.19)	*0.45*	0.11 (0.19)	*0.55*	1.06	1.04
No Vitamin D	87	2.69 (0.09)		87	2.60 (0.09)		87	2.33 (0.09)							
Vit. D + Calcium	55	2.30 (0.11)	*0.10*	54	2.31 (0.11)	*0.25*	54	2.08 (0.11)	*0.19*	0.08 (0.23)	*0.72*	0.06 (0.23)	*0.79*	1.03	1.01
Calcium only	54	2.57 (0.11)		54	2.50 (0.11)		55	2.29 (0.11)							
FLIC															
Calcium	69	3.45 (0.10)	*0.23*	69	3.36 (0.10)	*0.09*	68	2.98 (0.10)	*0.19*	-0.07 (0.21)	*0.73*	-0.02 (0.21)	*0.93*	0.98	0.99
No calcium	66	3.63 (0.11)		66	3.61 (0.11)		63	3.17 (0.11)							
Vitamin D	88	3.28 (0.09)	*0.25*	87	3.30 (0.09)	*0.64*	85	2.93 (0.10)	*0.40*	0.09 (0.19)	*0.63*	0.04 (0.19)	*0.84*	1.03	1.00
No Vitamin D	87	3.43 (0.09)		87	3.36 (0.09)		87	3.05 (0.09)							
Vit. D + Calcium	55	3.22 (0.11)	*0.76*	54	3.21 (0.11)	*0.52*	54	2.91 (0.11)	*0.78*	0.05 (0.23)	*0.81*	-0.01 (0.23)	*0.98*	1.02	1.00
Calcium only	54	3.17 (0.11)		54	3.11 (0.11)		55	2.86 (0.11)							
IgG															
Calcium	69	2.86 (0.09)	*0.28*	69	2.78 (0.09)	*0.07*	68	2.42 (0.09)	*0.06*	-0.09 (0.17)	*0.61*	-0.10 (0.18)	*0.57*	0.97	0.96
No calcium	66	3.00 (0.09)		66	3.00 (0.09)		63	2.65 (0.09)							
Vitamin D	88	2.70 (0.08)	*0.15*	87	2.72 (0.08)	*0.41*	85	2.43 (0.08)	*0.30*	0.07 (0.15)	*0.66*	0.04 (0.15)	*0.78*	1.03	1.01
No Vitamin D	87	2.86 (0.08)		87	2.81 (0.08)		87	2.54 (0.08)							

Vit. D + Calcium	55	2.59 (0.09)	*0.38*	54	2.59 (0.09)	*0.69*	54	2.36 (0.09)	*0.62*	0.06 (0.18)	*0.73*	0.05 (0.18)	*0.78*	1.03	1.02
Calcium only	54	2.71 (0.09)		54	2.64 (0.09)		55	2.43 (0.09)							
IgA															
Calcium	69	3.24 (0.15)	*0.36*	69	3.16 (0.15)	*0.24*	68	2.78 (0.15)	*0.47*	-0.05 (0.30)	*0.85*	0.04 (0.30)	*0.90*	0.98	1.00
No calcium	66	3.43 (0.15)		66	3.41 (0.15)		63	2.93 (0.15)							
Vitamin D	88	3.03 (0.13)	*0.19*	87	3.09 (0.13)	*0.69*	85	2.70 (0.13)	*0.48*	0.17 (0.25)	*0.51*	0.11 (0.26)	*0.67*	1.08	1.03
No Vitamin D	87	3.27 (0.13)		87	3.09 (0.13)		87	2.83 (0.13)							
Vit. D + Calcium	55	2.93 (0.16)	*0.65*	54	2.93 (0.16)	*0.89*	54	2.62 (0.16)	*0.65*	0.07 (0.31)	*0.82*	0.003 (0.31)	*0.99*	1.03	1.00
Calcium only	54	3.03 (0.16)		54	2.93 (0.16)		55	2.72 (0.16)							
FLIC-IgA															
Calcium	69	1.72 (0.08)	*0.38*	69	1.68 (0.08)	*0.22*	68	1.50 (0.08)	*0.50*	-0.04 (0.15)	*0.80*	0.02 (0.15)	*0.90*	0.98	1.01
No calcium	66	1.82 (0.08)		66	1.81 (0.08)		63	1.57 (0.08)							
Vitamin D	88	1.65 (0.07)	*0.31*	87	1.67 (0.07)	*0.70*	85	1.47 (0.07)	*0.42*	0.06 (0.13)	*0.66*	0.02 (0.13)	*0.89*	1.04	1.01
No Vitamin D	87	1.75 (0.07)		87	1.70 (0.07)		87	1.55 (0.07)							
Vit. D + Calcium	55	1.63 (0.08)	*0.98*	54	1.62 (0.08)	*0.85*	54	1.47 (0.08)	*0.86*	0.03 (0.17)	*0.88*	-0.02 (0.17)	*0.92*	1.02	0.99
Calcium only	54	1.63 (0.08)		54	1.59 (0.08)		55	1.49 (0.08)							
FLIC-IgG															
Calcium	69	1.73 (0.05)	*0.26*	69	1.69 (0.05)	*0.11*	68	1.48 (0.05)	*0.10*	-0.03 (0.10)	*0.74*	-0.04 (0.10)	*0.71*	0.98	0.97
No calcium	66	1.81 (0.05)		66	1.80 (0.05)		63	1.60 (0.05)							
Vitamin D	88	1.63 (0.05)	*0.38*	87	1.63 (0.05)	*0.70*	85	1.46 (0.05)	*0.56*	0.03 (0.09)	*0.73*	0.02 (0.09)	*0.84*	1.02	1.01
No Vitamin D	87	1.69 (0.05)		87	1.66 (0.05)		87	1.50 (0.05)							
Vit. D + Calcium	55	1.59 (0.05)	*0.49*	54	1.59 (0.06)	*0.30*	54	1.44 (0.06)	*0.40*	0.03 (0.11)	*0.80*	0.01 (0.11)	*0.91*	1.02	1.01
Calcium only	54	1.54 (0.06)		54	1.51 (0.06)		55	1.38 (0.05)							
LPS-IgA															
Calcium	69	1.51 (0.08)	*0.37*	69	1.48 (0.08)	*0.30*	68	1.28 (0.08)	*0.48*	-0.02 (0.15)	*0.92*	0.02 (0.16)	*0.90*	0.99	1.00
No calcium	66	1.61 (0.08)		66	1.60 (0.08)		63	1.36 (0.08)							
Vitamin D	88	1.38 (0.07)	*0.13*	87	1.42 (0.07)	*0.71*	85	1.23 (0.07)	*0.57*	0.11 (0.13)	*0.41*	0.09 (0.13)	*0.50*	1.07	1.06
No Vitamin D	87	1.52 (0.07)		87	1.46 (0.07)		87	1.28 (0.07)							
Vit. D + Calcium	55	1.30 (0.08)	*0.39*	54	1.32 (0.08)	*0.65*	54	1.16 (0.08)	*0.49*	0.05 (0.16)	*0.77*	0.02 (0.16)	*0.90*	1.04	1.01
Calcium only	54	1.40 (0.08)		54	1.37 (0.08)		55	1.24 (0.08)							
LPS-IgG															
Calcium	69	1.14 (0.05)	*0.50*	69	1.09 (0.05)	*0.17*	68	0.94 (0.05)	*0.15*	-0.05 (0.11)	*0.62*	-0.06 (0.11)	*0.57*	0.95	0.93
No calcium	66	1.19 (0.05)		66	1.20 (0.05)		63	1.05 (0.06)							

Vitamin D	88	1.07 (0.05)	*0.13*	87	1.08 (0.05)	*0.34*	85	0.97 (0.05)	*0.27*	0.04 (0.09)	*0.70*	0.03 (0.09)	*0.79*	1.03	1.01
No Vitamin D	87	1.17 (0.05)		87	1.15 (0.05)		87	1.05 (0.05)							
Vit. D + Calcium	55	1.00 (0.06)	*0.04*	54	1.00 (0.06)	*0.10*	54	0.92 (0.06)	*0.11*	0.04 (0.12)	*0.75*	0.04 (0.11)	*0.73*	1.04	1.03
Calcium only	54	1.17 (0.06)		54	1.13 (0.06)		55	1.05 (0.06)							

Abbreviations: Diff, difference; EOT, end of treatment; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; LPS, lipopolysaccharide; tx, treatment; Vit. D, vitamin D.

[a]The unadjusted effect of treatment agent on biomarker level was modeled using mixed linear models.

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 2: Mean levels of gut barrier biomarkers at baseline, year 1, and end of treatment according to treatment assignment[a]

	n	Baseline Mean (SE)	*P*	n	Year 1 Mean (SE)	*P*	n	EOT Mean (SE)	*P*	Abs. Tx Year 1[b] Mean (SE)	*P*	Abs. Tx EOT[c] Mean (SE)	*P*	Δ tx Year 1[d]	Δ tx EOT[e]
LPS															
4-arm Groups:															
Calcium	33	2.89 (0.16)	*0.99*	33	2.74 (0.16)	*0.87*	33	2.40 (0.16)	*0.99*	-0.03 (0.32)	*0.92*	0.002 (0.32)	*1.00*	0.99	1.00
Vitamin D	33	2.70 (0.16)	*0.39*	33	2.82 (0.16)	*0.84*	31	2.42 (0.16)	*0.94*	0.24 (0.31)	*0.45*	0.21 (0.32)	*0.51*	1.09	1.08
Vit. D + Calcium	36	2.43 (0.15)	*0.03*	36	2.43 (0.15)	*0.12*	35	2.05 (0.15)	*0.11*	0.13 (0.31)	*0.68*	0.11 (0.31)	*0.72*	0.99	1.02
Placebo	33	2.89 (0.16)		33	2.77 (0.16)		32	2.40 (0.16)							
2-arm Groups:															
Vitamin D	19	2.06 (0.15)	*0.98*	18	2.07 (0.15)	*0.79*	19	2.13 (0.15)	*0.95*	-0.05 (0.29)	*0.87*	0.02 (0.28)	*0.95*	0.98	1.00
Placebo	21	2.06 (0.14)		21	2.12 (0.14)		22	2.12 (0.14)							
FLIC															
4-arm Groups:															
Calcium	33	3.53 (0.15)	*0.11*	33	3.37 (0.15)	*0.05*	33	3.03 (0.15)	*0.12*	-0.07 (0.30)	*0.82*	0.01 (0.30)	*0.98*	0.98	0.99
Vitamin D	33	3.39 (0.15)	*0.02*	33	3.45 (0.15)	*0.12*	31	2.98 (0.15)	*0.07*	0.15 (0.30)	*0.62*	0.09 (0.30)	*0.76*	1.04	1.01
Vit. D + Calcium	36	3.38 (0.14)	*0.02*	36	3.36 (0.14)	*0.04*	35	2.93 (0.14)	*0.04*	0.06 (0.29)	*0.83*	0.05 (0.29)	*0.86*	1.02	1.00
Placebo	33	3.87 (0.15)		33	3.78 (0.15)		32	3.36 (0.15)							
2-arm Groups:															
Vitamin D	19	2.92 (0.17)	*0.20*	18	2.92 (0.18)	*0.36*	19	2.87 (0.17)	*0.28*	-0.08 (0.34)	*0.81*	-0.05 (0.34)	*0.89*	0.97	0.98
Placebo	21	2.61 (0.17)		21	2.69 (0.17)		22	2.61 (0.16)							
IgG															
4-arm Groups:															
Calcium	33	2.97 (0.12)	*0.44*	33	2.82 (0.12)	*0.15*	33	2.45 (0.12)	*0.10*	-0.12 (0.25)	*0.63*	-0.15 (0.25)	*0.54*	0.96	0.93
Vitamin D	33	2.89 (0.12)	*0.22*	33	2.93 (0.12)	*0.40*	31	2.55 (0.13)	*0.29*	0.07 (0.25)	*0.78*	0.03 (0.25)	*0.92*	1.02	1.00
Vit. D + Calcium	36	2.77 (0.12)	*0.05*	36	2.74 (0.12)	*0.06*	35	2.38 (0.12)	*0.04*	0.01 (0.24)	*0.97*	-0.03 (0.25)	*0.92*	1.00	0.97
Placebo	33	3.11 (0.12)		33	3.08 (0.12)		32	2.75 (0.13)							
2-arm Groups:															
Vitamin D	19	2.26 (0.12)	*0.84*	18	2.28 (0.13)	*0.62*	19	2.32 (0.12)	*0.73*	-0.05 (0.24)	*0.83*	-0.02 (0.24)	*0.92*	0.98	1.01
Placebo	21	2.29 (0.12)		21	2.36 (0.12)		22	2.38 (0.11)							
IgA															
4-arm Groups:															
Calcium	33	3.45 (0.21)	*0.49*	33	3.29 (0.21)	*0.53*	33	2.97 (0.21)	*0.88*	0.02 (0.42)	*0.96*	0.16 (0.43)	*0.70*	1.00	1.04
Vitamin D	33	3.20 (0.21)	*0.13*	33	3.34 (0.12)	*0.64*	31	2.84 (0.22)	*0.56*	0.32 (0.42)	*0.45*	0.28 (0.43)	*0.52*	1.10	1.08

Vit. D + Calcium	36	3.04 (0.20)	*0.04*	36	3.05 (0.20)	*0.14*	35	2.59 (0.21)	*0.15*	0.18 (0.41)	*0.66*	0.19 (0.42)	*0.65*	1.06	1.03
Placebo	33	3.66 (0.21)		33	3.48 (0.21)		32	3.02 (0.22)							
2-arm Groups:															
Vitamin D	19	2.71 (0.22)	*0.26*	18	2.71 (0.22)	*0.40*	19	2.68 (0.22)	*0.26*	-0.08 (0.43)	*0.85*	-0.01 (0.42)	*0.99*	0.97	1.00
Placebo	21	2.38 (0.21)		21	2.45 (0.21)		22	2.35 (0.20)							

Abbreviations: Diff, difference; EOT, end of treatment; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; LPS, lipopolysaccharide; Tx, treatment; Vit. D, vitamin D.

[a]The unadjusted effect of treatment agent on biomarker level was modeled using mixed linear models.

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 – placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 3: Mean levels of gut barrier biomarkers at baseline, year 1, and end of treatment according to treatment assignment[a]

	Baseline			Year 1			EOT			Abs. Tx Year 1[b]		Abs. Tx EOT[c]		Δ tx Year 1[d]	Δ tx EOT[e]
	n	Mean (SE)	*P*	N	Mean (SE)	*P*	n	Mean (SE)	*P*	Mean (SE)	*P*	Mean (SE)	*P*		
Permeability Score															
4-arm Groups:															
Calcium	33	6.42 (0.28)	*0.39*	33	6.11 (0.28)	*0.27*	33	5.43 (0.28)	*0.41*	-0.10 (0.57)	*0.86*	0.01 (0.57)	*0.99*	0.98	0.99
Vitamin D	33	6.09 (0.28)	*0.09*	33	6.27 (0.28)	*0.48*	31	5.39 (0.29)	*0.37*	0.38 (0.57)	*0.50*	0.30 (0.57)	*0.60*	1.06	1.04
Vit. D + Calcium	36	5.81 (0.27)	*0.02*	36	5.79 (0.27)	*0.05*	35	4.97 (0.28)	*0.05*	0.19 (0.56)	*0.73*	0.16 (0.56)	*0.77*	1.03	1.00
Placebo	33	6.76 (0.28)		33	6.55 (0.28)		32	5.76 (0.29)							
2-arm Groups:															
Vitamin D	19	4.97 (0.29)	*0.44*	18	4.98 (0.29)	*0.67*	19	5.00 (0.29)	*0.48*	-0.13 (0.56)	*0.82*	-0.03 (0.55)	*0.96*	0.97	0.99
Placebo	21	4.67 (0.27)		21	4.81 (0.27)		22	4.73 (0.27)							
FLIC-IgA															
4-arm Groups:															
Calcium	33	1.79 (0.11)	*0.35*	33	1.72 (0.11)	*0.29*	33	1.57 (0.11)	*0.59*	-0.02 (0.22)	*0.94*	0.06 (0.22)	*0.78*	0.99	1.03
Vitamin D	33	1.70 (0.11)	*0.11*	33	1.74 (0.11)	*0.39*	31	1.49 (0.11)	*0.28*	0.11 (0.22)	*0.61*	0.07 (0.22)	*0.74*	1.06	1.02
Vit. D + Calcium	36	1.66 (0.10)	*0.06*	36	1.64 (0.10)	*0.12*	35	1.43 (0.11)	*0.14*	0.05 (0.21)	*0.83*	0.05 (0.22)	*0.81*	1.02	1.01
Placebo	33	1.94 (0.11)		33	1.88 (0.11)		32	1.66 (0.11)							
2-arm Groups:															
Vitamin D	19	1.57 (0.14)	*0.29*	18	1.57 (0.14)	*0.39*	19	1.53 (0.13)	*0.32*	-0.03 (0.26)	*0.90*	-0.02 (0.26)	*0.95*	0.98	0.99
Placebo	21	1.37 (0.13)		21	1.40 (0.13)		22	1.35 (0.12)							
FLIC-IgG															
4-arm Groups:															
Calcium	33	1.73 (0.07)	*0.06*	33	1.65 (0.07)	*0.02*	33	1.46 (0.07)	*0.02*	-0.05 (0.15)	*0.72*	-0.05 (0.15)	*0.71*	0.97	0.95
Vitamin D	33	1.69 (0.07)	*0.02*	33	1.70 (0.07)	*0.05*	31	1.49 (0.08)	*0.04*	0.04 (0.15)	*0.80*	0.02 (0.15)	*0.89*	1.02	1.00
Vit. D + Calcium	36	1.72 (0.07)	*0.04*	36	1.71 (0.07)	*0.06*	35	1.50 (0.07)	*0.04*	0.02 (0.14)	*0.90*	-0.002 (0.14)	*0.99*	1.01	0.98
Placebo	33	1.93 (0.07)		33	1.90 (0.07)		32	1.71 (0.07)							
2-arm Groups:															
Vitamin D	19	1.35 (0.06)	*0.19*	18	1.35 (0.06)	*0.45*	19	1.34 (0.06)	*0.33*	-0.05 (0.12)	*0.70*	-0.03 (0.12)	*0.80*	0.95	0.98
Placebo	21	1.23 (0.06)		21	1.29 (0.06)		22	1.25 (0.06)							
LPS-IgA															

4-arm Groups:															
Calcium	33	1.65 (0.11)	*0.69*	33	1.57 (0.11)	*0.86*	33	1.40 (0.11)	*0.80*	0.04 (0.22)	*0.87*	0.10 (0.22)	*0.64*	0.97	0.86
Vitamin D	33	1.50 (0.11)	*0.17*	33	1.59 (0.11)	*0.97*	31	1.35 (0.11)	*0.96*	0.21 (0.22)	*0.35*	0.20 (0.22)	*0.36*	1.08	0.91
Vit. D + Calcium	36	1.38 (0.11)	*0.03*	36	1.40 (0.11)	*0.20*	35	1.16 (0.11)	*0.21*	0.14 (0.22)	*0.53*	0.14 (0.22)	*0.53*	1.03	0.85
Placebo	33	1.38 (0.11)		33	1.36 (0.11)		32	1.36 (0.11)							
2-arm Groups:															
Vitamin D	19	1.15 (0.10)	*0.31*	18	1.14 (0.10)	*0.49*	19	1.15 (0.10)	*0.27*	-0.05 (0.20)	*0.82*	0.01 (0.19)	*0.96*	0.94	1.01
Placebo	21	1.00 (0.10)		21	1.05 (0.10)		22	0.99 (0.09)							
LPS-IgG															
4-arm Groups:															
Calcium	33	1.24 (0.08)	*0.59*	33	1.16 (0.08)	*0.92*	33	1.00 (0.08)	*0.71*	-0.07 (0.15)	*0.65*	-0.10 (0.15)	*0.52*	0.94	0.92
Vitamin D	33	1.20 (0.08)	*0.86*	33	1.23 (0.08)	*0.63*	31	1.06 (0.08)	*0.82*	0.03 (0.15)	*0.83*	0.006 (0.16)	*0.97*	1.03	1.00
Vit. D + Calcium	36	1.04 (0.07)	*0.21*	36	1.03 (0.07)	*0.18*	35	0.88 (0.07)	*0.15*	-0.01 (0.15)	*0.95*	-0.02 (0.15)	*0.88*	1.00	0.96
Placebo	33	1.18 (0.08)		33	1.17 (0.08)		32	1.04 (0.08)							
2-arm Groups:															
Vitamin D	19	0.91 (0.09)	*0.24*	18	0.92 (0.09)	*0.24*	19	0.99 (0.09)	*0.26*	-0.003 (0.18)	*0.99*	0.008 (0.17)	*0.96*	1.00	1.02
Placebo	21	1.06 (0.09)		21	1.07 (0.09)		22	1.13 (0.08)							

Abbreviations: Diff, difference; EOT, end of treatment; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; LPS, lipopolysaccharide; Tx, treatment; Vit. D, vitamin D.

[a]The unadjusted effect of treatment agent on biomarker level was modeled using mixed linear models.

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 4: Mean baseline plasma levels of gut permeability biomarkers by demographic and lifestyle factors[a]

		LPS		FLIC		IgG		IgA	
Characteristics	**N**	**Mean (95% CI)**	***P***	**Mean (95% CI)**	***P***	**Mean (95% CI)**	***P***	**Mean (95% CI)**	***P***
Age, yrs									
≤55	57	2.57 (2.34-2.81)		3.38 (3.15-3.62)		2.79 (2.60-2.98)		3.17 (2.84-3.50)	
55.1-60	44	2.59 (2.32-2.85)		3.34 (3.07-3.60)		2.78 (2.57-2.99)		3.14 (2.78-3.51)	
60.1-65	38	2.42 (2.14-2.71)		3.21 (2.93-3.50)		2.61 (2.38-2.84)		3.03 (2.63-3.42)	
>65	36	2.63 (2.33-2.92)	*0.74*	3.37 (3.07-3.67)	*0.97*	2.73 (2.49-2.97)	*0.64*	3.27 (2.85-3.68)	*0.59*
Sex									
Men	109	2.71 (2.54-2.87)		3.52 (3.35-3.69)		2.94 (2.80-3.08)		3.29 (3.06-3.53)	
Women	66	2.40 (2.19-2.62)	*0.03*	3.14 (2.93-3.36)	*0.001*	2.72 (2.53-2.91)	*5E-04*	3.01 (2.71-3.30)	*0.13*
Center									
GA	103	2.43 (2.26-2.61)		3.22 (3.05-3.40)		2.71 (2.57-2.85)		2.94 (2.70-3.19)	
SC	72	2.68 (2.47-2.89)	*0.07*	3.44 (3.23-3.65)	*0.11*	2.76 (2.60-2.93)	*0.63*	3.36 (3.07-3.64)	*0.03*
BMI, kg/m^2									
≤25	32	2.28 (1.97-2.58)		3.26 (2.95-3.57)		2.63 (2.38-2.88)		2.91 (2.47-3.34)	
25.1-29.9	81	2.68 (2.47-2.88)		3.38 (3.18-3.58)		2.83 (2.67-2.99)		3.22 (2.94-3.50)	
≥30	62	2.56 (2.33-2.78)	*0.28*	3.31 (3.08-3.53)	*0.92*	2.67 (2.49-2.85)	*0.91*	3.19 (2.88-3.51)	*0.36*
BMI, kg/m^2									
<22.5	8	1.81 (1.22-2.40)		2.79 (2.19-3.39)		2.20 (1.72-2.68)		2.39 (1.56-3.23)	
22.5-25	24	2.43 (2.09-2.76)		3.41 (3.07-3.76)		2.77 (2.49-3.04)		3.07 (2.59-3.55)	
25-27.5	36	2.75 (2.46-3.03)		3.52 (3.23-3.82)		2.93 (2.70-3.16)		3.34 (2.93-3.75)	
27.5-30	45	2.62 (2.37-2.86)		3.27 (3.02-3.53)		2.76 (2.56-2.96)		3.13 (2.77-3.48)	
30-35	38	2.20 (1.93-2.47)		3.04 (2.76-3.32)		2.48 (2.26-2.70)		2.76 (2.37-3.14)	
>35	24	3.11 (2.78-3.45)	*0.06*	3.73 (3.38-4.07)	*0.54*	2.96 (2.69-3.24)	*0.72*	3.87 (3.40-4.35)	*0.11*
Smoking status									
Never	100	2.61 (2.43-2.79)		3.33 (3.15-3.51)		2.84 (2.70-2.98)		3.10 (2.85-3.35)	
Former	63	2.52 (2.29-2.75)		3.27 (3.04-3.51)		2.54 (2.36-2.72)		3.25 (2.93-3.58)	
Smoker	12	2.28 (1.78-2.78)	*0.56*	3.60 (3.10-4.11)	*0.50*	2.82 (2.42-3.21)	*0.13*	3.07 (2.37-3.77)	*0.57*
Alcohol intake, drinks/d									
Non-drinker	61	2.65 (2.42-2.88)		3.32 (3.09-3.55)		2.85 (2.67-3.03)		3.12 (2.80-3.44)	
0.1-0.5	38	2.52 (2.23-2.81)		3.41 (3.11-3.70)		2.79 (2.56-3.02)		3.14 (2.73-3.54)	
0.51-1.0	32	2.45 (2.12-2.78)		3.44 (3.11-3.77)		2.59 (2.33-2.85)		3.30 (2.84-3.76)	

1.01-2.0	26	2.51 (2.15-2.87)		3.32 (2.96-3.68)		2.76 (2.48-3.04)		3.07 (2.58-3.57)	
>2.0	18	2.45 (2.02-2.87)	*0.95*	3.05 (2.63-3.48)	*0.68*	2.34 (2.01-2.68)	*0.36*	3.16 (2.57-3.75)	*0.99*
Intake of red or procd meat, serv/d									
0	14	2.27 (1.79-2.75)		3.03 (2.55-3.50)		2.58 (2.19-2.97)		2.71 (2.05-3.37)	
0.1-0.5	50	2.57 (2.32-2.81)		3.18 (2.93-3.42)		2.70 (2.50-2.90)		3.04 (2.70-3.38)	
0.51-1.0	48	2.65 (2.39-2.90)		3.63 (3.38-3.88)		2.76 (2.55-2.96)		3.52 (3.17-3.86)	
>1	63	2.54 (2.30-2.77)	*0.18*	3.29 (3.06-3.52)	*0.01*	2.79 (2.60-2.97)	*0.31*	3.04 (2.72-3.36)	*0.01*
NSAID and/or aspirin use									
No	70	2.54 (2.33-2.75)		3.28 (3.07-3.49)		2.79 (2.62-2.96)		3.03 (2.74-3.32)	
Yes	105	2.57 (2.39-2.75)	*0.83*	3.36 (3.19-3.54)	*0.56*	2.70 (2.55-2.84)	*0.40*	3.24 (2.99-3.48)	*0.28*
NSAID and/or aspirin use ≥ 4 times/week									
No	54	2.54 (2.30-2.79)		3.33 (3.08-3.57)		2.77 (2.58-2.97)		3.10 (2.76-3.44)	
Yes	121	2.56 (2.40-2.72)	*0.92*	3.33 (3.17-3.49)	*0.97*	2.72 (2.59-2.85)	*0.65*	3.17 (2.95-3.40)	*0.72*
No. of adenomas									
1	126	2.58 (2.42-2.74)		3.37 (3.21-3.53)		2.75 (2.62-2.88)		3.21 (2.98-.343)	
≥2	49	2.48 (2.22-2.74)	*0.49*	3.22 (2.96-3.48)	*0.33*	2.70 (2.49-2.91)	*0.70*	3.00 (2.64-3.36)	*0.33*
Advanced adenoma									
No	128	2.58 (2.42-2.75)		3.34 (3.18-3.50)		2.79 (2.66-2.92)		3.14 (2.91-3.36)	
Yes	43	2.47 (2.19-2.75)	*0.48*	3.31 (3.03-3.59)	*0.87*	2.60 (2.38-2.82)	*0.15*	3.18 (2.79-3.57)	*0.85*
Serrated polyps									
No	390	2.57 (2.41-2.73)		3.29 (3.13-3.45)		2.73 (2.61-2.86)		3.12 (2.90-3.34)	
Yes	131	2.51 (2.25-2.78)	*0.72*	3.45 (3.19-3.72)	*0.30*	2.74 (2.53-2.96)	*0.95*	3.22 (2.85-3.59)	*0.65*
Diabetes									
No	153	2.57 (2.43-2.72)		3.34 (3.19-3.49)		2.74 (2.63-2.86)		3.17 (2.96-3.37)	
Yes	22	2.43 (2.05-2.81)	*0.48*	3.29 (2.90-3.67)	*0.81*	2.69 (2.38-2.99)	*0.73*	3.03 (2.50-3.56)	*0.63*
Cytokine IL-6									
≤379	46	2.55 (2.29-2.82)		3.47 (3.20-3.74)		2.81 (2.59-3.03)		3.21 (2.84-3.58)	
379-468	46	2.57 (2.31-2.83)		3.38 (3.11-3.64)		2.76 (2.55-2.98)		3.18 (2.82-3.54)	
468-625	45	2.58 (2.32-2.85)		3.30 (3.03-3.56)		2.75 (2.53-2.96)		3.13 (2.77-3.50)	
>625	45	2.56 (2.29-2.82)	*0.94*	3.27 (3.00-3.55)	*0.83*	2.72 (2.50-2.94)	*0.86*	3.11 (2.74-3.48)	*0.95*
Cytokine IL-10									
≤381	37	2.63 (2.34-2.92)		3.49 (3.19-3.78)		2.78 (2.54-3.01)		3.34 (2.95-3.73)	
381-446	36	2.67 (2.38-2.97)		3.42 (3.12-3.72)		2.73 (2.50-2.97)		3.36 (2.96-3.76)	

446-508	37	2.43 (2.14-2.71)		3.27 (2.98-3.57)		2.83 (2.60-3.06)		2.87 (2.48-3.26)	
508-607	36	2.44 (2.14-2.73)		3.19 (2.89-3.49)		2.57 (2.34-2.81)		3.05 (2.65-3.46)	
>607	36	2.66 (2.37-2.95)	*0.39*	3.40 (3.10-3.70)	*0.34*	2.88 (2.65-3.12)	*0.27*	3.18 (2.77-3.58)	*0.35*
Cytokine TNFA									
≤1118	46	2.61 (2.33-2.88)		3.43 (3.16-3.71)		2.86 (2.64-3.08)		3.18 (2.81-3.55)	
1118-1370	46	2.62 (2.36-2.88)		3.44 (3.18-3.71)		2.71 (2.50-2.92)		3.35 (3.00-3.71)	
1370-1785	45	2.55 (2.28-2.83)		3.37 (3.09-3.65)		2.78 (2.55-3.00)		3.15 (2.77-3.53)	
>1785	45	2.47 (2.20-2.74)	*0.89*	3.15 (2.88-3.43)	*0.35*	2.69 (2.47-2.90)	*0.53*	2.94 (2.57-3.31)	*0.57*

Abbreviations: BMI, body mass index; d, day; FLIC, flagellin; LPS, lipopolysaccharide; mg, milligram; mL, milliliters; ng, nanograms; No. number; NSAID, nonsteroidal anti-inflammatory drug; serv servings; procd, processed; yrs, years.

[a]Stratified by median levels of BMI, aspirin use, calcium intake, and vitamin d intake

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] – [treatment group baseline]) – ([placebo group year 1 – placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] – [treatment group baseline]) – ([placebo group EOT – placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/treatment group baseline])/ ([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 5: Mean baseline plasma levels of gut permeability biomarkers by demographic and lifestyle factors[a]

		LPS-IgG		LPS-IgA		FLIC-IgG		FLIC-IgA	
Characteristics	**N**	**Mean (95% CI)**	***P***	**Mean (95% CI)**	***P***	**Mean (95% CI)**	***P***	**Mean (95% CI)**	***P***
Age, yrs									
≤55	57	1.16 (1.05-1.28)		1.41 (1.24-1.58)		1.63 (1.51-1.74)		1.75 (1.58-1.93)	
55.1-60	44	1.13 (1.00-1.26)		1.45 (1.26-1.64)		1.65 (1.52-1.78)		1.69 (1.50-1.88)	
60.1-65	38	1.02 (0.88-1.16)		1.40 (1.20-1.61)		1.58 (1.45-1.72)		1.63 (1.42-1.83)	
>65	36	1.13 (0.99-1.28)	*0.56*	1.50 (1.28-1.71)	*0.39*	1.60 (1.45-1.74)	*0.86*	1.77 (1.56-1.98)	*0.86*
Sex									
Men	109	1.16 (1.08-1.24)		1.54 (1.42-1.67)		1.77 (1.69-1.85)		1.75 (1.63-1.87)	
Women	66	1.07 (0.97-1.18)	*0.20*	1.33 (1.18-1.48)	*0.03*	1.47 (1.36-1.57)	*0.0004*	1.68 (1.52-1.83)	*0.47*
Center									
GA	103	1.08 (1.00-1.17)		1.35 (1.22-1.48)		1.63 (1.54-1.71)		1.59 (1.47-1.72)	
SC	72	1.15 (1.05-1.26)	*0.29*	1.52 (1.38-1.67)	*0.08*	1.61 (1.51-1.71)	*0.78*	1.83 (1.68-1.98)	*0.02*
BMI, kg/m^2									
≤25	32	1.01 (0.86-1.16)		1.26 (1.04-1.49)		1.62 (1.47-1.76)		1.64 (1.42-1.87)	
25.1-29.9	81	1.19 (1.10-1.29)		1.48 (1.34-1.63)		1.64 (1.54-1.74)		1.74 (1.59-1.88)	
≥30	62	1.08 (0.97-1.19)	*0.82*	1.48 (1.32-1.64)	*0.18*	1.59 (1.48-1.70)	*0.68*	1.71 (1.55-1.88)	*0.67*
BMI, kg/m^2									
<22.5	8	0.80 (0.50-1.09)		1.01 (0.58-1.44)		1.41 (1.11-1.70)		1.38 (0.94-1.82)	
22.5-25	24	1.08 (0.92-1.25)		1.34 (1.09-1.59)		1.68 (1.51-1.85)		1.73 (1.48-1.98)	
25-27.5	36	1.21 (1.07-1.35)		1.54 (1.33-1.75)		1.72 (1.58-1.86)		1.80 (1.59-2.01)	
27.5-30	45	1.18 (1.05-1.30)		1.44 (1.26-1.62)		1.58 (1.46-1.71)		1.69 (1.50-1.87)	
30-35	38	0.96 (0.83-1.10)		1.24 (1.04-1.44)		1.52 (1.38-1.66)		1.52 (1.32-1.72)	
>35	24	1.26 (1.10-1.43)	*0.37*	1.85 (1.60-2.10)	*0.04*	1.70 (1.53-1.87)	*0.76*	2.03 (1.77-2.28)	*0.29*
Smoking status									
Never	100	1.19 (1.10-1.28)		1.42 (1.29-1.55)		1.65 (1.57-1.74)		1.68 (1.55-1.81)	
Former	63	1.03 (0.92-1.15)		1.49 (1.32-1.65)		1.51 (1.40-1.62)		1.77 (1.60-1.94)	
Smoker	12	0.94 (0.70-1.19)	*0.06*	1.34 (0.97-1.70)	*0.51*	1.87 (1.63-2.11)	*0.05*	1.73 (1.37-2.10)	*0.59*
Alcohol intake, drinks/d									
Non drinker	61	1.21 (1.10-1.32)		1.44 (1.27-1.61)		1.64 (1.53-1.75)		1.68 (1.51-1.85)	
0.1-0.5	38	1.11 (0.97-1.25)		1.41 (1.20-1.62)		1.68 (1.54-1.82)		1.73 (1.51-1.94)	

0.51-1.0	32	0.96 (0.80-1.12)		1.49 (1.26-1.73)		1.63 (1.47-1.78)		1.81 (1.57-2.05)	
1.01-2.0	26	1.12 (0.95-1.29)		1.39 (1.13-1.65)		1.63 (1.46-1.80)		1.69 (1.43-1.94)	
>2.0	18	0.98 (0.78-1.19)	*0.26*	1.46 (1.16-1.77)	*0.98*	1.36 (1.16-1.56)	*0.09*	1.69 (1.39-2.00)	*0.99*
Intake of red or procd meat, serv/d									
0	14	0.96 (0.73-1.20)		1.31 (0.96-1.65)		1.62 (1.38-1.85)		1.41 (1.07-1.75)	
0.1-0.5	50	1.16 (1.04-1.28)		1.41 (1.23-1.59)		1.55 (1.43-1.66)		1.63 (1.46-1.81)	
0.51-1.0	48	1.08 (0.95-1.20)		1.57 (1.39-1.75)		1.68 (1.56-1.80)		1.95 (1.77-2.13)	
>1	63	1.15 (1.04-1.27)	*0.25*	1.38 (1.22-1.55)	*0.12*	1.63 (1.52-1.75)	*0.16*	1.65 (1.49-1.82)	*0.01*
NSAID and/or aspirin use									
No	70	1.16 (1.06-1.26)		1.38 (1.23-1.53)		1.63 (1.53-1.73)		1.65 (1.50-1.80)	
Yes	105	1.09 (1.00-1.18)	*0.29*	1.48 (1.35-1.61)	*0.31*	1.61 (1.52-1.70)	*0.73*	1.76 (1.63-1.88)	*0.30*
NSAID and/or aspirin use ≥ 4 times/week									
No	54	1.14 (1.02-1.26)		1.41 (1.23-1.58)		1.64 (1.52-1.76)		1.69 (1.51-1.87)	
Yes	121	1.11 (1.03-1.19)	*0.71*	1.45 (1.33-1.57)	*0.69*	1.61 (1.53-1.69)	*0.70*	1.72 (1.60-1.84)	*0.76*
No. of adenomas									
1	126	1.12 (1.04-1.20)		1.47 (1.35-1.58)		1.63 (1.55-1.71)		1.74 (1.62-1.85)	
≥2	49	1.12 (0.99-1.25)	*0.99*	1.36 (1.17-1.55)	*0.33*	1.58 (1.46-1.71)	*0.51*	1.64 (1.45-1.83)	*0.36*
Advanced adenoma									
No	128	1.14 (1.07-1.22)		1.44 (1.32-1.56)		1.64 (1.57-1.72)		1.70 (1.58-1.81)	
Yes	43	1.04 (0.91-1.18)	*0.20*	1.43 (1.22-1.63)	*0.90*	1.56 (1.42-1.69)	*0.29*	1.75 (1.55-1.96)	*0.63*
Serrated polyps									
No	390	1.13 (1.06-1.21)		1.44 (1.32-1.55)		1.60 (1.52-1.68)		1.69 (1.57-1.80)	
Yes	131	1.07 (0.94-1.20)	*0.41*	1.44 (1.25-1.63)	*0.95*	1.67 (1.54-1.80)	*0.34*	1.78 (1.59-1.97)	*0.41*
Diabetes									
No	153	1.13 (1.05-1.20)		1.45 (1.34-1.56)		1.62 (1.55-1.69)		1.72 (1.61-1.83)	
Yes	22	1.07 (0.88-1.26)	*0.59*	1.36 (1.08-1.63)	*0.54*	1.62 (1.43-1.80)	*0.98*	1.67 (1.39-1.94)	*0.74*
Cytokine IL-6									
≤379	46	1.11 (0.98-1.24)		1.44 (1.25-1.64)		1.70 (1.57-1.83)		1.77 (1.57-1.96)	
379-468	46	1.13 (1.00-1.26)		1.43 (1.25-1.62)		1.63 (1.51-1.76)		1.75 (1.56-1.93)	
468-625	45	1.15 (1.02-1.29)		1.43 (1.24-1.62)		1.59 (1.46-1.72)		1.70 (1.51-1.89)	
>625	45	1.12 (0.98-1.25)	*0.97*	1.44 (1.25-1.63)	*0.87*	1.61 (1.48-1.74)	*0.52*	1.67 (1.47-1.86)	*0.95*

Cytokine IL-10									
≤381	37	1.11 (0.97-1.25)		1.52 (1.32-1.72)		1.67 (1.53-1.80)		1.82 (1.61-2.03)	
381-446	36	1.16 (1.01-1.30)		1.51 (1.31-1.72)		1.58 (1.43-1.72)		1.85 (1.63-2.06)	
446-508	37	1.13 (0.99-1.27)		1.30 (1.10-1.50)		1.70 (1.57-1.84)		1.57 (1.36-1.78)	
508-607	36	1.05 (0.90-1.19)		1.39 (1.18-1.60)		1.53 (1.39-1.67)		1.66 (1.45-1.87)	
>607	36	1.20 (1.05-1.34)	*0.49*	1.46 (1.26-1.67)	*0.47*	1.68 (1.54-1.83)	*0.30*	1.71 (1.50-1.92)	*0.30*
Cytokine TNFA									
≤1118	46	1.17 (1.03-1.30)		1.44 (1.24-1.63)		1.69 (1.56-1.82)		1.74 (1.55-1.94)	
1118-1370	46	1.08 (0.96-1.21)		1.53 (1.35-1.72)		1.63 (1.50-1.75)		1.82 (1.63-2.00)	
1370-1785	45	1.15 (1.01-1.28)		1.41 (1.21-1.60)		1.63 (1.50-1.76)		1.74 (1.54-1.94)	
>1785	45	1.11 (0.98-1.24)	*0.68*	1.36 (1.17-1.55)	*0.81*	1.58 (1.45-1.71)	*0.58*	1.58 (1.38-1.77)	*0.33*

Abbreviations: BMI, body mass index; d, day; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; IL_6, interleukin 6; IL_10, interleukin 10; LPS, lipopolysaccharide; No., number; NSAID, nonsteroidal anti-inflammatory drug; serv, servings; procd, processed; TNFA, tumor necrosis factors alpha; yrs, years.

Supplementary Table 6: Stratification of the mean levels of gut permeability biomarkers[a]

Characteristics	Baseline n	Baseline Mean (SE)	Baseline *P*	Year 1 n	Year 1 Mean (SE)	Year 1 *P*	EOT n	EOT Mean (SE)	EOT *P*	Abs. Tx Year 1[b] Mean (SE)	Abs. Tx Year 1[b] *P*	Abs. Tx EOT[c] Mean (SE)	Abs. Tx EOT[c] *P*	Δ tx Year 1[d]	Δ tx EOT[e]
Permeability Score *Stratified by Median BMI*															
< BMI of 28.50															
Calcium	29	5.91 (0.31)	*0.12*	29	5.79 (0.31)	*0.14*	28	4.75 (0.31)	*0.03*	0.03 (0.60)	*0.96*	-0.29 (0.61)	*0.64*	1.00	0.93
No calcium	31	6.57 (0.30)		31	6.42 (0.30)		31	5.70 (0.30)							
Vitamin D	40	5.69 (0.26)	*0.29*	40	5.73 (0.27)	*0.80*	39	4.97 (0.26)	*0.34*	0.30 (0.51)	*0.56*	0.04 (0.52)	*0.94*	1.05	1.00
No Vitamin D	40	6.08 (0.26)		41	5.82 (0.25)		41	5.32 (0.25)							
Vit. D + Calcium	26	5.38 (0.30)	*0.74*	28	5.37 (0.30)	*0.97*	29	4.69 (0.30)	*0.62*	0.13 (0.61)	*0.83*	-0.07 (0.61)	*0.91*	1.02	0.98
Calcium only	23	5.52 (0.32)		30	5.38 (0.31)		31	4.91 (0.31)							
≥ BMI of 28.50															
Calcium	40	6.24 (0.26)	*0.88*	40	6.05 (0.26)	*0.36*	40	5.50 (0.26)	*0.92*	-0.29 (0.53)	*0.58*	0.10 (0.54)	*0.86*	0.95	1.02
No calcium	35	6.29 (0.28)		35	6.40 (0.28)		32	5.46 (0.29)							
Vitamin D	48	5.77 (0.24)	*0.24*	47	5.87 (0.24)	*0.51*	46	5.27 (0.24)	*0.65*	0.17 (0.48)	*0.72*	0.25 (0.48)	*0.61*	1.03	1.04
No Vitamin D	47	6.17 (0.24)		46	6.09 (0.24)		46	5.42 (0.24)							
Vit. D + Calcium	29	5.65 (0.29)	*0.54*	28	5.67 (0.30)	*0.78*	29	5.23 (0.29)	*0.81*	0.13 (0.58)	*0.82*	0.15 (0.57)	*0.79*	1.02	1.02
Calcium only	31	5.90 (0.28)		30	5.78 (0.29)		31	5.33 (0.28)							
Stratified by Aspirin use Aspirin use, no. < 4/week															
Calcium	39	6.03 (0.27)	*0.18*	39	5.92 (0.27)	*0.28*	28	5.27 (0.27)	*0.54*	0.09 (0.53)	*0.87*	0.26 (0.53)	*0.62*	1.01	1.04
No calcium	41	6.53 (0.26)		41	6.33 (0.26)		31	5.51 (0.27)							
Vitamin D	62	5.75 (0.21)	*0.36*	61	5.80 (0.21)	*0.94*	39	5.22 (0.21)	*0.89*	0.31 (0.45)	*0.48*	0.33 (0.45)	*0.46*	1.05	1.06
No Vitamin D	48	6.05 (0.24)		48	5.80 (0.24)		41	5.18 (0.24)							
Vit. D + Calcium	39	5.47 (0.25)	*0.90*	38	5.51 (0.25)	*0.79*	25	5.07 (0.25)	*0.82*	0.15 (0.54)	*0.78*	0.13 (0.54)	*0.80*	1.01	1.03
Calcium only	30	5.52 (0.29)		30	5.41 (0.29)		24	4.98 (0.28)							
Aspirin use, no. ≥ 4/week															
Calcium	30	6.18 (0.30)	*0.88*	30	5.96 (0.30)	*0.19*	40	5.09 (0.30)	*0.17*	-0.51 (0.62)	*0.41*	-0.53 (0.62)	*0.39*	0.92	0.90
No calcium	25	6.25 (0.32)		25	6.54 (0.32)		32	5.69 (0.32)							

Vitamin D	26	5.68 (0.32)	*0.18*	26	5.81 (0.32)	*0.35*	46	4.94 (0.32)	*0.10*	0.16 (0.58)	*0.78*	-0.13 (0.58)	*0.82*	1.03	0.96
No Vitamin D	39	6.22 (0.26)		39	6.19 (0.26)		46	5.62 (0.26)							
Vit. D + Calcium	16	5.63 (0.38)	*0.44*	16	5.55 (0.38)	*0.54*	29	4.78 (0.38)	*0.24*	0.08 (0.69)	*0.91*	-0.20 (0.69)	*0.77*	1.01	0.95
Calcium only	24	6.00 (0.31)		24	5.85 (0.31)		31	5.36 (0.31)							
Stratified by Median Calcium Intake < median calcium intake of 162 mg/d															
Calcium	41	6.11 (0.25)	*0.06*	41	5.90 (0.25)	*0.03*	41	5.30 (0.25)	*0.13*	-0.10 (0.52)	*0.85*	0.14 (0.52)	*0.79*	0.98	1.01
No calcium	36	6.81 (0.27)		36	6.71 (0.27)		35	5.87 (0.27)							
Vitamin D	41	6.12 (0.26)	*0.61*	41	6.12 (0.26)	*0.89*	40	5.37 (0.27)	*0.62*	0.23 (0.51)	*0.65*	0.002 (0.51)	*1.00*	1.04	1.00
No Vitamin D	46	6.31 (0.25)		47	6.08 (0.25)		47	5.55 (0.25)							
Vit. D + Calcium	23	6.04 (0.32)	*0.32*	23	5.94 (0.32)	*0.28*	23	5.44 (0.32)	*0.31*	0.03 (0.62)	*0.96*	0.004 (0.62)	*0.99*	1.01	1.01
Calcium only	28	5.60 (0.29)		29	5.47 (0.29)		29	5.00 (0.29)							
≥ median calcium intake of 162 mg/d															
Calcium	28	6.09 (0.31)	*0.76*	28	5.99 (0.31)	*0.90*	27	5.03 (0.32)	*0.66*	-0.19 (0.61)	*0.76*	-0.33 (0.62)	*0.60*	0.97	0.94
No calcium	30	5.96 (0.30)		30	6.05 (0.30)		28	5.22 (0.31)							
Vitamin D	47	5.39 (0.23)	*0.11*	46	5.52 (0.23)	*0.35*	45	4.92 (0.23)	*0.47*	0.22 (0.47)	*0.64*	0.29 (0.47)	*0.54*	1.04	1.05
No Vitamin D	41	5.92 (0.24)		40	5.83 (0.25)		40	5.17 (0.25)							
Vit. D + Calcium	32	5.14 (0.27)	*0.06*	31	5.21 (0.27)	*0.18*	31	4.64 (0.27)	*0.10*	0.19 (0.57)	*0.74*	0.07 (0.56)	*0.90*	1.03	1.00
Calcium only	26	5.88 (0.29)		25	5.76 (0.30)		26	5.32 (0.29)							
Stratified by Median Vitamin D Intake < median vitamin D intake of 21.60 ng/mL															
Calcium	34	5.82 (0.28)	*0.03*	34	5.60 (0.28)	*0.003*	34	5.04 (0.28)	*0.05*	-0.32 (0.54)	*0.55*	0.06 (0.54)	*0.91*	0.95	0.99
No calcium	37	6.64 (0.26)		37	6.75 (0.26)		35	5.80 (0.27)							
Vitamin D	37	5.80 (0.27)	*0.20*	37	5.90 (0.27)	*0.54*	36	5.17 (0.27)	*0.40*	0.23 (0.50)	*0.64*	0.15 (0.50)	*0.76*	1.04	1.02
No Vitamin D	50	6.25 (0.23)		49	6.11 (0.23)		49	5.47 (0.23)							
Vit. D + Calcium	20	5.44 (0.32)	*0.45*	20	5.30 (0.32)	*0.50*	20	4.93 (0.32)	*0.70*	0.03 (0.59)	*0.95*	0.15 (0.59)	*0.79*	1.00	1.03
Calcium only	30	5.76 (0.26)		29	5.59 (0.27)		30	5.09 (0.26)							

≥ median vitamin D intake of 21.60 ng/mL														
Calcium	35	6.37 (0.28)	*0.59*	35	6.27 (0.28)	*0.48*	34	5.35 (0.28)	*0.92*	0.07 (0.58)	*0.90*	-0.18 (0.59)	*0.59*	1.01	0.97
No calcium	29	6.15 (0.30)		29	5.98 (0.30)		28	5.31 (0.31)							
Vitamin D	51	5.68 (0.23)	*0.45*	50	5.74 (0.23)	*0.93*	49	5.10 (0.24)	*0.69*	0.24 (0.50)	*0.64*	0.13 (0.50)	*0.80*	1.04	1.02
No Vitamin D	37	5.95 (0.27)		38	5.77 (0.27)		38	5.24 (0.27)							
Vit. D + Calcium	35	5.56 (0.28)	*0.73*	34	5.65 (0.28)	*0.95*	34	5.01 (0.28)	*0.65*	0.17 (0.61)	*0.78*	-0.05 (0.61)	*0.94*	1.03	0.99
Calcium only	24	5.71 (0.34)		25	5.63 (0.33)		25	5.21 (0.33)							

Abbreviations: BMI, body mass index; d, day; FLIC, flagellin; LPS, lipopolysaccharide; mg, milligram; mL, millilters; ng, nanograms; No. number; NSAID, nonsteroidal anti-inflammatory drug; serv servings; procd, processed; yrs, years.

[a]Stratified by median levels of BMI, aspirin use, calcium intake, and vitamin d intake

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]). [d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 7: Blood 25-(OH)-Vitamin D Concentrations at baseline, year 1, and end of treatment according to treatment assignment

	Baseline			Year 1			EOT			Abs. Tx Year 1[b]		Abs. Tx EOT[c]		Δ tx Year 1[d]	Δ tx EOT[e]
	n	Mean (SE)	*P*	n	Mean (SE)	*P*	n	Mean (SE)	*P*	Mean (SE)	*P*	Mean (SE)	*P*		
Vit. D Level, ng/mL															
Calcium	69	23.24 (0.68)	*0.61*	69	26.88 (0.68)	*0.33*	68	30.44 (0.68)	*0.00004*	0.45 (1.37)	*0.74*	2.97 (1.38)	*0.03*	1.01	1.10
No calcium	66	22.75 (0.70)		66	25.93 (0.70)		63	26.98 (0.70)							
Vitamin D	88	24.72 (0.59)	*0.01*	87	32.47 (0.59)	*<0.001*	85	34.48 (0.60)	*<0.001*	8.89 (1.18)	*<0.001*	7.82 (1.19)	*<0.001*	1.38	1.28
No Vitamin D	87	22.50 (0.59)		87	21.37 (0.59)		87	24.46 (0.59)							
Vit. D + Calcium	55	25.02 (0.77)	*0.10*	54	33.56 (0.77)	*<0.001*	54	36.24 (0.78)	*<0.001*	10.35 (1.55)	*<0.001*	8.96 (1.55)	*<0.001*	1.45	1.32
Calcium only	54	23.22 (0.77)		54	21.42 (0.78)		55	25.48 (0.77)							

Abbreviations: Abs, absolute; EOT, end of treatment; mL, milliliters; ng, nanograms; tx, treatment; Vit. D, vitamin D.

[a]The unadjusted effect of treatment agent on blood vitamin D levels was modeled using mixed linear models.

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 8: Mean levels of gut barrier biomarkers at baseline, year 1, and end of treatment according to treatment assignment[a]

	n	Baseline Mean (SE)	*P*	n	Year 1 Mean (SE)	*P*	n	EOT Mean (SE)	*P*	Abs. Tx Year 1[b] Mean (SE)	*P*	Abs. Tx EOT[c] Mean (SE)	*P*	Δ tx Year 1[d]	Δ tx EOT[e]
LPS															
4-arm Groups:															
Calcium	33	2.96 (0.16)	*0.98*	33	2.81 (0.16)	*0.86*	33	2.47 (0.16)	*0.99*	-0.03 (0.31)	*0.92*	0.002 (0.31)	*0.99*	0.99	1.00
Vitamin D	33	2.77 (0.16)	*0.37*	33	2.88 (0.16)	*0.86*	31	2.48 (0.16)	*0.97*	0.24 (0.31)	*0.45*	0.21 (0.32)	*0.51*	1.08	1.08
Vit. D + Calcium	36	2.48 (0.16)	*0.03*	36	2.49 (0.16)	*0.10*	35	2.11 (0.16)	*0.10*	0.13 (0.31)	*0.68*	0.12 (0.31)	*0.69*	1.05	1.02
Placebo	33	2.97 (0.16)		33	2.85 (0.16)		32	2.47 (0.16)							
2-arm Groups:															
Vitamin D	19	2.03 (0.14)	*0.59*	18	2.06 (0.14)	*0.51*	19	2.11 (0.14)	*0.65*	-0.03 (0.28)	*0.92*	0.02 (0.27)	*0.95*	0.94	0.96
Placebo	21	2.03 (0.14)		21	2.19 (0.14)		22	2.20 (0.13)							
FLIC															
4-arm Groups:															
Calcium	33	3.61 (0.15)	*0.12*	33	3.45 (0.15)	*0.06*	33	3.12 (0.15)	*0.13*	-0.07 (0.29)	*0.82*	0.01 (0.30)	*0.98*	0.98	0.99
Vitamin D	33	3.46 (0.15)	*0.02*	33	3.52 (0.15)	*0.11*	31	3.03 (0.15)	*0.06*	0.15 (0.29)	*0.61*	0.08 (0.30)	*0.79*	1.04	1.00
Vit. D + Calcium	36	3.44 (0.15)	*0.02*	36	3.42 (0.15)	*0.04*	35	2.99 (0.15)	*0.03*	0.06 (0.29)	*0.82*	0.05 (0.29)	*0.86*	1.02	1.00
Placebo	33	3.94 (0.15)		33	3.85 (0.15)		32	3.44 (0.15)							
2-arm Groups:															
Vitamin D	19	2.94 (0.18)	*0.22*	18	2.95 (0.18)	*0.37*	19	2.90 (0.18)	*0.30*	-0.08 (0.34)	*0.82*	-0.05 (0.34)	*0.89*	0.97	0.99
Placebo	21	2.64 (0.17)		21	2.72 (0.17)		22	2.64 (0.17)							
Permeability Score															
4-arm Groups:															
Calcium	33	6.57 (0.29)	*0.41*	33	6.26 (0.29)	*0.28*	33	5.58 (0.29)	*0.42*	-0.10 (0.56)	*0.86*	0.01 (0.56)	*0.99*	0.98	0.99
Vitamin D	33	6.22 (0.29)	*0.09*	33	6.40 (0.29)	*0.46*	31	5.51 (0.30)	*0.33*	0.39 (0.56)	*0.49*	0.29 (0.57)	*0.61*	1.06	1.04
Vit. D + Calcium	36	5.93 (0.28)	*0.01*	36	5.91 (0.28)	*0.05*	35	5.10 (0.28)	*0.04*	0.19 (0.55)	*0.73*	0.17 (0.55)	*0.76*	1.03	1.01
Placebo	33	6.91 (0.29)		33	6.70 (0.29)		32	5.91 (0.29)							
2-arm Groups:															
Vitamin D	19	4.97 (0.29)	*0.62*	18	5.00 (0.29)	*0.82*	19	5.00 (0.29)	*0.68*	-0.11 (0.56)	*0.85*	-0.03 (0.55)	*0.95*	0.98	0.99
Placebo	21	4.78 (0.27)		21	4.91 (0.27)		22	4.84 (0.27)							
FLIC-IgA															
4-arm Groups:															

Calcium	33	1.87 (0.11)	*0.37*	33	1.79 (0.11)	*0.31*	33	1.65 (0.11)	*0.62*	-0.02 (0.21)	*0.94*	0.06 (0.22)	*0.78*	0.99	1.03
Vitamin D	33	1.76 (0.11)	*0.11*	33	1.81 (0.11)	*0.37*	31	1.54 (0.11)	*0.23*	0.21 (0.60)	*0.60*	0.06 (0.22)	*0.78*	1.06	1.02
Vit. D + Calcium	36	1.72 (0.11)	*0.06*	36	1.71 (0.11)	*0.11*	35	1.50 (0.11)	*0.13*	0.05 (0.21)	*0.82*	0.05 (0.21)	*0.80*	1.02	1.01
Placebo	33	2.01 (0.11)		33	1.95 (0.11)		32	1.73 (0.11)							
2-arm Groups:															
Vitamin D	19	1.58 (0.13)	*0.37*	18	1.58 (0.14)	*0.46*	19	1.55 (0.13)	*0.42*	-0.03 (0.26)	*0.92*	-0.02 (0.26)	*0.95*	0.98	1.00
Placebo	21	1.42 (0.13)		21	1.45 (0.13)		22	1.40 (0.13)							
FLIC-IgG															
4-arm Groups:															
Calcium	33	1.74 (0.07)	*0.07*	33	1.66 (0.07)	*0.02*	33	1.47 (0.07)	*0.02*	-0.05 (0.15)	*0.73*	-0.05 (0.15)	*0.71*	0.97	0.95
Vitamin D	33	1.69 (0.07)	*0.02*	33	1.71 (0.07)	*0.06*	31	1.49 (0.08)	*0.04*	0.04 (0.15)	*0.80*	0.02 (0.15)	*0.91*	1.03	1.00
Vit. D + Calcium	36	1.72 (0.07)	*0.04*	36	1.71 (0.07)	*0.06*	35	1.50 (0.07)	*0.04*	0.02 (0.14)	*0.90*	-0.003 (0.14)	*0.98*	1.01	0.98
Placebo	33	1.93 (0.08)		33	1.90 (0.08)		32	1.71 (0.08)							
2-arm Groups:															
Vitamin D	19	1.36 (0.06)	*0.12*	18	1.36 (0.06)	*0.33*	19	1.35 (0.06)	*0.22*	-0.05 (0.12)	*0.68*	-0.03 (0.12)	*0.80*	0.96	0.98
Placebo	21	1.22 (0.06)		21	1.27 (0.06)		22	1.24 (0.06)							
LPS-IgA															
4-arm Groups:															
Calcium	33	1.71 (0.11)	*0.71*	33	1.63 (0.11)	*0.89*	33	1.46 (0.11)	*0.78*	0.04 (0.22)	*0.87*	0.10 (0.22)	*0.64*	1.02	1.06
Vitamin D	33	1.56 (0.11)	*0.16*	33	1.65 (0.11)	*0.95*	31	1.40 (0.11)	*0.91*	0.21 (0.22)	*0.34*	0.20 (0.22)	*0.37*	1.13	1.12
Vit. D + Calcium	36	1.43 (0.11)	*0.02*	36	1.45 (0.11)	*0.17*	35	1.21 (0.11)	*0.19*	0.14 (0.21)	*0.53*	0.14 (0.21)	*0.51*	1.08	1.05
Placebo	33	1.77 (0.11)		33	1.66 (0.11)		32	1.42 (0.11)							
2-arm Groups:															
Vitamin D	19	1.14 (0.10)	*0.45*	18	1.14 (0.10)	*0.63*	19	1.14 (0.10)	*0.40*	-0.04 (0.20)	*0.85*	0.01 (0.19)	*0.95*	0.96	1.01
Placebo	21	1.03 (0.10)		21	1.07 (0.09)		22	1.02 (0.09)							
LPS-IgG															
4-arm Groups:															
Calcium	33	1.17 (0.08)	*0.63*	33	1.01 (0.08)	*0.88*	33	1.17 (0.08)	*0.67*	-0.07 (0.15)	*0.65*	-0.10 (0.15)	*0.65*	0.86	1.12
Vitamin D	33	1.21 (0.08)	*0.88*	33	1.24 (0.08)	*0.65*	31	1.08 (0.08)	*0.81*	0.03 (0.15)	*0.83*	0.01 (0.16)	*0.95*	1.02	1.01
Vit. D + Calcium	36	1.05 (0.08)	*0.19*	36	1.04 (0.08)	*0.17*	35	0.90 (0.08)	*0.15*	-0.01 (0.15)	*0.95*	-0.02 (0.15)	*0.91*	0.99	0.97
Placebo	33	1.19 (0.08)		33	1.19 (0.08)		32	1.05 (0.08)							
2-arm Groups:															
Vitamin D	19	0.90 (0.09)	*0.08*	18	0.92 (0.09)	*0.10*	19	0.97 (0.09)	*0.09*	0.01 (0.17)	*0.95*	0.01 (0.17)	*0.98*	1.01	1.01
Placebo	21	1.11 (0.08)		21	1.12 (0.08)		22	1.18 (0.08)							

IgG														
4-arm Groups:														
Calcium	33	2.99 (0.13)	*0.44*	33	2.84 (0.13)	*0.15*	33	2.47 (0.13)	*0.10*	-0.12 (0.25)	*0.63*	-0.15 (0.25)	*0.54*	0.96	0.93
Vitamin D	33	2.90 (0.13)	*0.22*	33	2.94 (0.13)	*0.40*	31	2.57 (0.13)	*0.29*	0.07 (0.25)	*0.78*	0.03 (0.25)	*0.91*	1.02	1.00
Vit. D + Calcium	36	2.78 (0.13)	*0.05*	36	2.76 (0.13)	*0.05*	35	2.40 (0.13)	*0.04*	0.01 (0.24)	*0.97*	-0.02 (0.25)	*0.93*	1.00	0.98
Placebo	33	3.12 (0.13)		33	3.09 (0.13)		32	2.76 (0.13)							
2-arm Groups:														
Vitamin D	19	2.26 (0.12)	*0.65*	18	2.28 (0.12)	*0.50*	19	2.31 (0.12)	*0.55*	-0.04 (0.24)	*0.87*	-0.03 (0.23)	*0.91*	0.98	0.98
Placebo	21	2.33 (0.12)		21	2.40 (0.12)		22	2.42 (0.11)							
IgA														
4-arm Groups:														
Calcium	33	3.59 (0.21)	*0.51*	33	3.43 (0.21)	*0.55*	33	3.11 (0.21)	*0.91*	0.02 (0.42)	*0.96*	0.16 (0.42)	*0.70*	1.00	1.04
Vitamin D	33	3.32 (0.21)	*0.12*	33	3.46 (0.21)	*0.62*	31	2.94 (0.22)	*0.50*	0.32 (0.42)	*0.45*	0.26 (0.42)	*0.54*	1.09	1.07
Vit. D + Calcium	36	3.15 (0.21)	*0.03*	36	3.16 (0.21)	*0.12*	35	2.71 (0.21)	*0.14*	0.18 (0.41)	*0.66*	0.19 (0.41)	*0.64*	1.05	1.04
Placebo	33	3.78 (0.21)		33	3.60 (0.21)		32	3.14 (0.22)							
2-arm Groups:														
Vitamin D	19	2.72 (0.22)	*0.37*	18	2.72 (0.22)	*0.50*	19	2.68 (0.22)	*0.38*	-0.07 (0.43)	*0.88*	-0.01 (0.42)	*0.99*	0.97	1.00
Placebo	21	2.45 (0.21)		21	2.52 (0.21)		22	2.42 (0.21)							

Abbreviations: Abs., absolute; EOT, end of treatment; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; LPS, lipopolysaccharide; Tx, Treatment; Vit. D, vitamin D.

[a]The effect of treatment agent on biomarker level was modeled using mixed linear models adjusted by age, sex, study center, no. of baseline adenomas and follow up-period (mos.)

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 9: Mean levels of gut barrier biomarkers at baseline, year 1, and end of treatment according to treatment assignment[a]

	Baseline			Year 1			EOT			Abs. Tx Year 1[b]		Abs. Tx EOT[c]		Δ tx Year 1[d]	Δ tx EOT[e]
	n	Mean (SE)	*P*	n	Mean (SE)	*P*	n	Mean (SE)	*P*	Mean (SE)	*P*	Mean (SE)	*P*		
LPS															
Calcium	69	2.65 (0.11)	*0.33*	69	2.58 (0.11)	*0.16*	68	2.22 (0.11)	*0.23*	-0.07 (0.22)	*0.75*	-0.04 (0.22)	*0.87*	0.97	0.97
No calcium	66	2.80 (0.11)		66	2.80 (0.11)		63	2.41 (0.11)							
Vitamin D	88	2.44 (0.09)	*0.05*	87	2.49 (0.09)	*0.38*	85	2.20 (0.09)	*0.28*	0.14 (0.18)	*0.44*	0.12 (0.18)	*0.53*	1.06	1.04
No Vitamin D	87	2.70 (0.09)		87	2.61 (0.09)		87	2.34 (0.09)							
Vit. D + Calcium	55	2.28 (0.11)	*0.06*	54	2.29 (0.11)	*0.18*	54	2.07 (0.11)	*0.13*	0.08 (0.22)	*0.71*	0.06 (0.22)	*0.78*	1.03	1.02
Calcium only	54	2.58 (0.11)		54	2.51 (0.11)		55	2.30 (0.11)							
FLIC															
Calcium	69	3.45 (0.10)	*0.24*	69	3.37 (0.10)	*0.10*	68	2.98 (0.10)	*0.22*	-0.07 (0.21)	*0.72*	-0.01 (0.21)	*0.95*	0.98	0.99
No calcium	66	3.62 (0.11)		66	3.61 (0.11)		63	3.17 (0.11)							
Vitamin D	88	3.27 (0.09)	*0.16*	87	3.28 (0.09)	*0.47*	85	2.92 (0.09)	*0.25*	0.08 (0.18)	*0.64*	0.03 (0.18)	*0.86*	1.03	1.00
No Vitamin D	87	3.45 (0.09)		87	3.37 (0.09)		87	3.07 (0.09)							
Vit. D + Calcium	55	3.21 (0.11)	*0.86*	54	3.19 (0.11)	*0.62*	54	2.90 (0.11)	*0.88*	0.05 (0.21)	*0.82*	-0.004 (0.21)	*0.99*	1.01	1.00
Calcium only	54	3.18 (0.11)		54	3.12 (0.11)		55	2.88 (0.11)							
Permeability Score															
Calcium	69	6.10 (0.20)	*0.25*	69	5.94 (0.20)	*0.10*	68	6.10 (0.20)	*0.19*	-0.14 (0.39)	*0.72*	-0.05 (0.40)	*0.90*	0.98	1.15
No calcium	66	6.42 (0.20)		66	6.41 (0.20)		63	5.57 (0.20)							
Vitamin D	88	5.71 (0.17)	*0.07*	87	5.77 (0.17)	*0.38*	85	5.12 (0.17)	*0.23*	0.23 (0.33)	*0.50*	0.15 (0.33)	*0.66*	1.04	1.02
No Vitamin D	87	6.14 (0.17)		87	5.98 (0.17)		87	5.41 (0.17)							
Vit. D + Calcium	55	5.49 (0.20)	*0.34*	54	5.49 (0.20)	*0.63*	54	4.97 (0.20)	*0.46*	0.13 (0.40)	*0.74*	0.06 (0.39)	*0.88*	1.02	1.01
Calcium only	54	5.76 (0.20)		54	5.62 (0.20)		55	5.18 (0.20)							
FLIC-IgA															
Calcium	69	1.73 (0.07)	*0.40*	69	1.68 (0.07)	*0.22*	68	1.50 (0.07)	*0.55*	-0.04 (0.15)	*0.79*	0.03 (0.15)	*0.87*	0.98	1.01
No Calcium	66	1.82 (0.08)		66	1.81 (0.08)		63	1.57 (0.08)							
Vitamin D	88	1.65 (0.06)	*0.24*	87	1.66 (0.07)	*0.58*	85	1.47 (0.07)	*0.31*	0.06 (0.13)	*0.66*	0.01 (0.13)	*0.91*	1.03	1.00
No Vitamin D	87	1.75 (0.07)		87	1.71 (0.07)		87	1.56 (0.07)							

Vit. D + Calcium	55	1.61 (0.08)	*0.83*	54	1.60 (0.08)	*1.00*	54	1.46 (0.08)	*0.74*	0.03 (0.16)	*0.88*	-0.01 (0.16)	*0.93*	1.02	0.99
Calcium only	54	1.64 (0.08)		54	1.60 (0.08)		55	1.50 (0.08)							
FLIC-IgG															
Calcium	69	1.73 (0.05)	*0.27*	69	1.69 (0.05)	*0.12*	68	1.48 (0.05)	*0.11*	-0.03 (0.10)	*0.74*	-0.04 (0.10)	*0.71*	0.98	0.97
No calcium	66	1.81 (0.05)		66	1.80 (0.05)		63	1.60 (0.05)							
Vitamin D	88	1.62 (0.04)	*0.25*	87	1.62 (0.04)	*0.49*	85	1.45 (0.04)	*0.38*	0.03 (0.09)	*0.75*	0.02 (0.09)	*0.85*	1.01	1.00
No Vitamin D	87	1.69 (0.04)		87	1.67 (0.04)		87	1.51 (0.04)							
Vit. D + Calcium	55	1.59 (0.05)	*0.46*	54	1.59 (0.05)	*0.29*	54	1.44 (0.05)	*0.38*	0.02 (0.10)	*0.81*	0.01 (0.10)	*0.92*	1.02	1.01
Calcium only	54	1.54 (0.05)		54	1.51 (0.05)		55	1.38 (0.05)							
LPS-IgA															
Calcium	69	1.51 (0.08)	*0.37*	69	1.48 (0.08)	*0.30*	68	1.28 (0.08)	*0.50*	-0.02 (0.15)	*0.92*	0.02 (0.15)	*0.88*	0.99	1.01
No calcium	66	1.61 (0.08)		66	1.60 (0.08)		63	1.35 (0.08)							
Vitamin D	88	1.37 (0.06)	*0.09*	87	1.41 (0.06)	*0.60*	85	1.23 (0.06)	*0.47*	0.11 (0.13)	*0.41*	0.09 (0.13)	*0.49*	1.07	1.06
No Vitamin D	87	1.52 (0.06)		87	1.46 (0.06)		87	1.29 (0.06)							
Vit. D + Calcium	55	1.29 (0.08)	*0.29*	54	1.30 (0.08)	*0.51*	54	1.15 (0.08)	*0.40*	0.04 (0.15)	*0.78*	0.02 (0.15)	*0.89*	1.04	1.01
Calcium only	54	1.41 (0.08)		54	1.37 (0.08)		55	1.25 (0.08)							
LPS-IgG															
Calcium	69	1.13 (0.05)	*0.47*	69	1.09 (0.05)	*0.15*	68	0.94 (0.05)	*0.13*	-0.05 (0.11)	*0.62*	-0.06 (0.11)	*0.57*	0.96	0.94
No calcium	66	1.19 (0.05)		66	1.20 (0.05)		63	1.05 (0.06)							
Vitamin D	88	1.07 (0.05)	*0.12*	87	1.08 (0.05)	*0.31*	85	0.97 (0.05)	*0.26*	0.04 (0.09)	*0.70*	0.03 (0.09)	*0.77*	1.03	1.01
No Vitamin D	87	1.17 (0.05)		87	1.15 (0.05)		87	1.05 (0.05)							
Vit. D + Calcium	55	0.99 (0.06)	*0.03*	54	0.99 (0.06)	*0.08*	54	0.91 (0.06)	*0.08*	0.04 (0.11)	*0.73*	0.04 (0.11)	*0.73*	1.04	1.01
Calcium only	54	1.17 (0.06)		54	1.13 (0.06)		55	1.06 (0.06)							
IgG															
Calcium	69	2.86 (0.09)	*0.27*	69	2.78 (0.09)	*0.07*	68	2.42 (0.09)	*0.06*	-0.09 (0.17)	*0.61*	-0.10 (0.18)	*0.57*	0.97	0.96
No calcium	66	3.00 (0.09)		66	3.00 (0.09)		63	2.65 (0.09)							
Vitamin D	88	2.69 (0.07)	*0.10*	87	2.70 (0.07)	*0.30*	85	3.43 (0.07)	*0.22*	0.06 (0.15)	*0.66*	0.04 (0.15)	*0.77*	1.02	1.42
No Vitamin D	87	2.86 (0.07)		87	2.81 (0.07)		87	2.56 (0.07)							
Vit. D + Calcium	55	2.58 (0.09)	*0.31*	54	2.58 (0.09)	*0.62*	54	2.36 (0.09)	*0.54*	0.06 (0.18)	*0.72*	0.05 (0.18)	*0.78*	1.03	1.02
Calcium only	54	2.71 (0.09)		54	2.64 (0.09)		55	2.43 (0.09)							

IgA														
Calcium	69	3.24 (0.14)	*0.37*	69	3.16 (0.14)	*0.24*	68	2.78 (0.15)	*0.51*	-0.05 (0.29)	*0.85*	0.05 (0.29)	*0.87*	0.98	1.01
No calcium	66	3.43 (0.15)		66	3.41 (0.15)		63	2.92 (0.15)							
Vitamin D	88	3.02 (0.12)	*0.13*	87	3.07 (0.12)	*0.57*	85	2.69 (0.12)	*0.37*	0.16 (0.25)	*0.59*	0.10 (0.25)	*0.68*	1.05	1.03
No Vitamin D	87	3.28 (0.12)		87	3.17 (0.12)		87	2.85 (0.12)							
Vit. D + Calcium	55	2.91 (0.15)	*0.52*	54	2.91 (0.15)	*0.74*	54	2.61 (0.15)	*0.54*	0.07 (0.30)	*0.82*	0.01 (0.30)	*0.98*	1.02	0.99
Calcium only	54	3.05 (0.15)		54	2.98 (0.15)		55	2.75 (0.15)							

Abbreviations: Abs., absolute; EOT, end of treatment; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; LPS, lipopolysaccharide; Tx, treatment; Vit. D, vitamin D.

[a]The effect of treatment agent on biomarker level was modeled using mixed linear models adjusted by age, sex, study center, no. of baseline adenomas and follow up-period (mos.)

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]). [c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]). [d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 10: Mean levels of gut barrier biomarkers at baseline, year 1, and end of treatment according to treatment assignment[a]

	Baseline			Year 1			EOT			Abs. Tx Year 1[b]		Abs. Tx EOT[c]		Δ tx Year 1[d]	Δ tx EOT[e]
	n	Mean (SE)	*P*	n	Mean (SE)	*P*	n	Mean (SE)	*P*	Mean (SE)	*P*	Mean (SE)	*P*		
Permeability Score															
Calcium	69	6.09 (0.19)	*0.22*	69	5.94 (0.19)	*0.08*	68	5.18 (0.20)	*0.15*	-0.14 (0.39)	*0.72*	-0.06 (0.40)	*0.87*	0.98	0.98
No calcium	66	6.43 (0.20)		66	6.42 (0.20)		63	5.59 (0.20)							
Vitamin D	88	5.79 (0.17)	*0.21*	87	5.86 (0.18)	*0.75*	85	5.17 (0.18)	*0.49*	0.23 (0.35)	*0.51*	0.14 (0.35)	*0.69*	1.04	1.02
No Vitamin D	87	6.10 (0.17)		87	5.94 (0.17)		87	5.35 (0.17)							
Vit. D + Calcium	55	5.57 (0.21)	*0.62*	54	5.57 (0.21)	*0.94*	54	5.02 (0.21)	*0.71*	0.13 (0.42)	*0.76*	0.04 (0.42)	*0.93*	1.02	1.00

Abbreviations: Abs., absolute; EOT, end of treatment; FLIC, flagellin; IgA, immunoglobulin A; IgG, immunoglobulin G; LPS, lipopolysaccharide; Tx, treatment; Vit. D, vitamin D.

[a]The effect of treatment agent on biomarker level was modeled using mixed linear models adjusted by age, sex, study center, no. of baseline adenomas and follow up-period (mos.)

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT – placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Supplementary Table 11: Mean levels of gut barrier biomarkers at baseline, year 1, and end of treatment according to treatment assignment[a]

	Baseline			Year 1			EOT			Abs. Tx Year 1[b]		Abs. Tx EOT[c]		Δ tx Year 1[d]	Δ tx EOT[e]
	n	Mean (SE)	*P*	n	Mean (SE)	*P*	n	Mean (SE)	*P*	Mean (SE)	*P*	Mean (SE)	*P*		
Permeability Score															
Calcium	69	6.02 (0.20)	*0.06*	69	5.86 (0.20)	*0.06*	68	5.07 (0.20)	*0.07*	0.01 (0.40)	*0.99*	0.01 (0.41)	*0.97*	1.00	0.99
Calcium Placebo	66	6.58 (0.21)		66	6.41 (0.21)		63	5.61 (0.21)							
Vitamin D	88	5.76 (0.17)	*0.15*	87	5.73 (0.17)	*0.43*	85	5.06 (0.17)	*0.22*	0.16 (0.34)	*0.64*	0.05 (0.34)	*0.88*	1.03	1.00
Vitamin D Placebo	87	6.10 (0.17)		87	5.92 (0.17)		87	5.36 (0.17)							
Vit. D + Calcium	55	5.48 (0.20)	*0.48*	54	5.48 (0.20)	*0.82*	54	4.94 (0.20)	*0.60*	0.14 (0.39)	*1.00*	0.05 (0.39)	*0.05*	1.02	1.01
Calcium	54	5.68 (0.20)		54	5.55 (0.20)		55	5.09 (0.20)							

Abbreviations: Abs, absolute; EOT, end of treatment; FLIC, flagellin; LPS, lipopolysaccharide; tx, treatment; Vit. D, vitamin D.

[a]The effect of treatment agent on biomarker level was modeled using mixed linear models adjusted by age, sex, study center, no. of baseline adenomas, follow up period (mos.), BMI, and total calories.

[b]Abs. Tx Year 1= Absolute treatment effect at year 1= ([treatment group year 1] − [treatment group baseline]) − ([placebo group year 1 − placebo group baseline]).

[c]Abs. Tx EOT= Absolute treatment effect at the end of treatment= ([treatment group EOT] − [treatment group baseline]) − ([placebo group EOT − placebo group]).

[d]Δ tx Year 1= Relative treatment effect at year 1= ([treatment group year 1]/[treatment group baseline])/([placebo group year 1)/(placebo group baseline]).

[e]Δ tx EOT= Relative treatment effect at the end of treatment= ([treatment group EOT]/[treatment group baseline])/([placebo group EOT)/(placebo group baseline]).

Chapter III: Summary, Public Health Implications, Possible Future Directions

The results of this study demonstrated that daily supplementation with vitamin D_3 and/or calcium may not modify levels of gut permeability biomarkers, such as LPS, FLIC, IgG, or IgA. Although studies have suggested that vitamin D and calcium supplementation could prevent colorectal carcinogenesis, they may not significantly affect gut barrier function. Furthermore, we found that sex, BMI, and consumption of red or processed meat may play a role in affecting intestinal mucosal barrier integrity relevant to colorectal carcinogenesis. Thus, there should be further investigation on the relationship between vitamin D, calcium, and gut permeability along with these additional factors.

This study supports the need for further exploration of biomarkers that may improve the prevention of colorectal cancer. Additional observational and experimental studies are necessary to validate the use of biomarkers before they are used in a clinical setting. Biomarkers may not only help researchers understand how patients will respond to various treatments, but also gain insight into early detection, diagnosis, and the progression of colorectal cancer. Future discovery and insight into biomarkers and their related molecular mechanisms can lead to improvements in the clinical management of colorectal cancer.