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Effects of Calcium and Vitamin D Supplementation on Toll-Like Receptor 4 (TLR4) Expression in the Normal-Appearing Colorectal Mucosa of Individuals at High Risk for Colorectal Neoplasms

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Environmental Health and Epidemiology

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By

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B.A., Honors
University of Delaware
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Abstract

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By Rebecca Hodge

Inflammation plays an important role in colorectal carcinogenesis. Toll-like receptor 4 (TLR4)-mediated pro-inflammatory signaling in response to bacterial lipopolysaccharide (LPS) and other substrates has also been found to involved in colon carcinogenesis. Calcium and vitamin D, known to be promising chemopreventive agents for colorectal cancer, have been shown in cell culture and animal models to affect TLR4 regulation and expression, and it is possible their protective effects against carcinogenesis are at least in part mediated through inhibition of this pathway. Therefore, to test the effects of vitamin D and calcium supplementation on the TLR4 signaling pathway, we conducted a biomarker adjunct study nested within a larger randomized, double-blind, placebo-controlled, partial 2x2 factorial chemoprevention clinical trial of supplemental calcium (1,200 mg daily) and vitamin D (1000 IU daily), alone and in combination *versus* placebo. We assessed TLR4 expression in the normal-appearing colorectal mucosa of 105 patients at high risk of developing colorectal cancer at baseline and 1-yr follow up using standardized, automated immunohistochemistry and quantitative image analysis. Vitamin D_3 and calcium did not affect TLR4 expression in colorectal crypts. However, a combined treatment with both agents decreased the proportion of TLR4 expression in the upper portion of the crypt (differentiation zone) relative to the whole crypt (12% increase, p-value 0.039). At baseline, regular NSAID use, cigarette smoking, vitamin D deficiency status, intakes of fruits and vegetables, and dietary fiber, and having advanced or serrated adenomas were found to be associated with TLR4 expression. The results from our pilot biomarker study suggest that a combined treatment with vitamin D and calcium may modify the distribution of TLR4 within the colon crypts by reducing TLR4 expression in the differentiation zone relative to the whole crypt; and that several known risk factors for colorectal cancer risk might be associated with TLR4 expression. Future studies are needed to understand a combined effect of vitamin D and calcium supplementation, and a role of environmental and lifestyle modifiable factors on TLR4 signaling pathway.

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INTRODUCTION

Colorectal Cancer

In 2017, an estimated 135,430 new cases of colorectal cancers will occur, along with approximately 50,000 deaths[1], making colorectal cancer the second leading cause of cancer deaths in the United States[2]. The US Preventative Services Task Force (USPSTF) recommends screening in adults aged 50 to 75 using gualac-based fecal occult blood tests (gFOBT), fecal immunochemical tests (FIT), multitargeted stool DNA tests (FIT-DNA), flexible sigmoidoscopy, flexible sigmoidoscopy with FIT, colonoscopy, and CT colonography[2]. Risk factors for colorectal cancer include inflammatory bowel disease[3,4], age[5], smoking[3,5,6], body fatness[3-5], alcohol[3,4], and physical inactivity[4]. A diet high in dietary fiber and use of non-steroidal anti-inflammatory drugs were also shown to be associated with lower risk of colorectal cancer[4].

The carcinogenesis of colorectal cancer originates in colon crypts, which are epithelium lined invaginations in the colorectal mucosa[7]. The crypt base contains stem cells, which divide to produce progenitor cells that migrate upwards, where they then differentiate into colonocytes[8]. These stems cells are the main targets of neoplastic transformation[9], a transition in which normal epithelia transform into adenomas and then carcinomas following the accumulation of genetic mutations to oncogenes and tumor suppressor genes[10,11].

In individuals with genetic syndromes such as hereditary non-polyposis colorectal cancer (HNPCC) or familial adenomatous polyposis coli (FAP), these mutations are inherited rather than induced[10]. In colorectal cancers associated with inflammatory bowel disease, the adverse effects of chronic inflammation trigger these malignant changes[3,12]. In sporadic colorectal cancers, environmental factors may contribute to or favor these malignant changes[3,13], most likely through the development of chronic inflammation[3,14].

The Role of Inflammation and TLR4 in Colorectal Cancer Development

Our understanding of the ways in which chronic inflammation induces colorectal carcinogenesis comes from studying colitis-associated cancers (CACs)[12]. These cancer subtypes are associated with inflammatory bowel disease (IBD)[3] and are characterized by colitis (i.e., colonic inflammation) triggered by an adverse event, such as dysregulation of the gastrointestinal immune system[15] or a disturbance in the colon microbiome[14].

In colitis-associated cancers, the risk for developing colorectal cancer increases with both the duration and extent of the colitis[12,16,17]. Overexpression of inflammatory cytokines and chemokines are capable of inducing cancer in normal epithelia, and these signaling molecules are present in tumorous lesions[10]. Additionally, regular use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to be associated with the risk of colorectal cancer and reduce adenoma recurrence[10,18]. These observations have led to the hypothesis that the continuous release of reactive oxygen species and inflammatory cytokines in tissues with chronic inflammation can induce the genetic mutations that lead to colorectal cancer[5,12,19-21].

One of the pathways through which chronic inflammation can lead to oxidative stress and neoplastic transformation involves the Toll-like receptor 4 (TLR4) pathway. Associated with the Golgi apparatus of colonocytes[22], TLR4 is part of the body's innate immune response against gram-negative bacteria and plays a key role in maintaining intestinal homeostasis[11]. However, its overexpression has been observed in colorectal tumors[23], and polymorphisms in the *TLR4* gene are associated with ulcerative colitis and Crohn's disease[11,20].

TLR4 promotes inflammation and colon carcinogenesis by stimulating the production of the pro-inflammatory cytokine TNF-α and expression of nuclear factor-κB (NF-κB)[24,25], leading to increased expression of COX-2[23]. In colorectal cancer patients, elevated TNF-α levels are associated with neoplastic growth, higher tumor grade and increased risk of mortality[5]. The carcinogenic effects of TNF-α are mainly mediated through its induction of NF-κB[20], a protein complex which *via* control of DNA transcription promotes angiogenesis, inhibits apoptosis and generates the accumulation of reactive oxygen species[19]; this oxidative stress can induce genomic mutations that promote carcinogenesis[19-21]. NF-κB is also capable of prolonging inflammation[19] by inducing the production of additional pro- inflammatory cytokines[5] and the enzyme COX-2[19], which in turn also promotes inflammation and cell proliferation[18]. Both NF-κB and COX-2 are overexpressed in colorectal neoplasms[10].

Interestingly, a number of the known risk factors for colorectal cancer have been shown to impact TLR4 regulation and expression. In obese individuals, excess free fatty acids were shown to bind to TLR4, generating inflammation and activating NF-κB[26]. A high fat diet can lead to changes in the gut microbiome that are accompanied by increases in lipopolysaccharide (LPS) and increased TLR4 activation[27,28]. Outside of the colonic environment, chronic alcohol consumption and smoking has been shown to increase inflammation and TLR4 signaling in glial cells of the brain[29] and in lung CD8+ T cells[30], respectively. Lastly, physical activity has been shown to reduce chronic low-grade inflammation in mice by down-regulating the TLR4 signaling pathway in the liver, muscle and adipose tissue[28].

These observations demonstrate the multitude of ways in which various environmental exposures can impact TLR4 and thereby increase or decrease the risk for colorectal cancer. In this study we chose to focus on two environmental exposures that have previously

demonstrated efficacy against colorectal cancer, vitamin D and calcium. Our aim was to evaluate the effect of vitamin D and calcium supplementation on TLR4 expression in the normal colorectal mucosa of colorectal adenoma patients, who are at higher risk of developing colorectal cancer.

Vitamin D

Vitamin D is obtained through sun exposure, diet and supplements[7]. Upon exposure to ultraviolet radiation, the vitamin D substrate 7-dehydrocholesterol is converted in the skin to cholecalciferol[31], which is then converted by the liver enzyme CYP2R1 to the main circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D)[32]. This vitamin D metabolite provides the most accurate indicator of a person's total vitamin D exposure[7,32], reflecting both sun exposure, which provides 90-95% of a person's vitamin D, as well as dietary exposure, which accounts for the rest[7]. 25-hydroxyvitamin D is then hydroxylated by CYP27B1[7] in the kidney to yield the hormonally active form of vitamin D, calcitriol (1,25-$(OH)_2D$)[31,32], a prohormone which exerts its autocrine and paracrine[7] effects by binding to the vitamin D receptor (VDR)[32]. Interestingly, while CYP27B1 enzyme is normally also present in the colon[31,32] along with VDR[33], human colon tumors do not expression the CYP27B1 enzyme[34] and VDR is less prevalent in the normal colonic epithelium following malignant transformation[33].

Recently, vitamin D has gained increasing attention for its potential to prevent various cancers such as colorectal cancer[35]. By interacting with VDR, calcitriol directly and indirectly regulates up to 3-5% of the human genome[32], promoting bile acid degradation[36,37], regulating calcium homeostasis[21], bone growth and mineralization[31], and modulating growth factor signaling and DNA repair[36,37]. Cancer-specific effects of calcitriol include promoting

differentiation and apoptosis[4,31,38], inhibiting proliferation, angiogenesis and metastatic potential[31,39] and downregulating the Wnt signaling pathway[32,40], a pathway implicated in colorectal cancer[32]. Additionally, vitamin D is capable of down-regulating TLR4 expression[41], thereby decreasing the production of TNF- α and NF-κB[41-43]. It is likely one or more of these mechanisms account for the inverse associations observed between serum vitamin D levels and colorectal cancer risk and mortality[35,44].

Calcium

Obtained through diet or supplementation, calcium binds bile and free fatty acids in the colonic lumen[21,33], thereby reducing oxidative stress and inflammation[5] and decreasing NF-κB activation[8]. Through the creation of an intracellular gradient, calcium plays a pivotal role in the proliferation, differentiation and apoptosis of colonic crypts[4,7,33]. A deficiency in vitamin D can adversely impact this gradient, leading to increased proliferation, and reduced differentiation or apoptosis[33]. Intracellular calcium also acts as a second messenger[4]. One study by Racioppi *et al* found that calcium, by forming a complex with calmodulin (CaM) in macrophages, was capable of regulating the TLR4-mediated response to lipopolysaccharides and attenuating the inflammatory response in mice by reducing the accumulation of TNF-α and NF-κB[45]. Another study found that the calcium-calmodulin complex was required in TLR4's activation of a number of its target genes[46]. Therefore, it is possible that calcium intake may have an effect on TLR4 expression.

Higher intakes of calcium and vitamin D, separately and combined, have been shown to be associated with lower risk for colorectal neoplasms[7,13,21,37,47]. Additionally, calcium supplementation has been shown to reduce adenoma recurrence[37]. In its 2011 Continuous Update Report[4], the World Cancer Research Fund considered calcium to be a probable

preventive factor at a dose of 1200 mg/day, and vitamin D to be a possible preventive factor for colorectal cancer. The report noted that, "the effects of vitamin D and calcium are strongly interrelated because both restrain cellular proliferation, both induce differentiation and apoptosis in intestinal cells, and calcium-mediated effects are strongly dependent on vitamin D levels"[4]. For these reasons, this study's hypothesis was that the combined treatment of vitamin D and calcium supplementation would result in the greatest reduction in TLR4 expression, followed by the vitamin D group, and then the calcium group, as compared to the placebo group.

MATERIALS AND METHODS

Clinical Trial Protocol

Participants in this study were participating in a randomized, placebo-controlled, partial 2x2 factorial chemoprevention trial examining the efficacy of vitamin D and/or calcium supplementation for the prevention of colorectal adenomas[48]. Protocols for this parent study, including eligibility and exclusion criteria, have been previously published[48].

Protocols for the substudy have also been previously published[36]. Briefly, patients were eligible for this substudy if they visited two of the eleven clinical centers (South Carolina and Georgia) between May 2004 and July 2008 and agreed to provide rectal biopsy tissue at baseline and after one year of supplementation with the study agent. Out of 231 eligible participants, 105 met final eligibility, which included having had sufficient rectal biopsy tissue taken for biomarker measurements at both baseline and at the one-year follow up. Participants were enrolled and consented near the end of the placebo run-in period for the parent study without knowledge of the assigned treatment. The Institutional Review Boards at both clinical centers approved this research.

Upon enrollment into the parent study, information was collected regarding the participant's medical history, medication and nutritional supplement use, demographic data, lifestyle and diet (using the Block Brief 2000 food frequency questionnaire, Nutritionquest). Blood levels of the vitamin D metabolite 25-hydroxyvitamin D [25(OH)D] were obtained at both baseline and follow-up; calcium and the vitamin D metabolite calcitriol [$1,25(OH)_2D$] were obtained only at baseline.

Following the placebo run-in period, subjects were randomly assigned to the following four treatment groups: placebo, 1200 mg/d calcium supplementation (as calcium carbonate in equal doses twice daily), 1000 IU/d vitamin D_3 supplementation (500 IU twice daily), and 1200 mg/d elemental calcium plus 1000 IU/d vitamin D_3 supplementation. Women who declined to forego calcium supplementation were randomized to calcium or calcium plus vitamin D_3 using 2-arm randomization. Randomization was conducted 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-arm randomization. All study staff, as well as the participants, were blinded to treatment assignments.

During the trial, participants agreed to refrain from taking additional vitamin D or calcium supplements, although personal supplements up to 1000 IU vitamin D and/or 400 mg elemental calcium were permitted starting in April 2008. Bottles of study tablets were delivered to participants every four months. Telephone interviews were conducted every six months regarding participant adherence to the study treatment, illnesses, use of medications and supplements, and colorectal endoscopic or surgical procedures.

Rectal Biopsy Tissue Collection and Biomarker Quantification

Biopsies of normal-appearing rectal mucosa were collected from participants at baseline and at one year follow up without preceding bowel-cleansing preparation or procedure (i.e., "non-prep"). The exact rectal biopsy and immunohistochemistry protocol has been previously published for a different set of biomarkers[36]. Briefly, five slides with three levels of 3-um thick biopsy sections were taken 40um apart. Slides were placed in a preheated Pretreatment Module (Lab Vision Corp., Fremont, CA) wit 100x Citrate Buffer pH 6.0 (DAKO S1699, DAKO Corp., Carpinteria, CA) and steamed for forty minutes before being placed in a DakoCytomation Autostainer Plus System automated immunostainer. Slides were immunohistochemically processed using a labeled streptavidin-biotin method (LSAB2 Detection System [DAKO K0675]) for TLR4 by applying a rabbit polyclonal antibody (catalog no., ab47093; dilution, 1:325; Abcam, Cambridge, UK). Baseline and follow-up biopsy slides for the same study participant were included in the same immunohistochemistry batch, with each batch including a balance of participants from each treatment group as well as positive and negative controls.

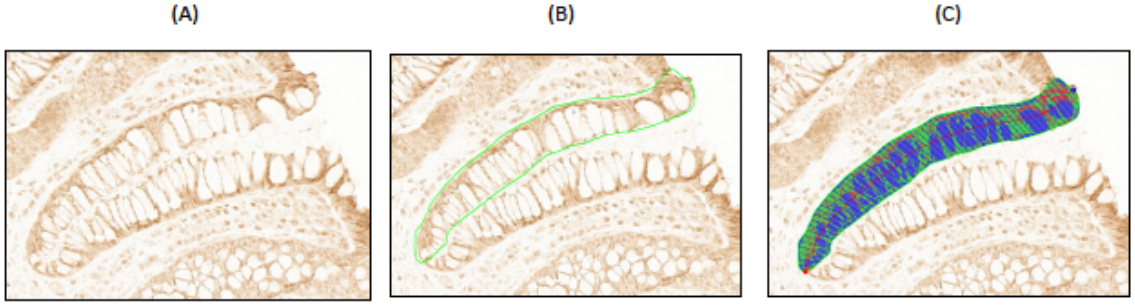


Figure 1. Measurement of TLR4 expression in crypts of normal appearing rectal mucosa using custom-designed quantitative image analysis software. The scoring process entailed (A) finding a full length hemicrypt, (B) tracing the hemicrypt, and (C) automated sectioning and quantification of TLR4 labeling optical density, overall and within each section of the hemicrypt.

To quantify the levels of the biomarker TLR4 in the samples, a quantitative image analysis method ("scoring") was used, in which the slides were first scanned using the Aperio

Scanscope CS digital scanner (3DHISTECH, Budapest, HU), and then the electronic images for all 105 participants were reviewed in a custom-developed software, the CellularEyes program. A single technician that was blinded to treatment assignment selected only "scorable" crypts for biomarker quantification (Figure 1a), which were defined as intact crypts extending from the muscularis mucosa to the colon lumen.

To score a selected hemicrypt (half a crypt), the technician traced its borders using a digital drawing board (Figure 1b). The CellularEyes program then divided the outline into equally spaced segments according to the average width of normal colonocytes. After measuring the background-corrected optical density of the biomarker labeling across the entire hemicrypt, as well as within each segment (Figure 1c), the program automatically transferred the data into the MySQL database. The technician continued this process until a total of 8 to 40 hemicrypts were selected among the five biopsy slides per baseline and follow-up visit. Whenever possible, both hemicrypts within a scorable crypt were selected. To assess intra-reader scoring reliability, a reliability control sample previously analyzed by the technician was re-analyzed during the course of the trial. The intra-class correlation coefficient was 0.98 for the biomarker.

Statistical Analyses

Baseline characteristics for participants assigned to each treatment group were compared within the four- and two-arm randomization groups using chi-squared tests for categorical variables and ANOVA for continuous variables. Baseline characteristics were also assessed for their correlation with baseline TLR4 expression levels in a generalized linear model controlling for age, sex (by study arm), study center and batch number, where appropriate. Vitamin D, dietary recommendations, and body mass index (BMI) cut points were chosen

according to the guidelines set forth by the Endocrine Society[49], the U.S. Departments of Agriculture and Health and Human Services[50], and the World Health Organization[51].

Treatment effects from baseline to follow-up on TLR4 expression in the entire crypt, TLR4 expression in the proliferation (the lower 60% of the crypt) or differentiation zone (the upper 40% of the crypt), or percent TLR4 expression in the differentiation zone to the whole crypt (ϕ_h), were evaluated using a general MIXED linear model in an intention-to-treat analysis and according to the treatment agent (i.e.; "vitamin D vs. no vitamin D", "calcium vs. no calcium", and "calcium vs. vitamin D plus calcium"). The model included the intercept, follow-up visit effects, time, treatment group, and the interaction of treatment with time. To test for possible confounding that could have arisen from an imbalanced distribution of certain baseline characteristics (e.g., dietary fiber) or from participant inclusion in this adjunct biomarker study (e.g., gender, age and study center), two additional models were run. The first controlled for age, gender (by study arm) and study center, while the second also controlled for smoking status (current and former vs. never), multivitamin use (yes vs. no), physical activity (continuous), dietary fiber (continuous), and total caloric intake (continuous). Adjustment for these potential confounders did not materially affect the treatment effects in either model.

The analysis of treatment effects on TLR4 expression was performed twice without stratification: once according to treatment assignment and once according to treatment agent. Secondary analyses to explore potential treatment effect modification were conducted by stratification by baseline characteristics identified as having an association with baseline TLR4 expression. Relative and absolute treatment effects were calculated according to the following: relative effect= [(treatment group follow-up)/(treatment group baseline)]/[(control group follow-up)/(control group baseline)]; absolute effect= [(treatment

group follow-up)-(treatment group baseline)]–[(control group follow-up)-(control group baseline)]. A relative effect of 1.3 would indicate a 30% increase in biomarker expression in the treatment group relative to the control group. In all analyses of randomized treatments, participants were retained in their originally assigned treatment group, regardless of adherence to study treatment and procedures.

All statistical analyses were conducted in SAS 9.4 (Cary, NC). A two-side p-value ≤ 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of Study Participants

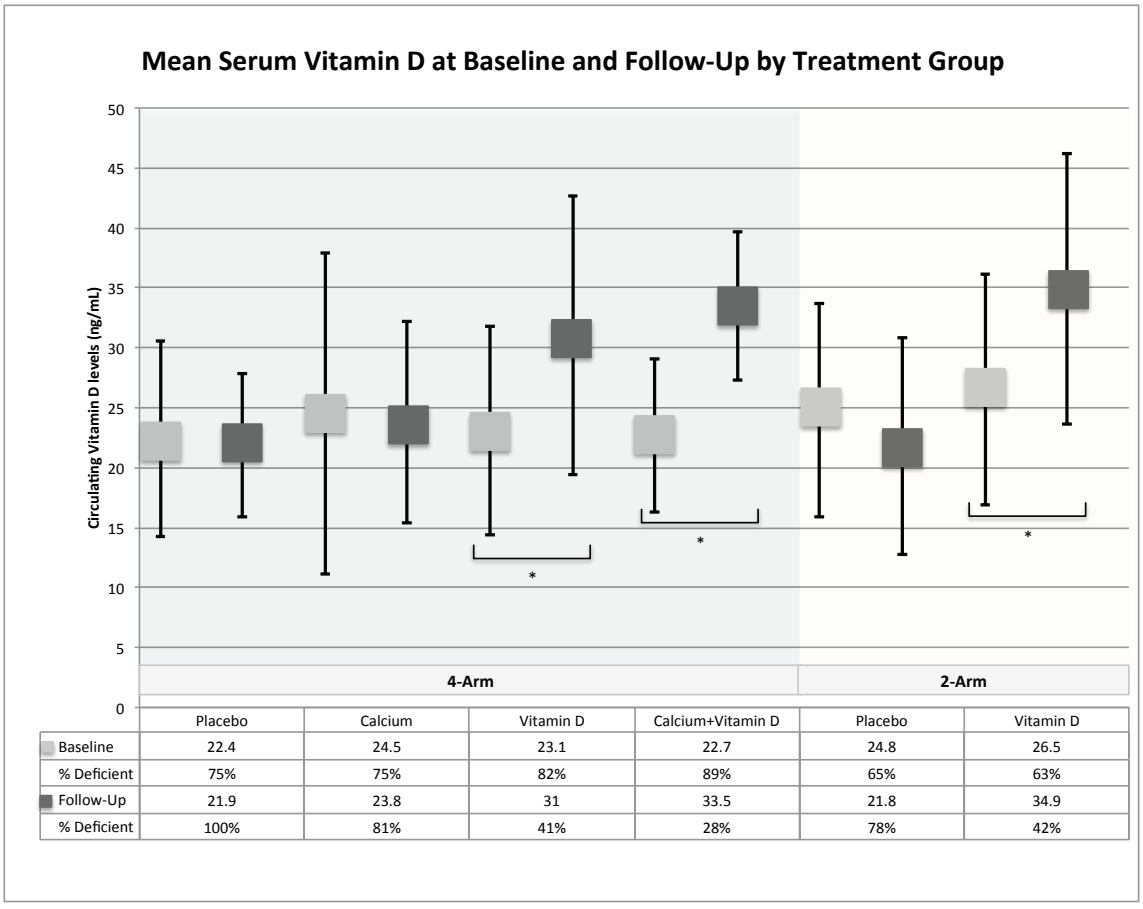
Select baseline characteristics for patients enrolled in the biomarker substudy are presented in Table 1. Mean age at enrollment for all participants was 59 years, and approximately half of participants were males. Most participants were white, overweight or obese, non-smokers, had no family history of colorectal cancer, and were vitamin D deficient. On average, 36% of participants' daily calories came from fat, and participants had an average of 9.4 mg/dL and 24.2 ng/mL of serum calcium and 25-OH-vitamin D_3, respectively. Physical activity and dietary fiber intake differed significantly among treatment groups in both study arms; fruit and vegetable intake differed significantly among women in the 2-arm group.

During the first year, 76% of participants reported taking 80% or more of their study tablets. Patients assigned to vitamin D had increased circulating 25(OH)D at follow-up compared to patients given placebo or calcium in both arms ($p<0.001$). The proportion of patients who were vitamin D deficient decreased for all treatment groups where vitamin D was assigned (Figure 2).

Vitamin D and/or Calcium Effects on TLR4 Expression

The effects of calcium and vitamin D supplementation, alone in combination, in accordance with initial treatment group assignment on the four measures of TLR4 expression from baseline to follow-up are shown in Table 2. There were no significant changes in TLR4 expression across the whole crypt, in the upper 40%, or in the lower 60% in any of the active treatment groups relative to the control group.

Participants in the four-arm group assigned to calcium or vitamin D combined with



	Placebo	Calcium	Vitamin D	Calcium+Vitamin D	Placebo	Vitamin D
Baseline	22.4	24.5	23.1	22.7	24.8	26.5
% Deficient	75%	75%	82%	89%	65%	63%
Follow-Up	21.9	23.8	31	33.5	21.8	34.9
% Deficient	100%	81%	41%	28%	78%	42%

Figure 2: Mean circulating 25(OH)D in participants according to treatment group
Vitamin D supplementation increased serum 25(OH)D levels in participants assigned to the four-arm group (vitamin D only, p-value 0.033; combined treatment, p-value <0.0001), as well as to women in the two-arm group (vitamin D, p-value 0.0189). This resulted in a decreased proportion of vitamin D deficiency at follow-up for participants in these treatment groups as compared to baseline.

calcium experienced non-significant increases in overall TLR4 expression across the crypt (respective increases of 3% and 3%; p-values 0.932 and 0.917; Table 2). Those in the four-arm group who received vitamin D only experienced a non-significant decrease in overall TLR4 expression (18% reduction; p-value 0.780); the same trend was observed for women assigned to vitamin D in the two-arm group (21% reduction; p-value 0.401).

In the upper 40% of the crypt, all treatment groups in both arms experienced non-significant decreases in TLR4 expression as compared to the placebo groups. In the four-arm group, calcium recipients experienced a 17% decrease (p-value 0.563), vitamin D recipients a 30% decrease (p-value 0.283), and vitamin D and calcium recipients a 3% decrease (p-value 0.935). In the two-arm group, women assigned to vitamin D experienced a 14% reduction in TLR4 expression in the differentiation zone (p-value 0.613).

In the lower 60% of the crypt, all participants randomized to the four-arm group experienced increases in TLR4 expression (calcium: 24%, vitamin D: 7%, vitamin D and calcium: 5%; respective p-values of 0.556, 0.847, 0.885). Women in the two-arm group that received vitamin D experienced a non-significant decrease in the TLR4 expression in the proliferation zone (25% reduction, p-value 0.323).

Participants in the four-arm group assigned to calcium supplementation or vitamin D supplementation, but not to the combined treatment, experienced a respective 19% and 22% decrease in percent TLR4 expression in the differentiation zone to the whole crypt as compared to participants assigned to the placebo group (p-values of 0.038 and 0.013, respectively; Table 2). Participants assigned to calcium or vitamin D in the 4-arm group had significantly different mean percentages of TLR4 expression in the differentiation zone at baseline (calcium: p-value 0.034; vitamin D: p-value 0.031) as compared to participants assigned to the placebo. Treatment with calcium or vitamin D served to 'normalize' the

percent of TLR4 expression in the differentiation zone; TLR4 expression in the differentiation zone was no longer statistically significantly different at follow-up, with participants assigned to calcium or vitamin D experiencing respective decreases of 20% and 22% (p-values of 0.038 and 0.013; Table 2) in the percent of TLR4 expression in the differentiation zone over the year long study period.

The effects of treatment agent, rather than treatment assignment, were examined in Table 3. There were no significant changes in TLR4 expression across the whole crypt, in the upper 40%, or in the lower 60% from treatment assignment for participants assigned to calcium (compared to no calcium), vitamin D (compared to no vitamin D), and vitamin D and calcium (compared to calcium only). However, there was a general trend of relative increases in TLR4 expression in the whole crypt, the upper 40% of the crypt, and the lower 60% of the crypt for participants assigned to calcium (respective increases of 9%, 11% and 9%; p-values 0.694, 0.639, 0.712), while participants assigned to vitamin D or the combined treatment experienced relative decreases of 9% and 10% in the whole crypt (p-values 0.597 and 0.624) and relative decreases in TLR4 expression in the proliferation zone of 11% and 18% (p-values 0.544 and 0.371). This trend was not observed for TLR4 expression in the upper 40% of the crypt, as the vitamin D group experienced a decrease of 8% (p-value 0.634) while the combined group experienced an increase of 1% (p-value 0.960).

The proportion of TLR4 expression in the crypt differentiation zone increased non-significantly for participants assigned to calcium and vitamin D (2% and 1% increase; p-values 0.753 and 0.919). Participants assigned to the combined treatment had a significantly lower proportion of TLR4 expression in the differentiation zone as compared to participants receiving calcium only (p-value 0.002). At follow-up, these groups were no longer statistically different, with the effect of vitamin D, when added to a calcium regiment,

causing a 12% increase in the percent of TLR4 expression in the differentiation zone (p-value of 0.039).

Stratified Analyses

Stratified analyses of the effects of treatment agent were performed to assess whether treatment effect may differ by sex, regular NSAID use, vitamin D deficiency status at baseline, fruit and vegetable consumption, smoking status, and presence of advanced and serrated adenomas (Tables 5-8).

In stratified analyses by sex, the effect of the combined vitamin D and calcium treatment was more pronounced in men (vs. women), as compared to men taking only calcium, and was a 19% increase in the percent of TLR4 expression in the differentiation zone (p-value: 0.029). No significant effects of treatment were observed for females with regards to the proportion of TLR4 expression in the differentiation zone. Neither gender experienced significant treatment effects for TLR4 expression in the whole crypt or in the differentiation or proliferation zone (Table 8).

When stratifying by NSAID use, only NSAID non-users taking both calcium and vitamin D experienced a 13% increase in the percent of TLR4 expression in the differentiation zone, as compared to irregular NSAID users taking calcium only (p-value: 0.029). Treatment with vitamin D and calcium, as compared to calcium only, mitigated initial baseline differences in irregular NSAID users (baseline: 29.5% vs. 33.8%, p-value of 0.002; follow-up: 32.0% vs. 32.3%, p-value of 0.794). No other treatment groups in either NSAID group experienced significant treatment effects (Table 8).

Having sufficient vitamin D (serum levels $\geq$ 30 ng/mL) was not associated with having a greater or lower proportion of expression in the differentiation zone relative to the whole

crypt as compared to having a deficiency (32.2% vs. 32.8%; p value of 0.726; Table 4). When stratifying on vitamin D deficiency, only participants with sufficient vitamin D incurred a significant treatment effect of calcium, experiencing a 105% increase in TLR4 expression in the proliferation zone as compared to non-calcium takers (p value: 0.035; Table 7).

Stratification by whether participants consumed the recommended daily fruit and vegetable servings amount did not result in any significant treatment effects of vitamin D and calcium on TLR4 expression (Tables 5-8); however, the combined effect of vitamin D and calcium, as compared to taking only calcium, was borderline significant (p-value of 0.083) and displayed a similar trend to those observed in the crude and stratified analyses (Table 8).

When stratifying by smoking status in a treatment effect analysis, there were no observed effects of treatment by any of the treatment agents in current and former smokers or never smokers (Tables 5-8). However, the effects of the combined calcium and vitamin D treatment, as compared to calcium only, in current and former smokers was borderline significant (p-value: 0.053; Table 8).

Patients with no advanced adenomas at baseline taking vitamin D and calcium incurred a 14% increase in the percent of TLR4 expression in the differentiation zone at follow-up relative to those taking calcium only (p value 0.035). The same trend was observed for patients with serrated adenomas at baseline: these patients receiving calcium and vitamin D experienced a 16% increase in the percent TLR4 expression in the differentiation zone relative to those receiving calcium only (p value 0.025; Table 8).

Predictors of Baseline TLR4 Expression

Lastly, this study examined whether baseline demographic, lifestyle, or dietary characteristics were potentially associated with TLR4 expression at baseline (Table 4). Among these, regular NSAID use, smoking status, fruit and vegetable intake, vitamin D deficiency, and presence of advanced or serrated adenomas were found to be associated with baseline TLR4 expression. Gender and daily dietary fiber recommendation achievement expressed borderline associations with TLR4.

DISCUSSION

Vitamin D_3 and calcium supplementation, alone or in combination, did not significantly impact the amount of TLR4 expression in the whole crypt, in the differentiation zone, or in the proliferation zone. However, our study suggests that a combined treatment of vitamin D_3 and elemental calcium, at respective doses of 1000 IU/day and 1200 mg/day, may significantly increase the proportion of TLR4 expression in the differentiation zone relative to TLR4 expression in the whole crypt in patients at high risk for colorectal neoplasms. The results of the stratified analyses suggest that there may be a stronger treatment effect for men, regular NSAID users, patients without advanced or serrated adenomas, and patients with sufficient vitamin D. This study also examined whether any baseline characteristics were associated with baseline TLR4 expression. Regular NSAIDS use, current or former smoking status, achieving the daily recommended fruit and vegetable intake of five servings or more, having sufficient vitamin D, and having had advanced or serrated adenomas at baseline were significantly associated with baseline TLR4 expression.

Previous studies have shown that calcitriol, the hormonally active form of vitamin D, can suppress TNF-α induced NF-κB activity[52,53], the main inflammatory products produced upon TLR4 stimulation by LPS[24,25]. The same effect has been observed for calcium[45]. This

would suggest that vitamin D and calcium supplementation would attenuate some of the harmful effects of TLR4 activation and should reduce inflammation. This effect has been described in a number of studies, which found that vitamin D and calcium promote differentiation and apoptosis and inhibit proliferation[4,31,33,38,39]. These anti-inflammatory effects were also observed in our study, which found that a combined treatment of vitamin D and calcium shifted the proportion of TLR4 expression from the proliferation zone to the differentiation zone. However, the null results observed for calcium or vitamin D treatment alone supports other studies demonstrating that these two agents depend on one another and act in concert[4,7,33].

The modifying effects of NSAIDs observed in this study also align with previous literature, which have found that NSAIDs decrease proliferation and increase apoptosis[18]. These anti-inflammatory effects are likely of equivalent magnitude to those observed from a combined treatment of vitamin D and calcium and may explain why only non-NSAID users experienced decreased TLR4 expression in the proliferation zone relative to the whole crypt. Additionally, gender has previously been shown to impact the effects of vitamin D and calcium on colorectal cancer. Estrogenic substances can enhance the expression of VDR in vivo[54], and a study by Aigner *et al* (2014) found an association between vitamin D concentrations and colorectal lesion characteristics in women, but not in men[55]. Males have also been shown to have increased TLR4[56] and LPS-induced TNF-α[57] as compared to females. These studies provide support for our observation that the combined treatment effects of vitamin D and calcium may be gender-dependent. Lastly, while our study found a stronger effect of calcium treatment in patients with sufficient vitamin D, this finding should be interpreted with caution given the low sample size. However, these results seem biologically plausible and are consistent with evidence suggesting that calcium and vitamin D

depend on one another to exert their affects and that vitamin D is involved in regulating calcium homeostasis[21,33].

Several of the baseline characteristics identified as being associated with baseline TLR4 expression in our study are also in line with previous research findings. Smoking status[4,6] and having advanced[58] and serrated[59] adenomas have been shown to increase the risk of colorectal cancer, while regular NSAID use[7,10], fruit and vegetable consumption[3], vitamin D status[7,13,33,59], and dietary fiber[4] have all been shown to reduce the risk of colorectal cancer. Of these, smoking has been shown to increase TLR4 expression levels[29,30,60], while certain components of fruits and vegetables have been shown to decrease TLR4 expression[61] and inhibit LPS-induced production of pro-inflammatory cytokines[62]. As previously mentioned, vitamin D can down-regulate TLR4 expression[41]; conversely, TLR4 activation has been shown to influence the expression of CYP27B1, the enzyme responsible for the local production of calcitriol in the intestine[53]. However, this study could not find evidence to support our finding that the presence of advanced or serrated adenomas are associated with TLR4 expression.

Strengths and Limitations

This study is an adjunct biomarker study of a larger clinical trial testing the efficacy of vitamin D and calcium supplementation on the prevention of colorectal adenomas. Patients in this parent clinical trial that received calcium or vitamin D alone or combined did not have a significantly reduced risk of colorectal adenoma recurrence over a period of 3 to 5 years[48]. Given this information and this study's use of TLR4 as a proxy for colorectal adenoma recurrence risk, it is not surprising that in this adjunct study neither vitamin D nor calcium supplementation altered overall TLR4 expression in the crypt.

Another possible reason for the minimal impact of vitamin D and calcium treatment on TLR4 expression is that TLR4's primary function is to recognize and bind lipopolysaccharide (LPS), which is a component of gram-negative bacteria[63]. It is likely that overall TLR4 expression in colonic mucosal epithelial cells is driven mainly by the gut microbiota, which have been implicated in tumor development and colorectal cancer[59,63-73]. However, vitamin D is capable of regulating the gut microbiome[74], and deficiencies in calcitriol or VDR can result in dysbiosis[74,75]. Thus, it is likely that vitamin D moderates the effects of the gut microbiome on TLR4 expression, and the potential limitation concerning a lack of information on the participants' microbiomes may be at least partially resolved.

This study's small sample size also presented a possible limitation. Due to low sample sizes in each treatment group, it is likely that this study lacked the statistical power to detect statistically significant changes in TLR4 expression over the study period that were due to vitamin D and/or calcium supplementation. Our small sample size presents additional limitations in the stratified analyses, where we experienced further reductions in power. As such, all findings in the stratified analyses should be considered exploratory and were primarily performed as a hypothesis-generating mechanism. For instance, when we performed a stratified analysis by vitamin D sufficiency, calcium treatments appeared to increase TLR4 expression in the proliferative zone in the six patients with sufficient vitamin D. The small sample size precluded us from drawing any conclusions, but the possibility of such an effect should be evaluated in future studies.

Lastly, this study was only able to obtain tissue samples from patients at baseline and year one, despite the parent clinical trial lasting three to five years. This prevented us from determining if any changes in TLR4 expression from baseline were directly associated with risks of colorectal polyp recurrence, regardless of treatment assignment. However, due to

our small sample sizes, it is likely that the numbers of patients with recurrent polyps in each treatment group would have been too small to detect significance even if we had had biomarker data at this time point. For our study this meant that changes in TLR4 were used as a proxy for the risk of polyp recurrence. A strength gained from this was the ability to determine a potential mechanism of the effects of the treatment agents on TLR4 expression, and therefore in reducing the risk of polyp recurrence, something the parent study was unable to do.

A major strength of this study was patient information on a number of known risk factors for colorectal cancer. While information about patients' gut microbiota was not available, it is unlikely the gut microbiota would have confounded the relationship between treatment assignment and TLR4 expression, since colorectal cancer is a polymicrobial disease[65,71] and the gut microbiota is relatively stable over time[76,77].

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study's findings suggest that a combined treatment of vitamin D and calcium may increase the proportion of TLR4 expression in the differentiation zone relative to the whole crypt over a one-year period for patients at higher risk of colorectal neoplasms. However, vitamin D and calcium treatments do not seem to affect the amount of TLR4 expression in the whole colon crypts, or their differentiation or proliferative zones. This study also found that NSAID use, smoking status, intakes of fruits and vegetables, and dietary fiber, vitamin D status, and having had advanced or serrated adenomas at baseline were associated with baseline TLR4 expression, suggesting that TLR4 expression might be a treatable target for colorectal neoplasms. Further studies are needed to understand a

combined effect of vitamin D and calcium supplementation, and a role of environmental and lifestyle modifiable factors on TLR4 signaling pathway.

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Table 1. Select Baseline Characteristics of Clinical Trial Participants by Treatment Group Assigment (n=105)[a]

	Full Factorial Randomization (4-Arm)					Two-Group Randomization (2-Arm)		
Baseline Characteristics	Placebo (n=12)	Calcium (n=16)	Vitamin D (n=17)	Calcium + Vitamin D (n=18)	*p value*[b]	Placebo (n=23)	Vitamin D (n=19)	*p value*[c]
Age, years	59.9 (7.2)	59.9 (6.5)	59.2 (7.8)	58.0 (7.1)	*0.857*	58.2 (5.3)	59.2 (7.3)	*0.598*
Men, %	75.0	81.3	70.6	83.3	*0.833*	0.0	0.0	--
White, %	83.3	75.0	70.6	94.4	*0.286*	69.6	84.2	*0.305*
College graduate[d], %	66.7	37.5	64.7	55.6	*0.374*	47.8	36.8	*0.542*
1° family history of CRC, %***	0.0	12.5	20.0	5.6	*0.367*	4.4	11.1	*0.573*
Regular[e] non-aspirin NSAID users, %	8.3	18.8	17.7	5.6	*0.585*	8.7	10.5	*0.841*
Regular[e] aspirin users, %	41.7	68.8	29.4	33.3	*0.098*	21.7	31.6	*0.470*
If woman (n=56), HRT users, %	100.0	0.0	20.0	33.3	*0.105*	21.7	31.6	*0.504*
Current smoker, %	25.0	6.3	0.0	5.6	*0.103*	0	15.8	*0.084*
Multivitamin users, %	41.7	81.3	47.1	66.7	*0.102*	69.57	89.5	*0.149*
Physical activity, MET-min/wk[f]*	1620 (1195)	2128 (2378)	2782 (2764)	4042 (2456)	*0.033*	1458 (1235)	3021 (3469)	*0.051*
BMI, kg/m^2	29.4 (4.9)	32.3 (7.6)	28.7 (5.5)	30.2 (4.4)	*0.313*	29.7 (5.6)	27.5 (4.7)	*0.178*
Adenomas removed at colonoscopy	1.58 (0.67)	1.63 (0.96)	1.35 (0.79)	1.39 (0.70)	*0.702*	1.17 (0.65)	1.58 (0.96)	*0.113*
Had advanced adenoma, %***	36.4	6.7	23.5	27.8	*0.295*	9.1	15.8	*0.649*
Dietary Intakes								
Total energy intake, kcal/d***	1314 (381)	1737 (556)	1437 (527)	1569 (565)	*0.213*	1254 (549)	1429 (595)	*0.327*
Total fat, g/d***	57.1 (22.3)	68.9 (25.6)	60.5 (27.3)	61.6 (26.8)	*0.688*	50.3 (25.9)	61.5 (36.1)	*0.249*
Calories from fat, %***	38.9 (8.1)	35.8 (7.0)	37.2 (6.6)	35.2 (8.0)	*0.583*	35.6 (7.7)	36.0 (8.3)	*0.610*
Dietary fiber, g/d***	9.5 (4.1)	15.8 (5.6)	13.7 (6.2)	15.1 (5.7)	*0.043*	13.8 (5.4)	17.2 (5.0)	*0.043*
Red and/or processed meat, servings/d	1.21 (0.86)	1.04 (0.72)	0.89 (0.76)	1.02 (0.71)	*0.740*	0.58 (0.54)	0.67 (0.55)	*0.595*
Fruits and vegetables, servings/d***	3.0 (1.7)	4.4 (2.0)	4.5 (2.5)	4.3 (1.7)	*0.278*	4.7 (1.7)	6.0 (2.4)	*0.045*
Alcohol intake, drinks/day	0.68 (0.74)	0.81 (1.0)	0.85 (0.95)	0.86 (0.90)	*0.950*	0.54 (0.98)	0.34 (0.50)	*0.422*
Total vitamin D[g], IU/d	354 (307)	457 (189)	313 (278)	421 (296)	*0.485*	521 (354)	634 (276)	*0.341*
Total calcium[h], mg/d	715 (455)	895 (264)	671 (278)	667 (255)	*0.143*	996 (498)	1232 (563)	*0.198*
Serum levels								
25-OH-vitamin D_3, ng/mL	22.4 (8.2)	24.5 (13.4)	23.1 (8.7)	22.7 (6.4)	*0.934*	24.8 (8.9)	26.5 (9.6)	*0.543*
Ca^{2+}, mg/dL	9.2 (0.19)	9.3 (0.32)	9.3 (0.34)	9.4 (0.29)	*0.249*	9.48 (0.33)	9.42 (0.32)	*0.516*

Abbreviations: CRC= colorectal cancer; HRT= hormone replacement therapy; NSAID= non-steroidal anti-inflammatory drug; BMI= body mass index; g= grams; IU= international units; kcal= kilocalories; d= day; wk= week; MET=metabolic

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

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

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

[d]Received a Bachelor's degree or higher

[e]At least four times a week

[f]Metabolic equivalent of task

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

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

*One patient missing per asterisk

Table 2. Comparison of TLR4 Expression in the Normal-Appearing Colorectal Mucosa of Study Participants (n=105) by Treatment Assignment[a]

	Baseline				1-Yr Follow-up				Relative Treatment Effect[d]			Absolute[e]
		Geometric[b]				Geometric			Geometric			
Treatment Group	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx Effec	(95% CI)	*p value*	Rx Effect
Whole crypts												
4-Arm												
Placebo	12	697.6	(465.4, 1,045.6)		12	737.2	(491.7, 1,105.2)					
Calcium	16	742.4	(522.9, 1,054.1)	*0.817*	16	807.1	(568.4, 1,146.2)	*0.736*	1.03	(0.5, 2.0)	*0.932*	25.1
Vitamin D	17	686.4	(488.5, 964.4)	*0.951*	17	662.0	(471.1, 930.2)	*0.685*	0.91	(0.5, 1.7)	*0.780*	-64.1
Calcium+Vitamin D	18	569.6	(409.3, 792.7)	*0.441*	18	622.7	(447.3, 866.6)	*0.520*	1.03	(0.5, 2.0)	*0.917*	13.4
2-Arm												
Placebo	23	580.0	(430.0, 782.3)		23	505.9	(341.5, 749.5)					
Vitamin D	19	486.5	(350.0, 676.1)	*0.429*	19	335.1	(217.4, 516.3)	*0.162*	0.79	(0.4, 1.4)	*0.401*	-77.4
Upper 40% of crypts												
4-Arm												
Placebo	12	212.0	(141.7, 317.4)		12	249.5	(159.0, 391.7)					
Calcium	16	271.5	(191.5, 385.1)	*0.357*	16	264.0	(178.7, 390.1)	*0.851*	0.83	(0.4, 1.6)	*0.563*	-45.0
Vitamin D	17	246.1	(175.4, 345.4)	*0.573*	17	203.9	(139.6, 297.8)	*0.495*	0.70	(0.4, 1.3)	*0.283*	-79.7
Calcium+Vitamin D	18	176.5	(127.0, 245.4)	*0.484*	18	202.4	(140.1, 292.4)	*0.474*	0.97	(0.5, 1.8)	*0.935*	-11.7
2-Arm												
Placebo	23	179.8	(130.3, 248.1)		23	157.9	(104.1, 239.5)					
Vitamin D	19	135.1	(94.8, 192.5)	*0.234*	19	102.0	(64.5, 161.3)	*0.162*	0.86	(0.5, 1.6)	*0.613*	-11.1
Lower 60% of crypts												
4-Arm												
Placebo	12	417.1	(268.4, 648.1)		12	422.5	(282.4, 632.1)					
Calcium	16	382.3	(261.0, 560.0)	*0.766*	16	479.7	(338.4, 680.0)	*0.636*	1.24	(0.6, 2.6)	*0.556*	92.0
Vitamin D	17	365.6	(252.5, 529.5)	*0.649*	17	396.8	(282.8, 556.6)	*0.812*	1.07	(0.5, 2.2)	*0.847*	25.8
Calcium+Vitamin D	18	345.6	(241.2, 495.4)	*0.511*	18	368.6	(265.3, 512.2)	*0.601*	1.05	(0.5, 2.1)	*0.885*	17.6
2-Arm												
Placebo	23	367.0	(272.4, 494.6)		23	314.7	(211.0, 469.3)					
Vitamin D	19	325.0	(234.1, 451.3)	*0.583*	19	209.7	(135.1, 325.4)	*0.175*	0.75	(0.4, 1.3)	*0.323*	-63.0

Table 2. *Cont*

	Baseline				1-Yr Follow-Up				Absolute Treatment Effect			Relative
	n	Mean	(95% CI)	p value	n	Mean	(95% CI)	*p value*	Rx Effec	(95% CI)	*p value*	Rx Effect
φh[c]												
4-Arm												
Placebo	12	31.3	(27.3, 35.2)		12	34.5	(30.8, 38.3)					
Calcium	16	37.0	(33.5, 40.4)	*0.034*	16	33.1	(29.9, 36.3)	*0.560*	-7.2	(-13.9, -0.4)	*0.038*	0.81
Vitamin D	17	37.0	(33.7, 40.4)	*0.031*	17	31.8	(28.6, 34.9)	*0.259*	-8.5	(-15.2, -1.9)	*0.013*	0.78
Calcium+Vitamin D	18	31.3	(28.0, 34.6)	*0.988*	18	32.9	(29.9, 36.0)	*0.501*	-1.7	(-8.3, 4.9)	*0.615*	0.95
2-Arm												
Placebo	23	31.6	(29.4, 33.7)		23	31.9	(29.1, 34.7)					
Vitamin D	19	28.1	(25.7, 30.5)	*0.038*	19	31.0	(27.9, 34.1)	*0.654*	2.5	(-2.3, 7.3)	*0.299*	1.09

[a]The effect of treatment assignment on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC)

[b]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[c]Defined as the percent of expression in the distribution zone (expression in upper 40% of crypts over expression in the whole crypts). Values are means of the optical density (not geometric means)

[d]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[e]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table 3. Comparison of TLR4 Expression in the Normal-Appearing Colorectal Mucosa of Study Participants (n=105) by Treatment Agent[a]

	Baseline				1-Yr Follow-up				Relative Treatment Effect[d]			Absolute[e]
		Geometric[f]				*Geometric*			*Geometric*			
Treatment Effect Comparisons	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Whole Crypts												
No calcium	29	691.0	(533.6, 894.8)		29	692.1	(534.3, 896.6)					
Calcium[b]	34	645.2	(508.2, 819.3)	*0.698*	34	703.5	(553.9, 893.5)	*0.927*	1.09	(0.7, 1.7)	*0.694*	65.5
No vitamin D	51	654.5	(538.5, 795.5)		51	640.0	(507.9, 806.5)					
Vitamin D[c]	54	571.5	(472.8, 690.7)	*0.325*	54	510.4	(407.7, 639.0)	*0.167*	0.91	(0.7, 1.3)	*0.597*	-17.2
Calcium only	39	641.8	(520.0, 792.1)		39	612.8	(461.2, 814.2)					
Vitamin D and calcium[d]	37	525.3	(423.3, 651.9)	*0.190*	37	453.0	(338.3, 606.4)	*0.144*	0.90	(0.6, 1.4)	*0.624*	-6.3
Upper 40% of crypts												
No calcium	29	231.4	(178.0, 300.9)		29	221.7	(166.1, 295.8)					
Calcium	34	216.2	(169.6, 275.5)	*0.705*	34	229.3	(175.7, 299.3)	*0.863*	1.11	(0.7, 1.7)	*0.639*	24.8
No vitamin D	51	212.7	(173.1, 261.5)		51	206.6	(160.8, 265.5)					
Vitamin D	54	178.4	(145.9, 218.0)	*0.228*	54	159.4	(124.9, 203.4)	*0.144*	0.92	(0.7, 1.3)	*0.634*	-1.5
Calcium only	39	213.0	(169.9, 266.9)		39	194.9	(144.2, 263.6)					
Vitamin D and calcium	37	153.9	(122.0, 194.0)	*0.049*	37	142.4	(104.4, 194.0)	*0.152*	1.01	(0.7, 1.5)	*0.960*	19.4
Lower 60% of crypts												
No calcium	29	386.1	(292.0, 510.6)		29	362.4	(314.8, 526.8)					
Calcium	34	362.4	(280.0, 469.2)	*0.741*	34	417.3	(328.9, 529.2)	*0.890*	1.09	(0.7, 1.7)	*0.712*	37.8
No vitamin D	51	383.1	(313.0, 469.0)		51	385.0	(305.7, 484.8)					
Vitamin D	54	344.3	(282.8, 419.1)	*0.454*	54	309.3	(247.3, 387.0)	*0.180*	0.89	(0.6, 1.3)	*0.544*	-19.7
Calcium only	39	373.2	(301.2, 462.4)		39	374.1	(281.3, 497.6)					
Vitamin D and calcium	37	334.9	(268.8, 417.3)	*0.484*	37	275.9	(205.8, 369.7)	*0.142*	0.82	(0.5, 1.3)	*0.371*	-37.1
	Baseline				**1-Yr Follow-Up**				**Absolute Treatment Effect**			**Relative**
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx Effect	(95% CI)	*p value*	Rx Effect
φh[e]												
No calcium	29	34.6	(31.9, 37.4)		29	32.9	(30.5, 35.3)					
Calcium	34	34.0	(31.4, 36.5)	*0.722*	34	33.0	(30.8, 35.2)	*0.959*	0.8	(-4.0, 5.5)	*0.753*	1.02
No vitamin D	51	33.2	(31.3, 35.1)		51	32.9	(31.1, 34.7)					
Vitamin D	54	32.0	(30.1, 33.8)	*0.374*	54	31.9	(30.1, 33.6)	*0.416*	0.2	(-3.2, 3.6)	*0.919*	1.01
Calcium only	39	33.8	(32.0, 35.5)		39	32.4	(30.5, 34.3)					
Vitamin D and calcium	37	29.7	(27.9, 31.4)	*0.002*	37	31.9	(30.0, 33.9)	*0.731*	3.6	(0.2, 7.1)	*0.039*	1.12

[a]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC)

[b]Calcium group were patients assigned to either calcium or to calcium+vitamin D (combined) in the 4-arm group. Patients in the 2-arm group were excluded

[c]Vitamin D group consisted of patients assigned to vitamin D or to calcium+vitamin D (combined) in the 4-arm group, or to vitamin D in the 2-arm group

[d]Vitamin D and Calcium group consisted of patients assigned to calcium+vitamin D (combined) of the 4-arm group, or to vitamin D of the 2-arm group

[e]Defined as the percent of expression in the upper 40% of crypts to expression over the whole crypts. Values are means of the optical density (not geometric means)

[f]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[g]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[h]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table 4. Categorical Baseline Predictors of TLR4 Expression at Baseline[a]

		Whole crypt				Upper 40% of crypt				Lower 60% of crypt				φh[c]			
		Geometric[b]				*Geometric*				*Geometric*							
Baseline Characteristics	n	Mean	(95% CI)	Diff[d]	*p value[e]*	Mean	95% CI	Diff	*p value*	Mean	95% CI	Diff	*p value*	Mean	95% CI	Diff	*p value*
Age																	
< 60 years	56	514.0	(409.8, 644.8)			159.2	(125.2, 202.5)			305.8	(241.3, 387.5)			32.3	(30.5, 34.1)		
≥ 60 years	49	482.9	(388.3, 600.5)	-6.1	*0.849*	157.7	(125.2, 198.8)	-0.9	*0.962*	282.4	(224.9, 354.7)	-7.6	*0.878*	32.9	(30.9, 34.8)	1.8	*0.675*
Sex																	
Men	49	895.1	(651.8, 1,229.3)			264.2	(188.8, 369.7)			567.7	(407.4, 791.0)			29.6	(26.1, 33.1)		
Women	56	625.0	(493.4, 791.6)	-30.2	*0.075*	191.3	(148.9, 245.7)	-27.6	*0.129*	383.1	(299.2, 490.5)	-32.5	*0.062*	31.6	(29.0, 34.2)	6.8	*0.361*
1° family history of CRC																	
No	93	510.1	(423.6, 614.4)			160.6	(131.5, 196.2)			305.9	(252.2, 371.0)			32.4	(31.0, 33.9)		
Yes	9	614.3	(398.8, 946.3)	20.4	*0.339*	191.8	(120.5, 305.4)	19.4	*0.418*	347.1	(221.6, 543.7)	13.5	*0.445*	31.8	(27.2, 36.3)	-2.1	*0.776*
Regular NSAID use (4 or more times per week)																	
No	93	535.1	(438.9, 652.3)			163.2	(131.9, 201.9)			328.8	(268.4, 402.7)			32.0	(30.4, 33.6)		
Yes	12	376.1	(253.1, 558.7)	-29.7	*0.119*	139.0	(90.9, 212.7)	-14.8	*0.506*	190.4	(126.9, 285.7)	-42.1	*0.020*	33.8	(31.3, 36.2)	5.4	*0.010*
Regular aspirin user (4 or more times per week)																	
No	67	502.7	(412.4, 612.9)			161.3	(130.8, 198.9)			298.3	(242.5, 367.0)			32.7	(30.9, 34.4)		
Yes	38	487.1	(374.1, 634.1)	-3.1	*0.097*	150.6	(113.9, 199.0)	-6.7	*0.145*	284.6	(216.0, 375.0)	-4.6	*0.133*	32.4	(30.4, 34.5)	-0.7	*0.865*
Current HRT user (women only, N=56)																	
No	40	552.5	(426.7, 715.3)			177.2	(133.9, 234.5)			325.6	(250.4, 423.4)			33.1	(30.6, 35.6)		
Yes	16	408.7	(262.7, 814.1)	-26.0	*0.807*	121.1	(75.0, 195.5)	-31.7	*0.697*	256.0	(163.3, 401.2)	-21.4	*0.533*	30.9	(26.5, 35.2)	-6.8	*0.399*
Smoking status																	
Never	61	576.8	(466.9, 712.8)			185.7	(148.4, 232.3)			336.1	(268.9, 420.1)			33.3	(30.8, 35.7)		
Former or current	8	417.7	(333.3, 523.4)	-27.6	*0.021*	131.4	(103.5, 166.8)	-29.3	*0.020*	249.7	(196.8, 316.8)	-25.7	*0.044*	32.4	(29.8, 35.0)	-2.6	*0.583*
Multivitamin user																	
No	34	476.4	(377.2, 601.7)			148.9	(116.3, 190.6)			289.4	(226.5, 369.6)			32.2	(29.6, 34.8)		
Yes	71	519.8	(412.6, 654.8)	9.1	*0.561*	167.3	(131.0, 213.5)	12.3	*0.463*	298.8	(234.6, 380.6)	3.3	*0.838*	33.2	(30.7, 35.8)	3.2	*0.537*
Physical activity (MET-min/wk)[t]*																	
Low	37	494.7	(386.7, 632.9)			162.0	(124.8, 210.3)			280.7	(217.1, 362.9)			33.8	(31.1, 36.5)		
Moderate	33	545.4	(414.1, 718.3)	10.2		169.9	(126.9, 227.4)	4.8		332.9	(249.8, 443.7)	18.6		32.2	(29.2, 35.2)	-4.7	
High	35	468.2	(363.2, 603.7)	-5.4	*0.729*	145.4	(111.1, 190.3)	-10.3	*0.519*	282.1	(216.5, 367.8)	0.5	*0.974*	31.9	(29.1, 34.7)	-5.7	*0.273*
BMI																	
Normal (<25)	22	433.5	(312.9, 600.6)			139.7	(98.9, 197.4)			249.3	(177.3, 350.6)			33.6	(30.0, 37.3)		
Overweight (25-30)	43	479.1	(376.8, 609.1)	10.5		150.1	(116.4, 193.7)	7.4		288.4	(224.3, 370.7)	15.7		32.2	(29.5, 34.9)	-4.4	
Obese (≥ 30)	40	548.9	(432.1, 697.2)	26.6	*0.212*	174.7	(135.5, 225.1)	25.0	*0.231*	323.0	(251.5, 414.9)	29.6	*0.241*	32.8	(30.1, 35.4)	-2.6	*0.971*
Total energy intake (kcal/d)[t]*																	
Low	35	523.0	(406.5, 672.9)			166.0	(127.1, 216.8)			310.7	(238.8, 404.4)			32.9	(30.1, 35.7)		
Medium	36	479.2	(362.6, 633.3)	-8.4		152.5	(113.5, 204.9)	-8.1		280.2	(209.3, 375.1)	-9.8		32.6	(29.5, 35.7)	-0.9	
High	34	489.1	(379.1, 631.1)	-6.5	*0.658*	154.7	(118.1, 202.7)	-6.8	*0.662*	289.6	(221.8, 378.0)	-6.8	*0.654*	32.6	(29.8, 35.4)	-1.0	*0.852*
Number of adenomas																	
1 polyp	76	502.1	(408.8, 616.6)			158.5	(127.5, 197.1)			298.9	(241.1, 370.5)			32.6	(30.3, 34.8)		
> 1 polyp	29	489.2	(369.5, 647.6)	-2.6	*0.871*	156.7	(116.4, 210.9)	-1.2	*0.945*	284.1	(211.9, 381.0)	-4.9	*0.762*	33.1	(30.0, 36.2)	1.6	*0.762*

Table 4. ***Cont***

		Whole crypt				Upper 40% of crypt				Lower 60% of crypt				φh[c]			
	n	Mean	(95% CI)		*p value*[d]	Mean	95% CI		*p value*	Mean	95% CI		*p value*	Mean	95% CI		*p value*
Had advanced adenomas																	
No	83	467.0	(381.6, 571.5)			152.9	(123.3, 189.8)			268.8	(218.3, 331.0)			33.8	(31.6, 36.0)		
Yes	19	590.9	(423.7, 823.9)	26.5	*0.209*	170.1	(119.2, 242.6)	11.2	*0.594*	382.0	(271.1, 538.2)	42.1	*0.071*	29.4	(25.8, 33.0)	-13.0	*0.031*
Had serrated adenomas																	
No	79	458.1	(376.5, 557.5)			148.1	(120.0, 182.7)			266.6	(217.4, 326.9)			33.3	(31.1, 35.5)		
Yes	26	611.6	(461.7, 810.2)	33.5	*0.067*	185.3	(137.1, 250.4)	25.2	*0.181*	374.8	(279.8, 501.9)	40.6	*0.038*	31.2	(28.1, 34.4)	-6.3	*0.227*
Percent daily calories from fat[t]*																	
Low	35	470.2	(362.4, 610.0)			144.5	(109.6, 190.5)			285.5	(217.7, 374.4)			31.7	(28.8, 34.7)		
Moderate	35	609.1	(458.9, 808.5)	29.5		193.4	(143.2, 261.2)	33.9		367.7	(273.8, 493.8)	28.8		32.6	(29.5, 35.8)	2.8	
High	35	462.6	(363.0, 589.4)	-1.6	*0.849*	150.7	(116.5, 194.9)	4.3	*0.881*	263.4	(204.6, 339.0)	-7.7	*0.576*	33.6	(30.9, 36.3)	5.9	*0.311*
Dietary fiber (g/1000kcal/d)[t]*																	
Low	35	428.5	(336.1, 546.2)			140.2	(108.1, 181.7)			246.5	(191.6, 317.2)			33.7	(31.0, 36.4)		
Moderate	35	565.8	(436.1, 734.0)	32.1		178.3	(135.0, 235.6)	27.2		338.7	(258.4, 443.9)	37.4		32.5	(29.6, 35.4)	-3.4	
High	35	548.1	(415.3, 723.3)	27.9	*0.154*	164.9	(122.6, 221.9)	17.7	*0.392*	334.2	(250.6, 445.9)	35.6	*0.088*	31.1	(28.0, 34.2)	-7.6	*0.152*
Achieved daily recommended dietary fiber intake (≥ 14 g/1000 kcal per day)*																	
No	87	483.4	(404.2, 578.0)			154.8	(127.8, 187.5)			283.8	(235.7, 341.8)			33.0	(31.0, 35.0)		
Yes	18	659.3	(455.7, 953.8)	36.4	*0.090*	191.1	(128.6, 283.9)	23.4	*0.280*	411.8	(280.5, 604.5)	45.1	*0.051*	30.0	(25.9, 34.1)	-9.2	*0.132*
Red and processed meat, servings/d																	
None	9	712.4	(438.4, 1,157.5)			249.0	(149.3, 415.3)			423.3	(254.5, 703.9)			33.5	(28.9, 38.1)		
1 or less	61	460.1	(372.6, 568.2)	-35.4		146.2	(117.1, 182.6)	-41.3		272.4	(218.3, 339.8)	-35.7		33.6	(31.2, 36.0)	0.3	
More than 1	35	512.2	(391.2, 670.7)	-28.1	*0.701*	157.3	(118.4, 209.0)	-36.8	*0.454*	300.9	(226.9, 399.1)	-28.9	*0.677*	32.6	(30.1, 35.2)	-2.5	*0.357*
Achieved daily recommended intake of fruit and vegetables (≥ 5 servings/d)[t]*																	
No	66	521.1	(424.4, 640.0)			172.3	(139.0, 213.6)			299.0	(241.0, 371.1)			33.9	(32.3, 35.6)		
Yes	39	458.9	(355.4, 592.5)	-11.9	*0.378*	135.3	(103.5, 176.7)	-21.5	*0.111*	285.5	(218.3, 373.4)	-4.5	*0.759*	30.3	(28.1, 32.4)	-10.8	*0.008*
Alcohol intake																	
None	36	482.2	(367.9, 632.2)			161.1	(120.9, 214.6)			282.3	(212.7, 374.7)			31.7	(29.4, 34.0)		
≤ 1 drink/wk	46	515.1	(394.2, 673.2)	6.8		159.8	(120.4, 212.2)	-0.8		304.7	(230.3, 403.0)	7.9		31.8	(29.3, 34.3)	0.1	
> 1 drink/wk	23	502.3	(365.3, 690.6)	4.2	*0.848*	150.8	(107.6, 211.3)	-6.4	*0.768*	300.5	(215.4, 419.2)	6.4	*0.779*	32.8	(30.0, 35.7)	3.4	*0.164*
Vitamin D deficiency (based on serum levels)																	
Deficient (< 30 ng/m	78	468.0	(391.4, 559.7)			149.1	(123.1, 180.5)			276.8	(229.4, 334.0)			32.8	(30.8, 34.9)		
Sufficient (≥ 30 ng/m	27	669.8	(499.4, 898.2)	43.1	*0.015*	208.3	(152.2, 285.0)	39.7	*0.034*	393.3	(288.9, 535.4)	42.1	*0.023*	32.2	(28.9, 35.6)	-1.8	*0.726*
Achieved daily recommended dietary calcium intake (≥ 1000 mg/d for males; ≥ 1200 for females)																	
No	72	464.2	(383.9, 561.3)			148.0	(120.5, 181.8)			271.8	(223.0, 331.3)			32.9	(30.8, 35.1)		
Yes	21	548.1	(395.0, 760.6)	18.1	*0.324*	177.4	(124.4, 253.0)	19.9	*0.320*	323.3	(229.9, 454.8)	18.9	*0.323*	33.2	(29.4, 37.0)	0.8	*0.891*

Abbreviations: Diff= proportional difference; CRC= colorectal cancer; HRT= hormone replacement therapy; NSAID= non-steroidal anti-inflammatory drug; BMI= body mass index; MET= metabolic equivalent of task; g= grams; IU= international units; kcal= kilocalories; d= day; wk= week

[a]The effect of the baseline characteristics on TLR4 expression was modeled using PROC GLM in SAS 9.4 (Cary, NC), controlling for age (continuous), gender (by study arm), study center and batch number, where appropriate.

[b]Reported means are geometric means (95% CI) and arithmatic means (95% CI) of optical density.

[c]Defined as the percent expression in the upper 40% of crypts to expression in whole crypts. Values are means (95% CI) of the optical density (not geometric means)

[d]Proportional Difference= (Comparison mean- reference mean)/reference mean*100%

[e]Reported p values are the Type III SS of the baseline characteristic. For baseline characteristics with more than two categories, the variables were treated as continuous and the reported p-value are of the overall trend

[t]Categorized by tertiles, according to gender and study-arm

*Missing values were replaced with treatment group- and sex-specific means

Table 5. Stratified Comparisons of TLR4 Expression in the Whole Crypt of the Normal-Appearing Colorectal Mucosa by Treatment Agent (n=105)[a]

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
		Geometric[e]				*Geometric*			*Geometric*			
Baseline Characteristics	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Gender												
Men												
No calcium	21	784.1	(591.8, 1,038.9)		21	707.1	(520.9, 959.9)					
Calcium[b]	28	665.2	(521.3, 848.8)	*0.379*	28	718.5	(551.4, 936.2)	*0.937*	1.20	(0.7, 1.9)	*0.456*	130.27
No vitamin D	22	702.6	(532.5, 927.0)		22	745.7	(553.5, 1,004.6)					
Vitamin D[c]	27	723.0	(563.0, 928.5)	*0.878*	27	688.5	(526.1, 901.1)	*0.692*	0.90	(0.6, 1.5)	*0.653*	-77.58
Calcium only	13	766.7	(534.8, 1,099.3)		13	813.6	(526.2, 1,258.2)					
Vitamin D and calcium[d]	15	588.1	(420.5, 822.5)	*0.278*	15	645.1	(429.9, 968.0)	*0.430*	1.03	(0.5, 2.1)	*0.926*	10.07
Women												
No calcium	8	496.0	(255.6, 962.3)		8	654.2	(366.8, 1,166.8)					
Calcium	6	559.8	(260.4, 1,203.4)	*0.799*	6	637.5	(326.9, 1,243.5)	*0.950*	0.86	(0.3, 2.6)	*0.778*	-80.51
No vitamin D	29	620.2	(474.4, 811.0)		29	570.0	(409.0, 794.4)					
Vitamin D	27	451.6	(342.1, 596.3)	*0.105*	27	378.4	(268.2, 533.8)	*0.092*	0.91	(0.6, 1.5)	*0.708*	-23.01
Calcium only	26	587.2	(450.2, 765.9)		26	531.8	(370.1, 764.1)					
Vitamin D and calcium	22	486.3	(364.3, 649.2)	*0.339*	22	355.9	(240.0, 527.8)	*0.138*	0.81	(0.5, 1.4)	*0.410*	-75.02
Regular NSAID use												
Less than every other day												
No calcium	25	733.0	(567.0, 947.6)		25	683.0	(512.7, 909.9)					
Calcium	30	678.6	(536.8, 857.8)	*0.658*	30	724.3	(557.5, 941.1)	*0.763*	1.15	(0.7, 1.8)	*0.561*	95.75
No vitamin D	45	672.7	(557.9, 811.1)		45	669.8	(522.7, 858.2)					
Vitamin D	48	620.5	(517.7, 743.7)	*0.539*	48	518.1	(407.5, 658.5)	*0.143*	0.84	(0.6, 1.2)	*0.337*	-99.53
Calcium only	34	678.9	(551.5, 835.8)		34	659.4	(485.2, 896.2)					
Vitamin D and calcium	34	558.3	(453.5, 687.3)	*0.189*	34	466.6	(343.3, 634.2)	*0.116*	0.86	(0.6, 1.3)	*0.502*	-72.22
Every other day or more												
No calcium	4	477.9	(132.9, 1,718.3)		4	752.1	(352.3, 1,605.4)					
Calcium	4	442.2	(123.0, 1,589.9)	*0.920*	4	565.3	(264.8, 1,206.5)	*0.539*	0.81	(0.2, 3.3)	*0.729*	-151.13
No vitamin D	6	533.0	(202.9, 1,400.2)		6	455.1	(215.5, 961.2)					
Vitamin D	6	295.7	(112.6, 776.9)	*0.359*	6	453.3	(214.6, 957.4)	*0.994*	1.80	(0.6, 5.0)	*0.230*	235.43
Calcium only	5	437.9	(154.5, 1,241.0)		5	371.9	(153.7, 900.2)					
Vitamin D and calcium	3	263.3	(68.6, 1,010.4)	*0.492*	3	323.4	(103.3, 1,012.4)	*0.821*	1.45	(0.3, 6.5)	*0.569*	126.08

Table 5. *Cont*

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Vitamin D deficiency (< 30 ng/mL)												
Deficient												
No calcium	23	645.2	(481.8, 863.8)		23	653.4	(488.8, 873.6)					
Calcium	28	619.7	(475.7, 807.3)	*0.838*	28	616.0	(473.4, 801.4)	*0.763*	0.98	(0.6, 1.6)	*0.942*	-11.95
No vitamin D	36	608.7	(477.7, 775.6)		36	594.7	(447.8, 789.7)					
Vitamin D	42	536.8	(429.0, 671.8)	*0.451*	42	481.7	(370.4, 626.4)	*0.281*	0.92	(0.6, 1.4)	*0.692*	-41.13
Calcium only	27	589.2	(450.5, 770.5)		27	560.4	(390.8, 803.8)					
Vitamin D and calcium	28	496.0	(381.1, 645.5)	*0.363*	28	424.8	(298.2, 605.3)	*0.363*	0.90	(0.5, 1.5)	*0.688*	-42.39
Sufficient												
No calcium	6	899.2	(472.5, 1,711.1)		6	862.8	(526.5, 1,413.9)					
Calcium	6	779.4	(409.6, 1,483.1)	*0.734*	6	1307.9	(798.2, 2,143.3)	*0.214*	1.75	(1.0, 3.2)	*0.065*	564.93
No vitamin D	15	779.1	(566.7, 1,071.2)		15	763.4	(508.6, 1,146.0)					
Vitamin D	12	711.1	(498.1, 1,015.1)	*0.697*	12	625.1	(396.9, 984.3)	*0.505*	0.90	(0.5, 1.5)	*0.684*	-70.34
Calcium only	12	778.1	(561.7, 1,077.7)		12	749.0	(470.2, 1,193.4)					
Vitamin D and calcium	9	628.0	(431.1, 914.9)	*0.379*	9	552.8	(322.9, 946.4)	*0.383*	0.91	(0.5, 1.9)	*0.794*	-46.22
Daily Fruit and Vegetable Consumption												
Below recommendations												
No calcium	21	668.1	(482.1, 925.9)		21	771.5	(573.1, 1,038.5)					
Calcium	23	676.7	(495.4, 924.3)	*0.955*	23	687.2	(517.3, 912.9)	*0.573*	0.88	(0.5, 1.4)	*0.592*	-92.90
No vitamin D	35	733.8	(577.4, 932.6)		35	751.7	(585.6, 964.7)					
Vitamin D	31	526.6	(408.2, 679.4)	*0.063*	31	565.9	(434.1, 737.7)	*0.124*	1.05	(0.7, 1.6)	*0.763*	21.43
Calcium only	25	736.1	(572.0, 947.1)		25	763.1	(555.2, 1,048.8)					
Vitamin D and calcium	20	482.4	(363.9, 639.4)	*0.029*	20	462.2	(323.9, 659.6)	*0.040*	0.92	(0.5, 1.6)	*0.763*	-47.17
Met recommendations												
No calcium	8	755.0	(480.4, 1,186.7)		8	520.5	(297.3, 911.3)					
Calcium	11	584.2	(397.3, 859.0)	*0.375*	11	738.9	(458.2, 1,191.2)	*0.330*	1.83	(0.7, 4.8)	*0.196*	389.16
No vitamin D	16	509.6	(362.9, 715.7)		16	450.2	(279.5, 725.2)					
Vitamin D	23	638.0	(480.6, 846.8)	*0.310*	23	444.2	(298.4, 661.0)	*0.965*	0.79	(0.4, 1.5)	*0.448*	-134.42
Calcium only	14	502.5	(344.9, 732.2)		14	414.2	(240.7, 712.7)					
Vitamin D and calcium	17	580.7	(412.7, 817.1)	*0.565*	17	442.3	(270.2, 723.8)	*0.856*	0.92	(0.5, 1.9)	*0.823*	-50.10
Smoking Status												
Current or former												
No calcium	13	517.4	(314.5, 851.2)		13	763.8	(526.5, 1,108.1)					
Calcium	15	533.1	(335.4, 847.5)	*0.929*	15	801.0	(566.5, 1,132.5)	*0.849*	1.02	(0.5, 1.9)	*0.955*	21.40
No vitamin D	17	597.9	(408.4, 875.5)		17	691.3	(483.0, 989.5)					
Vitamin D	27	479.3	(354.1, 648.6)	*0.364*	27	646.3	(486.2, 859.0)	*0.768*	1.17	(0.7, 1.9)	*0.515*	73.60
Calcium only	12	575.0	(379.8, 870.4)		12	690.4	(435.5, 1,094.2)					
Vitamin D and calcium	19	494.2	(355.4, 687.0)	*0.563*	19	587.3	(407.3, 846.9)	*0.579*	0.99	(0.5, 1.8)	*0.972*	-22.23
Never												
No calcium	16	874.1	(694.2, 1,100.7)		16	638.9	(439.3, 929.1)					
Calcium	19	750.2	(607.2, 926.9)	*0.327*	19	635.0	(450.3, 895.5)	*0.981*	1.16	(0.7, 2.0)	*0.589*	120.05
No vitamin D	34	684.8	(551.5, 850.2)		34	615.8	(455.3, 832.9)					
Vitamin D	27	681.4	(534.5, 868.6)	*0.975*	27	403.1	(287.3, 565.7)	*0.067*	0.66	(0.4, 1.0)	*0.068*	-209.26
Calcium only	27	674.0	(526.1, 863.3)		27	581.1	(403.2, 837.7)					
Vitamin D and calcium	18	560.3	(413.7, 758.8)	*0.347*	18	344.3	(220.0, 538.8)	*0.075*	0.71	(0.4, 1.3)	*0.239*	-123.13

Table 5. *Cont*

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Presence of Advanced Adenomas												
None present												
No calcium	21	606.0	(440.6, 833.5)		21	735.9	(541.6, 999.8)					
Calcium	28	665.2	(504.7, 876.6)	*0.659*	28	685.1	(525.3, 893.4)	*0.724*	0.85	(0.5, 1.4)	*0.484*	-110.04
No vitamin D	44	658.9	(534.5, 812.1)		44	638.0	(492.9, 825.8)					
Vitamin D	42	542.7	(438.2, 672.3)	*0.201*	42	496.6	(381.3, 646.6)	*0.181*	0.94	(0.7, 1.4)	*0.757*	-25.35
Calcium only	36	660.3	(531.0, 821.1)		36	611.5	(450.6, 829.7)					
Vitamin D and calcium	29	527.2	(413.6, 672.1)	*0.173*	29	421.9	(300.3, 592.8)	*0.110*	0.86	(0.6, 1.3)	*0.506*	-56.53
One or more present												
No calcium	8	975.3	(650.2, 1,462.8)		8	589.2	(336.7, 1,031.1)					
Calcium	6	559.9	(350.6, 894.1)	*0.075*	6	796.5	(417.4, 1,519.7)	*0.457*	2.35	(0.8, 6.8)	*0.106*	622.65
No vitamin D	7	627.8	(346.5, 1,137.5)		7	652.6	(365.1, 1,166.7)					
Vitamin D	12	684.4	(434.6, 1,077.5)	*0.811*	12	562.1	(360.6, 875.9)	*0.672*	0.79	(0.3, 2.2)	*0.639*	-147.15
Calcium only	3	456.3	(175.4, 1,187.6)		3	628.5	(235.1, 1,680.4)					
Vitamin D and calcium	8	518.4	(288.6, 931.1)	*0.803*	8	585.9	(320.9, 1,070.1)	*0.894*	0.82	(0.2, 4.4)	*0.796*	-104.60
Presence of Serrated Adenomas												
None present												
No calcium	24	693.8	(515.0, 934.7)		24	699.5	(531.1, 921.1)					
Calcium	22	617.2	(452.1, 842.5)	*0.587*	22	660.4	(495.3, 880.3)	*0.772*	1.06	(0.6, 1.8)	*0.825*	37.52
No vitamin D	37	648.6	(514.3, 817.8)		37	609.1	(464.4, 798.9)					
Vitamin D	42	520.1	(418.4, 646.6)	*0.171*	42	496.4	(384.8, 640.3)	*0.277*	1.02	(0.7, 1.5)	*0.938*	15.75
Calcium only	27	598.3	(463.8, 771.8)		27	558.2	(394.6, 789.8)					
Vitamin D and calcium	28	475.2	(370.1, 610.1)	*0.201*	28	432.9	(307.9, 608.7)	*0.299*	0.98	(0.6, 1.6)	*0.926*	-2.23
One or more present												
No calcium	5	677.7	(364.6, 1,259.7)		5	657.9	(305.9, 1,415.0)					
Calcium	12	700.0	(469.2, 1,044.5)	*0.927*	12	790.1	(481.9, 1,295.3)	*0.675*	1.16	(0.5, 2.6)	*0.699*	109.81
No vitamin D	14	670.5	(465.5, 965.8)		14	729.5	(452.2, 1,176.9)					
Vitamin D	12	794.3	(535.6, 1,178.0)	*0.521*	12	562.7	(335.7, 943.2)	*0.454*	0.65	(0.4, 1.2)	*0.161*	-290.70
Calcium only	12	751.5	(513.8, 1,099.3)		12	755.7	(442.7, 1,289.9)					
Vitamin D and calcium	9	717.6	(462.6, 1,113.1)	*0.869*	9	521.4	(281.2, 966.7)	*0.354*	0.72	(0.3, 1.5)	*0.381*	-200.35

[a]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC)

[b]Calcium group were patients assigned to either calcium or to calcium+vitamin D (combined) in the 4-arm group. Patients in the 2-arm group were excluded

[c]Vitamin D group consisted of patients assigned to vitamin D or to calcium+vitamin D (combined) in the 4-arm group, or to vitamin D in the 2-arm group

[d]Vitamin D and Calcium group consisted of patients assigned to calcium+vitamin D (combined) of the 4-arm group, or to vitamin D of the 2-arm group

[e]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[f]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[g]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table 6. Stratified Comparisons of TLR4 Expression in the Crypt Differentiation Zone in the Normal-Appearing Colorectal Mucosa by Treatment Agent (n=105)[a]

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	Geometric[e]				*Geometric*				*Geometric*			
Baseline Characteristics	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Gender												
Men												
No calcium	21	267.4	(197.9, 361.3)		21	227.3	(162.9, 317.3)					
Calcium[b]	28	224.1	(172.6, 290.8)	*0.376*	28	238.1	(178.4, 317.8)	*0.834*	1.25	(0.8, 2.1)	*0.382*	54.10
No vitamin D	22	249.2	(185.3, 335.1)		22	249.3	(180.2, 345.0)					
Vitamin D[c]	27	235.7	(180.4, 308.0)	*0.781*	27	221.2	(165.0, 296.6)	*0.585*	0.94	(0.6, 1.6)	*0.801*	-14.65
Calcium only	13	287.8	(200.8, 412.4)		13	273.4	(174.2, 429.2)					
Vitamin D and calcium[d]	15	180.4	(129.0, 252.1)	*0.996*	15	211.2	(138.8, 321.4)	*0.397*	1.23	(0.6, 2.5)	*0.547*	45.17
Women												
No calcium	8	158.4	(89.4, 280.6)		8	207.5	(104.8, 411.0)					
Calcium	6	182.9	(94.5, 354.0)	*0.725*	6	192.6	(87.5, 423.9)	*0.879*	0.80	(0.3, 2.0)	*0.619*	-39.49
No vitamin D	29	188.7	(143.4, 248.3)		29	179.1	(125.3, 256.2)					
Vitamin D	27	135.0	(101.6, 179.4)	*0.095*	27	114.8	(79.3, 166.4)	*0.089*	0.90	(0.5, 1.5)	*0.657*	-10.59
Calcium only	26	183.2	(137.5, 244.1)		26	164.6	(112.0, 241.9)					
Vitamin D and calcium	22	138.0	(101.1, 188.6)	*0.185*	22	108.8	(71.6, 165.3)	*0.150*	0.88	(0.5, 1.5)	*0.629*	-10.68
Regular NSAID use												
Less than every other day												
No calcium	25	236.3	(179.3, 311.3)		25	218.2	(159.8, 298.0)					
Calcium	30	224.4	(174.4, 288.7)	*0.784*	30	238.1	(179.1, 316.4)	*0.681*	1.15	(0.7, 1.9)	*0.569*	31.67
No vitamin D	45	218.1	(177.7, 267.8)		45	216.1	(165.2, 282.7)					
Vitamin D	48	189.2	(155.1, 230.7)	*0.324*	48	161.7	(124.7, 209.7)	*0.127*	0.86	(0.6, 1.3)	*0.437*	-25.41
Calcium only	34	225.4	(180.0, 282.4)		34	209.9	(151.1, 291.5)					
Vitamin D and calcium	34	162.8	(129.9, 203.9)	*0.045*	34	146.7	(105.6, 203.7)	*0.128*	0.97	(0.6, 1.5)	*0.884*	-0.61
Every other day or more												
No calcium	4	203.4	(66.8, 618.9)		4	244.3	(85.2, 700.8)					
Calcium	4	163.4	(53.7, 497.2)	*0.745*	4	173.3	(60.4, 497.1)	*0.593*	0.88	(0.3, 2.6)	*0.787*	-31.04
No vitamin D	6	176.3	(66.1, 469.8)		6	147.5	(64.7, 336.1)					
Vitamin D	6	111.6	(41.9, 297.3)	*0.479*	6	142.1	(62.3, 323.8)	*0.944*	1.52	(0.6, 4.0)	*0.361*	59.30
Calcium only	5	144.6	(47.2, 442.4)		5	117.9	(50.3, 276.4)					
Vitamin D and calcium	3	81.2	(19.2, 344.0)	*0.469*	3	101.5	(33.8, 304.9)	*0.801*	1.53	(0.3, 8.4)	*0.562*	47.00

Table 6. *Cont*

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Vitamin D deficiency (< 30 ng/mL)												
Deficient												
No calcium	23	216.5	(160.8, 291.4)		23	204.0	(147.5, 282.2)					
Calcium	28	204.9	(156.5, 268.3)	*0.785*	28	197.6	(147.3, 265.1)	*0.884*	1.02	(0.6, 1.7)	*0.929*	5.10
No vitamin D	36	195.3	(151.7, 251.5)		36	188.4	(138.6, 256.2)					
Vitamin D	42	172.0	(136.1, 217.3)	*0.465*	42	150.5	(113.3, 200.0)	*0.288*	0.91	(0.6, 1.4)	*0.657*	-14.60
Calcium only	27	193.7	(146.0, 257.1)		27	175.6	(120.0, 256.9)					
Vitamin D and calcium	28	149.5	(113.2, 197.4)	*0.196*	28	134.9	(92.9, 196.0)	*0.326*	1.00	(0.6, 1.7)	*0.987*	3.51
Sufficient												
No calcium	6	298.8	(158.3, 564.3)		6	304.6	(182.2, 509.1)					
Calcium	6	277.4	(146.9, 523.7)	*0.857*	6	459.7	(275.0, 768.4)	*0.236*	1.63	(0.8, 3.3)	*0.165*	176.55
No vitamin D	15	261.2	(181.5, 375.9)		15	257.6	(165.6, 400.9)					
Vitamin D	12	202.7	(134.9, 304.5)	*0.348*	12	194.7	(118.8, 319.2)	*0.393*	0.97	(0.6, 1.7)	*0.922*	-4.39
Calcium only	12	263.5	(179.5, 386.9)		12	246.6	(148.4, 409.7)					
Vitamin D and calcium	9	168.2	(108.0, 262.1)	*0.126*	9	168.2	(93.6, 302.3)	*0.315*	1.07	(0.6, 2.1)	*0.836*	16.92
Daily Fruit and Vegetable Consumption												
Below recommendations												
No calcium	21	237.1	(171.2, 328.4)		21	247.1	(177.6, 343.7)					
Calcium	23	226.9	(166.2, 309.7)	*0.844*	23	227.1	(165.6, 311.3)	*0.712*	0.96	(0.6, 1.6)	*0.869*	-9.72
No vitamin D	35	248.4	(194.2, 317.8)		35	252.8	(192.0, 332.9)					
Vitamin D	31	171.4	(132.0, 222.7)	*0.043*	31	171.7	(128.2, 230.0)	*0.059*	0.98	(0.7, 1.5)	*0.939*	-4.08
Calcium only	25	254.3	(195.7, 330.6)		25	256.2	(181.9, 360.9)					
Vitamin D and calcium	20	142.6	(106.4, 191.1)	*0.005*	20	139.9	(95.4, 205.2)	*0.022*	0.97	(0.6, 1.7)	*0.923*	-4.54
Met recommendations												
No calcium	8	217.1	(133.1, 353.9)		8	166.8	(88.4, 314.4)					
Calcium	11	195.4	(128.8, 296.5)	*0.735*	11	234.2	(136.3, 402.1)	*0.402*	1.56	(0.6, 4.1)	*0.351*	89.01
No vitamin D	16	151.5	(104.3, 220.1)		16	132.8	(80.3, 219.8)					
Vitamin D	23	188.2	(137.8, 257.0)	*0.372*	23	144.1	(94.7, 219.4)	*0.802*	0.87	(0.5, 1.7)	*0.681*	-25.35
Calcium only	14	155.1	(102.7, 234.0)		14	119.6	(68.6, 208.6)					
Vitamin D and calcium	17	168.3	(115.8, 244.5)	*0.766*	17	145.3	(87.7, 240.5)	*0.601*	1.12	(0.5, 2.3)	*0.752*	12.41
Smoking Status												
Current or former												
No calcium	13	174.0	(106.0, 285.4)		13	237.5	(156.4, 360.8)					
Calcium	15	180.5	(113.8, 286.2)	*0.912*	15	252.9	(171.4, 373.2)	*0.823*	1.03	(0.6, 1.9)	*0.930*	8.84
No vitamin D	17	192.8	(130.4, 284.9)		17	209.1	(141.6, 308.8)					
Vitamin D	27	154.3	(113.1, 210.3)	*0.372*	27	206.1	(151.3, 280.9)	*0.954*	1.23	(0.8, 1.9)	*0.348*	35.57
Calcium only	12	191.9	(122.4, 300.8)		12	204.9	(125.4, 334.9)					
Vitamin D and calcium	19	151.1	(105.7, 216.1)	*0.402*	19	190.2	(128.7, 281.0)	*0.809*	1.18	(0.7, 2.1)	*0.564*	25.99
Never												
No calcium	16	291.8	(226.9, 375.3)		16	209.6	(137.7, 318.9)					
Calcium	19	249.3	(197.8, 314.0)	*0.355*	19	212.3	(144.4, 312.1)	*0.963*	1.19	(0.7, 2.1)	*0.563*	45.27
No vitamin D	34	223.5	(175.8, 284.0)		34	205.4	(148.0, 284.9)					
Vitamin D	27	206.3	(157.6, 269.9)	*0.658*	27	123.3	(85.4, 178.0)	*0.042*	0.65	(0.4, 1.1)	*0.081*	-64.88
Calcium only	27	223.1	(171.2, 290.7)		27	190.7	(129.5, 280.8)					
Vitamin D and calcium	18	156.8	(113.4, 216.8)	*0.097*	18	104.8	(65.2, 168.5)	*0.055*	0.78	(0.4, 1.4)	*0.418*	-19.54

Table 6. *Cont*

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Presence of Advanced Adenomas												
None present												
No calcium	21	210.2	(152.3, 290.2)		21	236.5	(168.9, 331.3)					
Calcium	28	225.7	(170.8, 298.4)	*0.738*	28	227.4	(169.8, 304.4)	*0.859*	0.90	(0.6, 1.4)	*0.638*	-24.70
No vitamin D	44	215.0	(171.9, 268.9)		44	207.8	(157.3, 274.5)					
Vitamin D	42	171.5	(136.4, 215.6)	*0.163*	42	158.9	(119.5, 211.3)	*0.184*	0.96	(0.7, 1.4)	*0.824*	-5.30
Calcium only	36	218.2	(171.8, 277.0)		36	196.9	(142.1, 272.7)					
Vitamin D and calcium	29	154.7	(118.5, 201.8)	*0.059*	29	137.2	(95.4, 197.2)	*0.144*	0.98	(0.6, 1.5)	*0.940*	3.80
One or more present												
No calcium	8	297.8	(189.2, 468.8)		8	186.9	(96.7, 361.3)					
Calcium	6	176.6	(104.6, 298.2)	*0.126*	6	238.8	(111.6, 511.1)	*0.606*	2.15	(0.7, 6.8)	*0.170*	173.03
No vitamin D	7	199.0	(108.4, 365.4)		7	199.4	(103.4, 384.8)					
Vitamin D	12	204.9	(128.8, 325.9)	*0.937*	12	161.1	(97.5, 266.1)	*0.593*	0.78	(0.3, 2.3)	*0.636*	-44.20
Calcium only	3	159.4	(64.3, 395.4)		3	173.3	(61.6, 487.7)					
Vitamin D and calcium	8	151.0	(86.6, 263.2)	*0.910*	8	162.8	(86.4, 306.8)	*0.910*	0.99	(0.2, 4.9)	*0.991*	-2.03
Presence of Serrated Adenomas												
None present												
No calcium	24	234.6	(175.1, 314.3)		24	220.9	(161.5, 302.2)					
Calcium	22	205.8	(151.6, 279.3)	*0.536*	22	215.3	(155.2, 298.7)	*0.910*	1.11	(0.6, 1.9)	*0.700*	23.24
No vitamin D	37	211.7	(166.4, 269.2)		37	195.0	(145.2, 261.8)					
Vitamin D	42	162.5	(129.7, 203.7)	*0.115*	42	153.9	(116.7, 203.0)	*0.248*	1.03	(0.7, 1.6)	*0.896*	8.09
Calcium only	27	201.4	(154.2, 263.0)		27	174.9	(121.1, 252.5)					
Vitamin D and calcium	28	136.8	(105.3, 177.9)	*0.043*	28	136.5	(95.2, 195.8)	*0.339*	1.15	(0.7, 1.9)	*0.596*	26.19
One or more present												
No calcium	5	216.9	(107.9, 436.0)		5	225.4	(99.6, 510.1)					
Calcium	12	236.7	(150.8, 371.5)	*0.826*	12	257.4	(151.9, 436.2)	*0.775*	1.05	(0.5, 2.3)	*0.903*	12.27
No vitamin D	14	215.6	(142.0, 327.3)		14	240.8	(143.9, 402.9)					
Vitamin D	12	247.1	(157.4, 387.8)	*0.652*	12	180.0	(103.3, 313.9)	*0.436*	0.65	(0.4, 1.2)	*0.160*	-92.19
Calcium only	12	241.5	(156.5, 372.7)		12	249.0	(140.6, 440.8)					
Vitamin D and calcium	9	221.5	(134.2, 365.6)	*0.788*	9	162.1	(83.8, 313.6)	*0.317*	0.71	(0.3, 1.5)	*0.350*	-66.82

[a]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC)

[b]Calcium group were patients assigned to either calcium or to calcium+vitamin D (combined) in the 4-arm group. Patients in the 2-arm group were excluded

[c]Vitamin D group consisted of patients assigned to vitamin D or to calcium+vitamin D (combined) in the 4-arm group, or to vitamin D in the 2-arm group

[d]Vitamin D and Calcium group consisted of patients assigned to calcium+vitamin D (combined) of the 4-arm group, or to vitamin D of the 2-arm group

[e]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[f]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[g]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table 7. Stratified Comparisons of TLR4 Expression in the Crypt Proliferation Zone in the Normal-Appearing Colorectal Mucosa by Treatment Agent (n=105)[a]

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	Geometric[e]				*Geometric*				*Geometric*			
Baseline Characteristics	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Gender												
Men												
No calcium	21	441.2	(329.7, 590.5)		21	418.7	(307.8, 569.7)					
Calcium[b]	28	368.1	(286.0, 473.8)	*0.349*	28	419.2	(321.1, 547.3)	*0.996*	1.20	(0.7, 2.0)	*0.472*	73.54
No vitamin D	22	370.5	(278.4, 493.2)		22	432.5	(320.2, 584.1)					
Vitamin D[c]	27	421.5	(325.6, 545.7)	*0.504*	27	408.3	(311.3, 535.6)	*0.776*	0.83	(0.5, 1.4)	*0.459*	-75.23
Calcium only	13	381.6	(257.4, 565.6)		13	472.5	(302.7, 737.5)					
Vitamin D and calcium[d]	15	356.8	(247.3, 514.8)	*0.800*	15	377.9	(249.7, 572.0)	*0.457*	0.86	(0.4, 1.9)	*0.685*	-69.87
Women												
No calcium	8	272.1	(124.1, 596.3)		8	378.6	(218.2, 656.9)					
Calcium	6	337.1	(136.2, 834.3)	*0.704*	6	408.2	(216.0, 771.4)	*0.849*	0.87	(0.2, 3.5)	*0.831*	-35.41
No vitamin D	29	393.0	(295.7, 522.1)		29	352.4	(252.6, 491.7)					
Vitamin D	27	281.2	(209.4, 377.5)	*0.107*	27	234.3	(165.9, 330.9)	*0.094*	0.93	(0.5, 1.6)	*0.785*	-6.33
Calcium only	26	369.1	(282.9, 481.5)		26	332.9	(230.6, 480.5)					
Vitamin D and calcium	22	320.7	(240.2, 428.2)	*0.475*	22	222.6	(149.4, 331.7)	*0.142*	0.77	(0.5, 1.3)	*0.325*	-61.90
Regular NSAID use												
Less than every other day												
No calcium	25	425.1	(326.0, 554.4)		25	403.1	(302.1, 538.0)					
Calcium	30	388.1	(304.5, 494.6)	*0.613*	30	428.8	(329.5, 558.0)	*0.753*	1.16	(0.7, 1.9)	*0.538*	62.67
No vitamin D	45	397.0	(328.8, 479.3)		45	403.6	(315.4, 516.6)					
Vitamin D	48	382.8	(318.9, 459.4)	*0.783*	48	315.5	(248.4, 400.7)	*0.158*	0.81	(0.6, 1.2)	*0.278*	-73.93
Calcium only	34	399.3	(323.5, 493.0)		34	403.8	(297.4, 548.1)					
Vitamin D and calcium	34	356.5	(288.8, 440.1)	*0.450*	34	285.3	(210.1, 387.3)	*0.114*	0.79	(0.5, 1.3)	*0.321*	-75.65
Every other day or more												
No calcium	4	211.6	(49.5, 904.3)		4	433.8	(226.9, 829.5)					
Calcium	4	217.0	(50.8, 927.6)	*0.977*	4	340.1	(177.9, 650.3)	*0.540*	0.76	(0.1, 4.8)	*0.733*	-99.07
No vitamin D	6	293.3	(106.4, 808.3)		6	270.0	(128.1, 569.0)					
Vitamin D	6	147.4	(53.5, 406.1)	*0.310*	6	264.0	(125.2, 556.4)	*0.963*	1.95	(0.5, 6.9)	*0.270*	139.97
Calcium only	5	235.6	(82.3, 674.4)		5	222.8	(86.2, 576.1)					
Vitamin D and calcium	3	164.9	(42.4, 640.9)	*0.629*	3	188.9	(55.4, 643.8)	*0.803*	1.21	(0.2, 6.3)	*0.785*	36.79

Table 7. *Cont*

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Vitamin D deficiency (< 30 ng/mL)												
Deficient												
No calcium	23	359.5	(262.1, 493.2)		23	392.9	(294.6, 524.1)					
Calcium	28	354.4	(266.1, 472.0)	*0.946*	28	367.0	(282.6, 476.4)	*0.725*	0.95	(0.5, 1.7)	*0.848*	-20.76
No vitamin D	36	360.7	(280.6, 463.7)		36	361.0	(271.9, 479.2)					
Vitamin D	42	317.6	(251.7, 400.7)	*0.461*	42	291.6	(224.3, 379.0)	*0.274*	0.92	(0.6, 1.4)	*0.707*	-26.28
Calcium only	27	346.6	(263.9, 455.4)		27	343.3	(238.8, 493.4)					
Vitamin D and calcium	28	310.5	(237.5, 405.9)	*0.566*	28	256.5	(179.6, 366.3)	*0.256*	0.83	(0.5, 1.4)	*0.504*	-50.64
Sufficient												
No calcium	6	507.6	(250.7, 1,027.9)		6	467.1	(274.5, 794.7)					
Calcium	6	402.5	(198.8, 815.2)	*0.616*	6	759.6	(446.5, 1,292.5)	*0.180*	2.05	(1.1, 3.9)	*0.035*	397.59
No vitamin D	15	442.7	(319.4, 613.7)		15	449.3	(300.1, 672.8)					
Vitamin D	12	456.7	(317.0, 657.9)	*0.897*	12	380.4	(242.2, 597.5)	*0.576*	0.82	(0.4, 1.5)	*0.505*	-82.91
Calcium only	12	440.8	(316.0, 614.7)		12	453.9	(286.1, 720.0)					
Vitamin D and calcium	9	423.9	(288.6, 622.3)	*0.874*	9	346.0	(203.1, 589.4)	*0.430*	0.79	(0.4, 1.8)	*0.547*	-91.02
Daily Fruit and Vegetable Consumption												
Below recommendations												
No calcium	21	358.0	(251.4, 509.8)		21	455.7	(339.2, 612.3)					
Calcium	23	382.0	(272.5, 535.4)	*0.791*	23	407.5	(307.3, 540.3)	*0.583*	0.84	(0.5, 1.4)	*0.498*	-72.18
No vitamin D	35	420.4	(326.8, 540.7)		35	442.8	(345.6, 567.3)					
Vitamin D	31	306.6	(234.6, 400.7)	*0.091*	31	349.7	(268.8, 455.1)	*0.197*	1.08	(0.7, 1.7)	*0.714*	20.69
Calcium only	25	416.9	(321.2, 541.1)		25	453.7	(330.1, 623.5)					
Vitamin D and calcium	20	308.2	(230.3, 412.5)	*0.127*	20	289.1	(202.6, 412.6)	*0.064*	0.86	(0.5, 1.5)	*0.584*	-55.84
Met recommendations												
No calcium	8	470.7	(292.9, 756.4)		8	303.1	(174.0, 528.2)					
Calcium	11	324.8	(216.7, 486.7)	*0.226*	11	438.4	(273.1, 704.0)	*0.301*	2.10	(0.7, 5.9)	*0.152*	281.29
No vitamin D	16	312.8	(221.7, 441.3)		16	283.5	(175.4, 458.1)					
Vitamin D	23	402.5	(302.0, 536.4)	*0.262*	23	262.1	(175.6, 391.2)	*0.801*	0.72	(0.4, 1.4)	*0.321*	-111.03
Calcium only	14	306.2	(208.7, 449.3)		14	265.1	(152.4, 461.1)					
Vitamin D and calcium	17	369.3	(260.8, 522.9)	*0.465*	17	261.1	(158.0, 431.5)	*0.967*	0.82	(0.4, 1.8)	*0.591*	-67.10
Smoking Status												
Current or former												
No calcium	13	291.3	(173.2, 490.0)		13	467.5	(320.0, 682.9)					
Calcium	15	299.3	(184.5, 485.7)	*0.938*	15	477.9	(335.8, 680.1)	*0.931*	1.00	(0.5, 2.0)	*0.989*	2.43
No vitamin D	17	351.0	(235.9, 522.1)		17	435.3	(303.1, 625.1)					
Vitamin D	27	285.9	(208.6, 391.8)	*0.419*	27	386.8	(290.3, 515.5)	*0.609*	1.09	(0.6, 1.9)	*0.749*	16.57
Calcium only	12	333.0	(219.6, 505.0)		12	445.0	(278.9, 709.9)					
Vitamin D and calcium	19	308.0	(221.2, 428.7)	*0.766*	19	345.6	(238.5, 501.0)	*0.394*	0.84	(0.4, 1.6)	*0.594*	-74.28
Never												
No calcium	16	485.4	(366.4, 643.0)		16	364.1	(253.0, 523.8)					
Calcium	19	421.5	(325.6, 545.6)	*0.457*	19	374.8	(268.4, 523.4)	*0.905*	1.19	(0.7, 2.1)	*0.559*	74.62
No vitamin D	34	400.3	(320.2, 500.3)		34	362.1	(268.4, 488.4)					
Vitamin D	27	414.6	(322.7, 532.5)	*0.835*	27	247.4	(176.8, 346.2)	*0.096*	0.66	(0.4, 1.1)	*0.082*	-128.98
Calcium only	27	392.6	(304.4, 506.3)		27	346.3	(239.9, 500.0)					
Vitamin D and calcium	18	365.9	(268.0, 499.6)	*0.726*	18	217.4	(138.7, 341.0)	*0.113*	0.67	(0.4, 1.2)	*0.182*	-102.17

Table 7. *Cont*

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[f]			Absolute[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Presence of Advanced Adenomas												
None present												
No calcium	21	326.0	(231.4, 459.4)		21	433.1	(318.3, 589.4)					
Calcium	28	369.0	(274.2, 496.6)	*0.585*	28	400.5	(306.6, 522.9)	*0.701*	0.82	(0.5, 1.4)	*0.441*	-75.66
No vitamin D	44	382.8	(308.2, 475.4)		44	379.4	(293.1, 491.2)					
Vitamin D	42	323.9	(259.5, 404.4)	*0.288*	42	298.1	(228.9, 388.3)	*0.198*	0.93	(0.6, 1.4)	*0.709*	-22.48
Calcium only	36	384.1	(308.0, 479.0)		36	369.6	(272.3, 501.7)					
Vitamin D and calcium	29	336.1	(262.8, 429.9)	*0.423*	29	251.2	(178.7, 353.0)	*0.096*	0.78	(0.5, 1.2)	*0.273*	-70.47
One or more present												
No calcium	8	601.9	(393.4, 921.0)		8	346.5	(204.6, 586.8)					
Calcium	6	333.2	(203.9, 544.5)	*0.071*	6	505.5	(275.1, 928.7)	*0.327*	2.64	(0.9, 7.8)	*0.077*	427.76
No vitamin D	7	385.3	(208.8, 710.9)		7	421.5	(241.1, 737.1)					
Vitamin D	12	426.2	(267.0, 680.4)	*0.786*	12	351.9	(229.7, 539.3)	*0.595*	0.75	(0.3, 2.2)	*0.592*	-110.52
Calcium only	3	264.5	(97.2, 719.7)		3	432.2	(164.7, 1,134.4)					
Vitamin D and calcium	8	330.4	(179.0, 610.0)	*0.678*	8	387.6	(214.6, 699.8)	*0.832*	0.72	(0.1, 4.1)	*0.679*	-110.65
Presence of Serrated Adenomas												
None present												
No calcium	24	384.6	(276.7, 534.5)		24	417.7	(318.3, 548.0)					
Calcium	22	350.1	(248.2, 493.7)	*0.693*	22	388.4	(292.5, 515.8)	*0.711*	1.02	(0.6, 1.8)	*0.942*	5.26
No vitamin D	37	383.5	(300.7, 489.2)		37	370.2	(282.6, 485.0)					
Vitamin D	42	310.2	(246.9, 389.8)	*0.209*	42	300.7	(233.4, 387.5)	*0.267*	1.00	(0.6, 1.6)	*0.985*	3.83
Calcium only	27	351.3	(270.7, 455.9)		27	345.5	(244.2, 488.8)					
Vitamin D and calcium	28	302.9	(234.5, 391.2)	*0.419*	28	261.3	(185.8, 367.4)	*0.255*	0.88	(0.5, 1.5)	*0.621*	-35.77
One or more present												
No calcium	5	393.5	(211.9, 730.7)		5	360.8	(167.4, 777.7)					
Calcium	12	386.2	(259.0, 575.8)	*0.957*	12	475.8	(289.8, 781.1)	*0.529*	1.34	(0.5, 3.4)	*0.501*	122.33
No vitamin D	14	382.1	(267.4, 545.8)		14	426.8	(263.8, 690.6)					
Vitamin D	12	496.0	(337.4, 729.0)	*0.315*	12	341.4	(203.0, 574.0)	*0.521*	0.62	(0.3, 1.2)	*0.156*	-199.31
Calcium only	12	427.7	(290.9, 628.9)		12	447.6	(259.7, 771.5)					
Vitamin D and calcium	9	457.8	(293.3, 714.5)	*0.812*	9	326.7	(174.2, 612.5)	*0.438*	0.68	(0.3, 1.6)	*0.357*	-150.95

[a]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC)

[b]Calcium group were patients assigned to either calcium or to calcium+vitamin D (combined) in the 4-arm group. Patients in the 2-arm group were excluded

[c]Vitamin D group consisted of patients assigned to vitamin D or to calcium+vitamin D (combined) in the 4-arm group, or to vitamin D in the 2-arm group

[d]Vitamin D and Calcium group consisted of patients assigned to calcium+vitamin D (combined) of the 4-arm group, or to vitamin D of the 2-arm group

[e]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[f]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[g]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table 8. Stratified Comparisons of the Percent TLR4 Expression in the Crypt Differentiation Zone[a] in the Normal-Appearing Colorectal Mucosa by Treatment Agent (n=105)[b]

	Baseline				1-Yr Follow-Up				Absolute Treatment Effect[f]			Relative[g]
Baseline Characteristics	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Gender												
Men												
No calcium	21	34.8	(32.0, 37.5)		21	33.0	(30.2, 35.8)					
Calcium[c]	28	34.1	(31.8, 36.5)	*0.732*	28	33.5	(31.1, 35.9)	*0.791*	1.11	(-3.4, 5.7)	*0.627*	1.03
No vitamin D	22	35.9	(33.3, 38.5)		22	33.9	(31.2, 36.6)					
Vitamin D[d]	27	33.2	(30.8, 35.5)	*0.124*	27	32.8	(30.4, 35.3)	*0.571*	1.70	(-2.8, 6.2)	*0.453*	1.05
Calcium only	13	37.8	(30.9, 36.9)		13	33.9	(30.9, 36.9)					
Vitamin D and calcium[e]	15	31.0	(35.2, 40.4)	*0.001*	15	33.2	(30.4, 36.0)	*0.741*	6.12	(0.7, 11.6)	*0.029*	1.19
Women												
No calcium	8	34.3	(25.7, 42.9)		8	32.6	(27.3, 38.0)					
Calcium	6	33.1	(23.2, 43.1)	*0.851*	6	30.6	(24.4, 36.8)	*0.597*	-0.88	(-17.8, 16.1)	*0.912*	0.97
No vitamin D	29	31.1	(28.4, 33.8)		29	32.2	(29.7, 34.6)					
Vitamin D	27	30.8	(28.0, 33.6)	*0.861*	27	30.9	(28.4, 33.5)	*0.477*	-0.92	(-6.1, 4.3)	*0.724*	0.97
Calcium only	26	31.8	(29.7, 33.9)		26	31.7	(29.2, 34.2)					
Vitamin D and calcium	22	28.7	(26.5, 31.0)	*0.055*	22	31.1	(28.3, 33.8)	*0.742*	2.42	(-2.1, 7.0)	*0.291*	1.08
Regular NSAID use												
Less than every other day												
No calcium	25	33.2	(30.5, 35.9)		25	32.8	(30.3, 35.3)					
Calcium	30	33.5	(31.1, 36.0)	*0.847*	30	33.2	(30.9, 35.5)	*0.801*	0.07	(-4.5, 4.6)	*0.976*	1.00
No vitamin D	45	33.1	(31.2, 35.0)		45	32.8	(31.0, 34.7)					
Vitamin D	48	31.1	(29.2, 32.9)	*0.125*	48	31.8	(30.0, 33.6)	*0.444*	1.05	(-2.3, 4.4)	*0.529*	1.03
Calcium only	34	33.8	(31.9, 35.7)		34	32.3	(30.3, 34.3)					
Vitamin D and calcium	34	29.5	(27.7, 31.4)	*0.002*	34	32.0	(30.0, 34.0)	*0.794*	3.91	(0.4, 7.4)	*0.029*	1.13
Every other day or more												
No calcium	4	43.7	(32.9, 54.5)		4	33.8	(22.9, 44.6)					
Calcium	4	37.2	(26.4, 47.9)	*0.335*	4	31.4	(20.6, 42.3)	*0.724*	4.21	(-20.6, 29.0)	*0.692*	1.09
No vitamin D	6	33.5	(25.0, 42.1)		6	33.5	(25.8, 41.1)					
Vitamin D	6	39.1	(30.6, 47.7)	*0.327*	6	32.3	(24.6, 39.9)	*0.806*	-6.82	(-24.4, 10.8)	*0.409*	0.83
Calcium only	5	33.6	(26.6, 40.5)		5	32.9	(23.7, 42.1)					
Vitamin D and calcium	3	31.1	(22.1, 40.1)	*0.616*	3	31.6	(19.7, 43.5)	*0.835*	1.11	(-19.0, 21.2)	*0.897*	1.03

Table 8. *Cont*

	Baseline				1-Yr Follow-Up				Absolute Treatment Effect[f]			Relative[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Vitamin D deficiency (< 30 ng/mL)												
Deficient												
No calcium	23	34.9	(31.6, 38.1)		23	32.1	(29.4, 34.8)					
Calcium	28	33.5	(30.6, 36.5)	*0.542*	28	32.5	(30.1, 34.9)	*0.833*	1.72	(-3.6, 7.0)	*0.518*	1.05
No vitamin D	36	32.7	(30.4, 35.0)		36	32.3	(30.2, 34.4)					
Vitamin D	42	32.8	(30.7, 35.0)	*0.928*	42	31.9	(29.9, 33.8)	*0.771*	-0.57	(-4.6, 3.5)	*0.780*	0.98
Calcium only	27	33.3	(31.4, 35.3)		27	31.9	(29.6, 34.2)					
Vitamin D and calcium	28	30.5	(28.6, 32.4)	*0.040*	28	32.2	(30.0, 34.5)	*0.839*	3.19	(-0.9, 7.3)	*0.122*	1.11
Sufficient												
No calcium	6	33.7	(28.4, 38.9)		6	36.0	(30.2, 41.9)					
Calcium	6	36.0	(30.7, 41.2)	*0.511*	6	35.4	(29.6, 41.3)	*0.869*	-2.90	(-15.4, 9.6)	*0.615*	0.92
No vitamin D	15	34.4	(30.9, 37.9)		15	34.4	(30.7, 38.0)					
Vitamin D	12	29.0	(25.1, 32.9)	*0.043*	12	31.9	(27.8, 36.0)	*0.358*	2.89	(-4.0, 9.8)	*0.398*	1.10
Calcium only	12	34.8	(30.9, 38.6)		12	33.6	(29.6, 37.6)					
Vitamin D and calcium	9	27.1	(22.7, 31.5)	*0.013*	9	31.0	(26.4, 35.6)	*0.396*	5.10	(-2.2, 12.4)	*0.158*	1.18
Daily Fruit and Vegetable Consumption												
Below recommendations												
No calcium	21	36.5	(33.3, 39.7)		21	33.0	(30.1, 35.8)					
Calcium	23	33.9	(30.9, 37.0)	*0.248*	23	33.3	(30.6, 36.1)	*0.854*	2.93	(-2.7, 8.5)	*0.298*	1.09
No vitamin D	35	34.4	(32.0, 36.7)		35	34.2	(32.0, 36.3)					
Vitamin D	31	33.4	(30.9, 35.9)	*0.595*	31	31.0	(28.8, 33.3)	*0.048*	-2.21	(-6.3, 1.9)	*0.291*	0.93
Calcium only	25	35.0	(33.0, 37.0)		25	34.0	(31.8, 36.2)					
Vitamin D and calcium	20	29.9	(27.6, 32.1)	*0.001*	20	30.7	(28.3, 33.2)	*0.049*	1.84	(-1.9, 5.6)	*0.324*	1.06
Met recommendations												
No calcium	8	29.7	(24.4, 34.9)		8	32.8	(27.8, 37.7)					
Calcium	11	34.0	(29.5, 38.5)	*0.204*	11	32.3	(28.1, 36.5)	*0.883*	-4.78	(-14.2, 4.6)	*0.299*	0.86
No vitamin D	16	30.6	(27.4, 33.8)		16	30.2	(26.9, 33.5)					
Vitamin D	23	30.0	(27.3, 32.7)	*0.772*	23	33.0	(30.3, 35.8)	*0.188*	3.44	(-2.6, 9.5)	*0.259*	1.12
Calcium only	14	31.6	(28.3, 34.9)		14	29.6	(26.1, 33.1)					
Vitamin D and calcium	17	29.4	(26.5, 32.4)	*0.321*	17	33.4	(30.2, 36.5)	*0.109*	5.98	(-0.8, 12.8)	*0.083*	1.21
Smoking Status												
Current or former												
No calcium	13	34.6	(30.4, 38.7)		13	31.9	(28.1, 35.6)					
Calcium	15	34.3	(30.5, 38.2)	*0.926*	15	32.1	(28.6, 35.6)	*0.936*	0.46	(-8.4, 9.3)	*0.915*	1.01
No vitamin D	17	33.1	(29.6, 36.6)		17	30.6	(27.7, 33.5)					
Vitamin D	27	32.8	(30.1, 35.6)	*0.888*	27	32.5	(30.3, 34.8)	*0.292*	2.25	(-4.1, 8.6)	*0.478*	1.07
Calcium only	12	34.4	(30.8, 37.9)		12	30.0	(26.9, 33.1)					
Vitamin D and calcium	19	30.9	(28.1, 33.7)	*0.127*	19	32.8	(30.4, 35.3)	*0.155*	6.32	(-0.1, 12.7)	*0.053*	1.22
Never												
No calcium	16	34.7	(30.8, 38.6)		16	33.8	(30.5, 37.0)					
Calcium	19	33.7	(30.1, 37.3)	*0.706*	19	33.7	(30.7, 36.7)	*0.988*	0.96	(-4.4, 6.3)	*0.719*	1.03
No vitamin D	34	33.2	(30.8, 35.6)		34	34.1	(31.8, 36.4)					
Vitamin D	27	31.1	(28.5, 33.8)	*0.246*	27	31.2	(28.6, 33.8)	*0.109*	-0.76	(-4.8, 3.3)	*0.705*	0.98
Calcium only	27	33.5	(31.6, 35.5)		27	33.5	(31.0, 36.0)					
Vitamin D and calcium	18	28.4	(26.0, 30.7)	*0.002*	18	31.0	(28.0, 34.0)	*0.209*	2.67	(-1.5, 6.8)	*0.201*	1.09

Table 8. *Cont*

	Baseline				1-Yr Follow-Up				Absolute Treatment Effect[f]			Relative[g]
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Presence of Advanced Adenomas												
None present												
No calcium	21	35.9	(32.6, 39.1)		21	33.1	(30.2, 36.0)					
Calcium	28	34.3	(31.5, 37.1)	*0.472*	28	33.6	(31.1, 36.1)	*0.776*	2.08	(-3.7, 7.8)	*0.471*	1.06
No vitamin D	44	33.3	(31.1, 35.4)		44	33.3	(31.3, 35.2)					
Vitamin D	42	32.4	(30.3, 34.6)	*0.582*	42	32.6	(30.6, 34.6)	*0.638*	0.18	(-3.8, 4.2)	*0.928*	1.01
Calcium only	36	33.7	(31.8, 35.5)		36	32.8	(30.8, 34.8)					
Vitamin D and calcium	29	29.7	(27.6, 31.7)	*0.005*	29	33.0	(30.7, 35.2)	*0.912*	4.15	(0.3, 8.0)	*0.035*	1.14
One or more present												
No calcium	8	31.4	(25.7, 37.1)		8	32.5	(27.9, 37.1)					
Calcium	6	32.3	(25.7, 38.9)	*0.828*	6	30.1	(24.8, 35.4)	*0.473*	-3.27	(-12.2, 5.6)	*0.440*	0.90
No vitamin D	7	32.7	(27.5, 37.9)		7	30.8	(25.9, 35.7)					
Vitamin D	12	30.4	(26.4, 34.4)	*0.474*	12	29.4	(25.7, 33.2)	*0.637*	0.86	(-6.1, 7.8)	*0.796*	1.03
Calcium only	3	35.2	(27.7, 42.7)		3	27.7	(21.8, 33.7)					
Vitamin D and calcium	8	29.6	(25.0, 34.2)	*0.186*	8	28.2	(24.5, 31.8)	*0.890*	6.04	(-1.3, 13.4)	*0.096*	1.21
Presence of Serrated Adenomas												
None present												
No calcium	24	35.1	(31.9, 38.2)		24	32.6	(29.7, 35.4)					
Calcium	22	33.7	(30.4, 37.0)	*0.546*	22	33.1	(30.1, 36.1)	*0.813*	1.88	(-4.1, 7.9)	*0.531*	1.06
No vitamin D	37	33.2	(30.9, 35.6)		37	32.8	(30.4, 35.1)					
Vitamin D	42	32.1	(29.9, 34.3)	*0.492*	42	31.8	(29.6, 34.0)	*0.548*	0.15	(-4.1, 4.4)	*0.943*	1.00
Calcium only	27	34.0	(32.1, 36.0)		27	32.1	(29.5, 34.6)					
Vitamin D and calcium	28	29.2	(27.3, 31.1)	*0.001*	28	32.1	(29.6, 34.6)	*0.959*	4.94	(0.7, 9.2)	*0.025*	1.17
One or more present												
No calcium	5	32.5	(26.1, 38.9)		5	34.6	(29.9, 39.3)					
Calcium	12	34.5	(30.3, 38.6)	*0.592*	12	32.9	(29.9, 35.9)	*0.528*	-3.65	(-12.2, 4.9)	*0.375*	0.90
No vitamin D	14	33.1	(29.5, 36.7)		14	33.4	(30.8, 35.9)					
Vitamin D	12	31.5	(27.6, 35.4)	*0.543*	12	32.2	(29.5, 34.9)	*0.526*	0.44	(-5.1, 6.0)	*0.873*	1.01
Calcium only	12	33.2	(29.1, 37.3)		12	33.2	(30.6, 35.9)					
Vitamin D and calcium	9	31.1	(26.4, 35.8)	*0.492*	9	31.3	(28.3, 34.3)	*0.325*	0.15	(-5.9, 6.2)	*0.959*	1.00

[a]Defined as the upper 40% of the crypt

[b]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC)

[c]Calcium group were patients assigned to either calcium or to calcium+vitamin D (combined) in the 4-arm group only

[d]Vitamin D group consisted of patients assigned to vitamin D or to calcium+vitamin D (combined) in the 4-arm group, or to vitamin D in the 2-arm group

[e]Vitamin D and Calcium group consisted of patients assigned to calcium+vitamin D (combined) of the 4-arm group, or to vitamin D of the 2-arm group

[f]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

[g]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

Supplementary Tables and Figures

Table S2.A. Comparison of TLR4 Expression in the Normal-Appearing Colorectal Mucosa of Study Participants (n=105) by Treatment Assignment[a]

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[d]			Absolute[e]
		Geometric[b]				Geometric				Geometric		
Treatment Group	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx Effec	(95% CI)	*p value*	Rx Effect
Whole crypts												
4-Arm												
Placebo	12	630.2	(394.3, 1,007.3)		12	666.0	(426.5, 1,039.9)					
Calcium	16	607.3	(389.3, 947.6)	*0.901*	16	660.2	(431.0, 1,011.4)	*0.976*	1.03	(0.5, 2.0)	*0.932*	17.1
Vitamin D	17	573.6	(370.2, 888.8)	*0.742*	17	553.2	(363.3, 842.4)	*0.492*	0.91	(0.5, 1.8)	*0.780*	-56.2
Calcium+Vitamin D	18	468.0	(303.1, 722.6)	*0.321*	18	511.6	(336.9, 776.7)	*0.354*	1.03	(0.5, 2.0)	*0.917*	7.8
2-Arm												
Placebo	23	1079.9	(664.7, 1,754.4)		23	942.0	(553.0, 1,604.6)					
Vitamin D	19	764.9	(486.4, 1,202.8)	*0.151*	19	526.8	(315.4, 880.0)	*0.049*	0.79	(0.4, 1.4)	*0.402*	-100.1
Upper 40% of crypts												
4-Arm												
Placebo	12	182.6	(113.7, 293.1)		12	214.9	(132.5, 348.5)					
Calcium	16	213.6	(135.7, 336.1)	*0.601*	16	207.7	(130.9, 329.5)	*0.912*	0.83	(0.4, 1.6)	*0.563*	-38.2
Vitamin D	17	194.0	(124.1, 303.4)	*0.832*	17	160.7	(102.0, 253.3)	*0.325*	0.70	(0.4, 1.3)	*0.284*	-65.6
Calcium+Vitamin D	18	135.4	(86.8, 211.1)	*0.323*	18	155.2	(98.8, 243.8)	*0.293*	0.97	(0.5, 1.9)	*0.935*	-12.5
2-Arm												
Placebo	23	348.8	(209.3, 581.3)		23	306.2	(174.7, 536.6)					
Vitamin D	19	226.5	(140.7, 364.9)	*0.090*	19	171.1	(99.6, 293.7)	*0.061*	0.86	(0.5, 1.3)	*0.614*	-12.8
Lower 60% of crypts												
4-Arm												
Placebo	12	381.6	(229.3, 635.1)		12	386.5	(245.4, 608.8)					
Calcium	16	322.0	(199.4, 520.0)	*0.603*	16	404.1	(261.4, 624.7)	*0.877*	1.24	(0.6, 2.6)	*0.556*	77.1
Vitamin D	17	314.6	(196.4, 504.0)	*0.538*	17	341.4	(222.2, 524.6)	*0.651*	1.07	(0.5, 2.2)	*0.847*	21.9
Calcium+Vitamin D	18	295.7	(185.4, 471.4)	*0.433*	18	315.3	(205.7, 483.2)	*0.481*	1.05	(0.5, 2.1)	*0.885*	14.7
2-Arm												
Placebo	23	668.3	(407.4, 1,096.1)		23	573.0	(331.3, 991.1)					
Vitamin D	19	494.3	(311.7, 783.8)	*0.214*	19	318.8	(187.9, 540.9)	*0.054*	0.75	(0.4, 1.3)	*0.325*	-80.2

Table S2.A. *Cont*

	Baseline				1-Yr Follow-Up				Absolute Treatment Effect			Relative
	n	Mean	(95% CI)	p value	n	Mean	(95% CI)	*p value*	Rx Effec	(95% CI)	*p value*	Rx Effect
φh[c]												
4-Arm												
Placebo	12	30.23	(25.6, 34.8)		12	33.53	(29.5, 37.6)					
Calcium	16	36.47	(32.2, 40.8)	*0.038*	16	32.59	(28.7, 36.4)	*0.715*	-7.17	(-14.0, -0.4)	*0.039*	0.81
Vitamin D	17	35.93	(31.7, 40.2)	*0.049*	17	30.68	(26.9, 34.5)	*0.247*	-8.55	(-15.3, -1.8)	*0.014*	0.77
Calcium+Vitamin D	18	29.89	(25.7, 34.1)	*0.907*	18	31.51	(27.7, 35.3)	*0.433*	-1.67	(-8.3, 5.0)	*0.615*	0.95
2-Arm												
Placebo	23	32.61	(28.9, 36.3)		23	33.00	(28.9, 37.1)					
Vitamin D	19	29.97	(26.5, 33.4)	*0.153*	19	32.87	(28.9, 36.9)	*0.954*	2.52	(-2.3, 7.4)	*0.300*	1.08

[a]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC), controlling for age, gender (if 4-arm), study center, smoking status (ever vs. never), multivitamin use (yes vs. no), physical activity (MET-mins/wk), dietary fiber (g/d) and total calories (kcal/d)

[b]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[c]Defined as the percent of expression in the distribution zone (expression in upper 40% of crypts over expression in the whole crypts). Values are means of the optical density (not geometric means)

[d]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[e]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table S2.B. Comparison of TLR4 Expression in the Normal-Appearing Colorectal Mucosa of Study Participants (n=105) by Treatment Assignment[a]

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[d]			Absolute[e]
	Geometric[b]				*Geometric*				*Geometric*			
Treatment Group	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx Effec	(95% CI)	*p value*	Rx Effect
Whole crypts												
4-Arm												
Placebo	12	629.0	(421.7, 938.1)		12	664.7	(445.7, 991.5)					
Calcium	16	641.0	(448.8, 915.6)	*0.942*	16	696.9	(487.9, 995.5)	*0.856*	1.03	(0.5, 2.0)	*0.932*	20.13
Vitamin D	17	613.0	(437.5, 858.9)	*0.920*	17	591.2	(421.9, 828.3)	*0.647*	0.91	(0.5, 1.7)	*0.780*	-57.49
Calcium+Vitamin D	18	525.5	(375.5, 735.5)	*0.480*	18	574.4	(410.4, 803.9)	*0.566*	1.03	(0.5, 2.0)	*0.917*	13.22
2-Arm												
Placebo	23	561.9	(392.6, 804.2)		23	490.2	(342.5, 701.5)					
Vitamin D	19	491.5	(333.6, 724.3)	*0.612*	19	338.6	(229.8, 498.9)	*0.166*	0.79	(0.4, 1.4)	*0.401*	-81.21
Upper 40% of crypts												
4-Arm												
Placebo	12	187.6	(123.8, 284.5)		12	220.8	(145.6, 334.8)					
Calcium	16	228.1	(157.3, 330.8)	*0.470*	16	221.8	(152.9, 321.6)	*0.987*	0.83	(0.4, 1.6)	*0.563*	-39.54
Vitamin D	17	214.8	(151.2, 305.3)	*0.611*	17	178.0	(125.3, 252.9)	*0.419*	0.70	(0.4, 1.3)	*0.283*	-70.07
Calcium+Vitamin D	18	159.1	(112.1, 225.9)	*0.534*	18	182.4	(128.5, 259.0)	*0.471*	0.97	(0.5, 1.8)	*0.935*	-9.90
2-Arm												
Placebo	23	172.1	(117.6, 251.8)		23	151.1	(103.3, 221.0)					
Vitamin D	19	136.6	(90.5, 206.2)	*0.411*	19	103.2	(68.4, 155.7)	*0.178*	0.86	(0.5, 1.6)	*0.613*	-12.44
Lower 60% of crypts												
4-Arm												
Placebo	12	380.7	(249.2, 581.5)		12	385.6	(252.4, 589.0)					
Calcium	16	336.2	(230.5, 490.3)	*0.652*	16	421.9	(289.3, 615.2)	*0.744*	1.24	(0.6, 2.6)	*0.556*	80.77
Vitamin D	17	331.8	(232.1, 474.3)	*0.613*	17	360.0	(251.8, 514.7)	*0.800*	1.07	(0.5, 2.2)	*0.847*	23.37
Calcium+Vitamin D	18	323.6	(226.7, 461.9)	*0.548*	18	345.1	(241.8, 492.6)	*0.681*	1.05	(0.5, 2.1)	*0.885*	16.59
2-Arm												
Placebo	23	357.3	(248.7, 513.4)		23	306.3	(213.2, 440.2)					
Vitamin D	19	328.3	(221.8, 485.9)	*0.751*	19	211.7	(143.1, 313.4)	*0.171*	0.75	(0.4, 1.3)	*0.323*	-65.57

Table S2.B. *Cont*

	Baseline				1-Yr Follow-Up				Absolute Treatment Effect			Relative
	n	Mean	(95% CI)	p value	n	Mean	(95% CI)	*p value*	Rx Effec	(95% CI)	*p value*	Rx Effect
φh[c]												
4-Arm												
Placebo	12	30.76	(26.9, 34.7)		12	34.05	(30.2, 38.0)					
Calcium	16	36.23	(32.8, 39.7)	*0.034*	16	32.36	(28.9, 35.8)	*0.504*	-7.17	(-13.9, -0.4)	*0.038*	0.81
Vitamin D	17	36.38	(33.1, 39.7)	*0.028*	17	31.14	(27.8, 34.4)	*0.246*	-8.55	(-15.2, -1.9)	*0.013*	0.77
Calcium+Vitamin D	18	30.70	(27.4, 34.0)	*0.983*	18	32.33	(29.1, 35.6)	*0.488*	-1.67	(-8.3, 4.9)	*0.615*	0.95
2-Arm												
Placebo	23	31.14	(28.6, 33.7)		23	31.54	(29.0, 34.0)					
Vitamin D	19	28.15	(25.4, 30.9)	*0.111*	19	31.06	(28.3, 33.8)	*0.796*	2.52	(-2.3, 7.3)	*0.299*	1.09

[a]The effect of treatment assignment on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC), controlling for age, gender (if 4-arm) and study center

[b]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[c]Defined as the percent of expression in the distribution zone (expression in upper 40% of crypts over expression in the whole crypts). Values are means of the optical density (not geometric means)

[d]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[e]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table S3.A. Comparison of TLR4 Expression in the Normal-Appearing Colorectal Mucosa of Study Participants (n=105) by Treatment Agent[a]

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[g]			Absolute[h]
	Geometric[f]				*Geometric*				*Geometric*			
Treatment Effect Comparisons	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Whole Crypts												
No calcium	29	604.3	(425.7, 857.8)		29	605.3	(432.6, 847.0)					
Calcium[b]	34	546.0	(386.6, 771.2)	*0.597*	34	595.3	(426.8, 830.5)	*0.926*	1.09	(0.7, 1.7)	*0.694*	48.3
No vitamin D	51	673.4	(506.3, 895.8)		51	658.5	(491.2, 882.7)					
Vitamin D[c]	54	601.5	(455.0, 795.1)	*0.450*	54	537.2	(403.3, 715.5)	*0.196*	0.91	(0.7, 1.3)	*0.597*	-49.4
Calcium only	39	862.2	(596.4, 1,246.4)		39	823.2	(556.4, 1,217.9)					
Vitamin D and calcium[d]	37	675.1	(482.8, 943.9)	*0.155*	37	582.1	(405.3, 835.9)	*0.080*	0.90	(0.6, 1.4)	*0.624*	-54.0
Upper 40% of crypts												
No calcium	29	191.7	(133.2, 276.0)		29	183.7	(127.3, 264.9)					
Calcium	34	172.6	(120.3, 247.6)	*0.590*	34	183.1	(127.4, 263.1)	*0.988*	1.11	(0.7, 1.7)	*0.639*	18.6
No vitamin D	51	215.3	(159.8, 290.1)		51	209.1	(153.1, 285.6)					
Vitamin D	54	184.0	(137.4, 246.3)	*0.310*	54	164.4	(121.2, 223.0)	*0.153*	0.92	(0.7, 1.3)	*0.634*	-13.4
Calcium only	39	303.5	(206.9, 445.2)		39	277.8	(184.8, 417.5)					
Vitamin D and calcium	37	207.2	(146.3, 293.5)	*0.034*	37	191.7	(131.6, 279.2)	*0.071*	1.01	(0.7, 1.5)	*0.960*	10.2
Lower 60% of crypts												
No calcium	29	345.1	(237.6, 501.2)		29	364.0	(258.6, 512.3)					
Calcium	34	317.7	(220.3, 458.2)	*0.691*	34	365.8	(260.6, 513.3)	*0.979*	1.09	(0.7, 1.8)	*0.712*	29.1
No vitamin D	51	396.0	(294.1, 533.1)		51	397.9	(294.9, 537.1)					
Vitamin D	54	367.5	(274.8, 491.6)	*0.637*	54	330.2	(246.3, 442.7)	*0.246*	0.89	(0.6, 1.3)	*0.544*	-39.3
Calcium only	39	488.7	(333.3, 716.7)		39	489.9	(326.7, 734.5)					
Vitamin D and calcium	37	425.5	(300.2, 603.0)	*0.439*	37	350.5	(241.1, 509.6)	*0.102*	0.82	(0.5, 1.3)	*0.372*	-76.2
	Baseline				**1-Yr Follow-Up**				**Absolute Treatment Effect**			**Relative**
	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx Effect	(95% CI)	*p value*	Rx Effect
φh[e]												
No calcium	29	33.6	(30.0, 37.1)		29	31.9	(28.8, 34.9)					
Calcium	34	32.9	(29.4, 36.3)	*0.739*	34	31.9	(28.9, 35.0)	*0.964*	0.8	(-4.0, 5.5)	*0.753*	1.02
No vitamin D	51	33.0	(30.5, 35.4)		51	32.7	(30.3, 35.1)					
Vitamin D	54	31.6	(29.2, 34.0)	*0.328*	54	31.5	(29.2, 33.9)	*0.373*	0.2	(-3.3, 3.6)	*0.919*	1.01
Calcium only	39	35.6	(33.1, 38.1)		39	34.2	(31.4, 37.1)					
Vitamin D and calcium	37	31.1	(28.8, 33.4)	*0.000*	37	33.4	(30.8, 36.0)	*0.567*	3.6	(0.2, 7.1)	*0.039*	1.12

[a]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC), controlling for age, gender (if 4-arm), study center, smoking status (ever vs. never), multivitamin use (yes vs. no), physical activity (MET-mins/wk), dietary fiber (g/d) and total calories (kcal/d)

[b]Calcium group were patients assigned to either calcium or to calcium+vitamin D (combined) in the 4-arm group. Patients in the 2-arm group were excluded

[c]Vitamin D group consisted of patients assigned to vitamin D or to calcium+vitamin D (combined) in the 4-arm group, or to vitamin D in the 2-arm group

[d]Vitamin D and Calcium group consisted of patients assigned to calcium+vitamin D (combined) of the 4-arm group, or to vitamin D of the 2-arm group

[e]Defined as the percent of expression in the differentiation zone over the whole crypts. Values are means of the optical density (not geometric means)

[f]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[g]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[h]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Table S3.B. Comparison of TLR4 Expression in the Normal-Appearing Colorectal Mucosa of Study Participants (n=105) by Treatment Agent[a]

	Baseline				1-Yr Follow-Up				Relative Treatment Effect[g]			Absolute[h]
	Geometric[f]				*Geometric*				*Geometric*			
Treatment Effect Comparisons	n	Mean	(95% CI)	*p value*	n	Mean	(95% CI)	*p value*	Rx effect	(95% CI)	*p value*	Rx Effect
Whole Crypts												
No calcium	29	617.7	(475.6, 802.2)		29	618.7	(476.4, 803.4)					
Calcium[b]	34	576.2	(447.7, 741.7)	*0.684*	34	628.2	(488.1, 808.7)	*0.928*	1.09	(0.7, 1.7)	*0.694*	51.01
No vitamin D	51	625.4	(501.6, 779.7)		51	611.6	(490.5, 762.4)					
Vitamin D[c]	54	545.1	(442.3, 671.8)	*0.345*	54	486.9	(395.1, 600.1)	*0.118*	0.91	(0.7, 1.3)	*0.597*	-44.34
Calcium only	39	643.4	(482.3, 858.2)		39	614.2	(460.5, 819.3)					
Vitamin D and calcium[d]	37	534.3	(402.8, 708.8)	*0.297*	37	460.7	(347.3, 611.2)	*0.108*	0.90	(0.6, 1.4)	*0.624*	-44.49
Upper 40% of crypts												
No calcium	29	202.3	(153.7, 266.3)		29	193.8	(147.2, 255.1)					
Calcium	34	188.2	(144.3, 245.6)	*0.687*	34	199.7	(153.0, 260.6)	*0.867*	1.11	(0.7, 1.7)	*0.639*	19.97
No vitamin D	51	200.9	(159.3, 253.4)		51	195.1	(154.7, 246.1)					
Vitamin D	54	168.2	(135.0, 209.5)	*0.244*	54	150.3	(120.6, 187.2)	*0.089*	0.92	(0.7, 1.3)	*0.634*	-12.09
Calcium only	39	213.5	(157.7, 289.1)		39	195.4	(144.3, 264.6)					
Vitamin D and calcium	37	156.6	(116.3, 210.7)	*0.099*	37	144.9	(107.6, 194.9)	*0.110*	1.01	(0.7, 1.5)	*0.960*	6.36
Lower 60% of crypts												
No calcium	29	350.4	(266.0, 461.7)		29	369.6	(280.5, 487.0)					
Calcium	34	329.1	(252.2, 429.3)	*0.728*	34	378.8	(290.4, 494.2)	*0.891*	1.09	(0.7, 1.7)	*0.712*	30.57
No vitamin D	51	366.4	(291.5, 460.4)		51	368.1	(292.9, 462.6)					
Vitamin D	54	330.3	(265.9, 410.2)	*0.492*	54	296.8	(239.0, 368.6)	*0.155*	0.89	(0.6, 1.3)	*0.544*	-35.28
Calcium only	39	373.6	(278.1, 501.8)		39	374.5	(278.8, 503.1)					
Vitamin D and calcium	37	341.7	(255.8, 456.6)	*0.625*	37	281.5	(210.7, 376.1)	*0.121*	0.82	(0.5, 1.3)	*0.371*	-61.13
	Baseline				**1-Yr Follow-Up**				**Absolute Treatment Effect**			**Relative**
	n	Mean		*p value*	n	Mean		*p value*	Rx Effect		*p value*	Rx Effect
φh[e]												
No calcium	29	34.0	(31.4, 36.7)		29	32.3	(29.7, 35.0)					
Calcium	34	33.3	(30.8, 35.9)	*0.679*	34	32.3	(29.8, 34.9)	*0.986*	0.8	(-4.0, 5.5)	*0.753*	1.02
No vitamin D	51	32.9	(31.0, 34.8)		51	32.6	(30.7, 34.6)					
Vitamin D	54	31.7	(29.9, 33.5)	*0.336*	54	31.6	(29.8, 33.4)	*0.410*	0.2	(-3.2, 3.6)	*0.919*	1.01
Calcium only	39	33.7	(31.7, 35.8)		39	32.4	(30.3, 34.4)					
Vitamin D and calcium	37	29.6	(27.6, 31.7)	*0.002*	37	31.9	(29.9, 33.9)	*0.725*	3.6	(0.2, 7.1)	*0.039*	1.12

[a]The effect of treatment agent on TLR4 expression was modeled using PROC MIXED in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Calcium group were patients assigned to either calcium or to calcium+vitamin D (combined) in the 4-arm group. Patients in the 2-arm group were excluded

[c]Vitamin D group consisted of patients assigned to vitamin D or to calcium+vitamin D (combined) in the 4-arm group, or to vitamin D in the 2-arm group

[d]Vitamin D and Calcium group consisted of patients assigned to calcium+vitamin D (combined) of the 4-arm group, or to vitamin D of the 2-arm group

[e]Defined as the percent of expression in the differentiation zone. Values are means of the optical density (not geometric means)

[f]Data were log-transformed. Reported values are geometric means (95% CI) of optical density

[g]Relative Treatment effect= [(Tx Y1)/(Tx BL)]/[(Pl Y1)/(Pl BL)]

[h]Absolute Treatment effect= [(Tx Y1)-(Tx BL)]-[(Pl Y1)-(Pl BL)]

Figure S2.A: Change in Overall TLR4 Expression across the Crypt According to Treatment Assignment

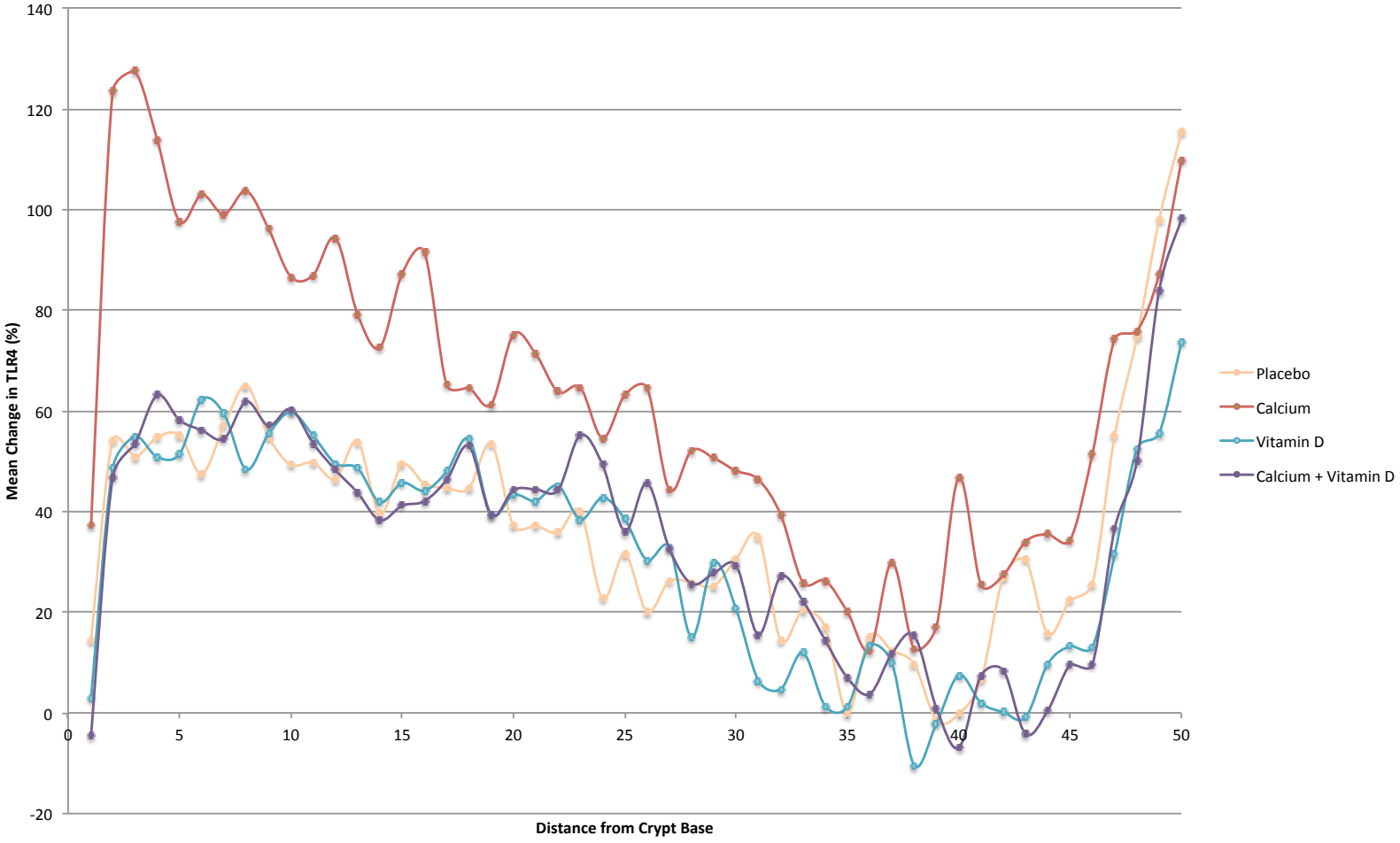

Figure S2.B: Change in Overall TLR4 Expression across the Crypt According to Treatment Assignment