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Effects of Vitamin D and Calcium Supplementation on Toll-Like Receptor 4 (TLR4) Expression in the Stroma of Normal-Appearing Rectal Mucosa of Colorectal Adenoma Patients

By

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Master of Science in Public Health

Epidemiology

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Abstract

Effects of Vitamin D and Calcium Supplementation on Toll-Like Receptor 4 (TLR4) Expression in the Stroma of Normal-Appearing Rectal Mucosa of Colorectal Adenoma Patients

By Stephen Ray

Colorectal cancer (CRC) has the third highest mortality among cancers within the United States. A variety of lifestyle and dietary factors are known to contribute to the risk of CRC, including high BMI, tobacco smoking, red or processed meat consumption, physical inactivity, low vitamin D exposure and calcium intake. TLR4 signaling pathway has been shown to contribute to the inflammatory processes in the colon. Therefore, understanding how this pathway could be beneficially modulated by dietary and lifestyle changes could have potential implications for future prevention of CRC. We conducted a biomarker adjunct study nested within a randomized clinical trial (RCT) testing the effect of vitamin D, calcium, and combined treatment on the expression of the TLR4 biomarker in the stroma of normal-appearing rectal epithelium of colorectal adenoma patients. One hundred and five participants were recruited into the adjunct biomarker sub study and had their baseline characteristics recorded and rectal biopsies taken for TLR4 expression measurement at baseline and at year one follow-up. Our results indicated that neither of the treatments had a statistically significant effect on TLR4 expression in the stroma of normal-appearing rectal epithelium of colorectal adenoma patients. There was however a modest inverse reduction in TLR4 expression that was the most profound with vitamin D treatment. Vitamin D treatment reduced TLR4 expression by 18% ($p = 0.394$), and the combined treatment of vitamin D and calcium resulted in a 21% reduction in TLR4 expression ($p = 0.425$). Additional analyses examining the associations between baseline characteristics and TLR4 expression identified being overweight ($p = 0.006$), being a regular aspirin user ($p = 0.046$), having low total calcium intake ($p = 0.033$) and high vitamin D intake ($p = 0.003$) as factors associated with TLR4 expression in the stroma of normal-appearing rectal mucosa. In conclusion, supplementation with vitamin D and to a lesser extent calcium combined with vitamin D has a modest effect at lowering TLR4 expression within the stroma of normal-appearing rectal epithelium of colorectal adenoma patients.

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Background

Colorectal Cancer

Colorectal cancer is the third leading cause of deaths due to cancer in the United States, behind prostate cancer for men, breast cancer for women, and the lung(1). It is estimated that 49,190 people will die from colorectal cancer, accounting for 8.3% of all cancer deaths. Colorectal cancer incidence is also ranked third, with an estimated 134,490 estimated number of new cases in 2016 accounting for 8.0% of all new cancer cases(1). Some of the widely accepted and common risk factors for colorectal cancer include age, male sex, obesity, low physical activity, inflammatory bowel disease (IBD), and Western-style diet (including high intake of red and processed meats)(2, 3). Other common risk factors include diabetes, excessive alcohol consumption, smoking, and family history of colorectal cancer(3). High intake of dietary fiber and total dairy products has been shown to have a protective effect against colorectal cancer, along with non-steroidal anti-inflammatory drugs (NSAIDs), high exposure to vitamin D, and high intake of calcium(4-9).

The colon mucosa is lined with epithelial cells that form invaginations of the cell line called colon crypts. At the bottom of each crypt are colonic stem cells, whose purpose is to consistently divide to renew the epithelial cell line. Through cell mitosis, new epithelial cells are formed and then propagate from the bottom of the crypt to the outside by pushing up the luminal surface of the crypt(10). Disruption of this normal proliferation process can disrupt function in adhesion, migration, and proliferation while eventually leading to the formation of polyps. Many colorectal tumors start out as colon polyps. There are three main types of colon polyps, hyperplastic polyps, serrated polyps, and adenomatous polyps. Adenomatous polyps are the most likely to transition into a pre-cancer state and the size of the polyp also has a

significant impact on the likelihood of an adenomatous polyp progressing into colorectal cancer(3, 11). Progression from an adenomatous polyp to adenocarcinoma involves multiple pathways, typically involving activation of various oncogenes and knockout of tumor suppressor genes(12). Chronic inflammation induced cancers are often referred to as colitis-associated cancers (CAC), colitis referring to inflammation of the colon(13). Colitis-associated cancers differ from the adenoma cancer pathway primarily in the sequence of molecular events leading to adenocarcinoma(12). While the single-layered epithelial cell surface is exposed to extremely high concentrations of commensurate bacteria, the body is normally able to regulate the activation of cells involved in immune response and inflammation to prevent overactivation by normal bacterial flora. In response to a pathogen, receptors on the epithelial cell surface activate downstream pathways of the immune system such as leukocyte recruitment and inflammation response. Dendritic cells, located under the surface of the epithelium, can also detect abnormal bacteria present within the lumen, and are also part of the immune response mechanism(14). The lamina propria also normally houses immune cells such as macrophages, T-cells, and B-cells. Abnormal signaling between epithelial cells and the immune cells within the lamina propria can lead to chronic inflammation and impaired epithelial cell function, risk factors known to be associated with colorectal neoplasm formation(12, 14). Chronic inflammation within the colon is often initiated by pro-inflammatory immune cells within the lamina propria and epithelial layer(15).

Inflammation and the Toll-like Receptor 4 Pathway in Colorectal Carcinogenesis

Inflammation is also a key risk factor in the formation of colorectal carcinogenesis; chronic exposure to inflammatory factors has been shown to be a contributing factor to the

progression of cell lineages into cancer tumors(12). Colitis can be triggered by various factors, such as the colon microbiome, or hyper-activation of the immune system(15, 16). Inflammation is associated with the release of inflammatory cytokines and chemokines which both have the potential to progress normal epithelia into colon cancer(12). It is likely that this increased risk of colorectal cancer is due to cellular damage caused by reactive oxygen species (ROS)(12, 17). ROS can cause damage to cellular DNA, RNA, lipids, and proteins by nitration and oxidation mechanisms(18). Of significant importance in cancer development is DNA damage, which can cause mutations in transcription for mRNA responsible for the regulation of cell proliferation or apoptosis(18). Cytokines are small proteins that act as downstream regulators of immune cells responsible for the regulation of cell cycle processes such as proliferation and apoptosis(15). Epithelial cells produce and can be acted upon by cytokines; because cytokines are regulators for multiple aspects of cell function such as cell growth and inflammation response, they can prevent apoptosis signaling, promote proliferation, and suppress or induce inflammation(19). Numerous cytokines are involved in colorectal carcinogenesis such as for example tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1)(15, 19). Development of colorectal carcinoma is often preceded by the production of cytokines that promote cellular growth and inhibition of apoptosis(15). Within the stroma of the colon, cytokines are involved in wound healing and tissue structure. Located in the stroma, fibroblasts and myofibroblasts provide structural support that is needed by other cells for support and growth. Development of colorectal carcinoma is often fueled by the recruitment and activation of stromal fibroblasts and myofibroblasts providing the tumor cells with structural support; activated fibroblasts can also contribute additional cytokines, such as transforming growth factor beta (TGF-β), to allow the carcinoma to grow while avoiding apoptosis(19, 20). It is unsurprising that serum levels of

several cytokines involved in cell growth regulation are elevated in colorectal carcinoma patients(19).

The Toll-like receptor 4 (TLR4) pathway has been shown to play an important role in CAC due to its link with chronic inflammation. TLR4, like all members of the toll-like receptor class of proteins, plays a crucial role in the inflammation response and innate host defense against microorganisms by detecting lipopolysaccharides (LPS) of gram-negative bacteria(17). TLR4, upon detecting the presence of LPS, will recruit Myeloid Differentiation Primary Response Gene 88 (MyD88), an adaptor protein involved in the signaling cascade that ultimately activates the NF-κB signaling pathway. MyD88 therefore supports the production of proinflammatory cytokines such as IL-1 in response to activation of TLR4 receptors, which in turn promotes immune cell recruitment and activation at the site of TLR4 receptor activation(21). Other proinflammatory pathways mediated by TLR4 receptors include the recruitment of type 1 interferons *via* TIR-domain containing adaptor inducing INF-β (TRIF) and TRIF-related Adaptor Molecule (TRAM). Both TRIF and TRAM are involved in a signaling cascade that produces INF-β and TRIF also activates dendritic cells within the stroma(17). The TRIF and TRAM signaling pathway are independent of MyD88 in proinflammatory response, but is important in activating the NF-κB signaling pathway(17, 22). TLR4 can therefore elicit a proinflammatory response *via* a MyD88-dependent pathway leading to a downstream activation of the NF-κB signaling pathway and *via* a MyD88-independent pathway through the adaptor molecules TRIF and TRAM that induce the production of INF-β(17, 22). The immune system within the intestine must serve a dual function: it must first protect the host from pathogenic functions, second it must coexist with the commensal organisms within the lumen. In normal conditions, TLR4 is part of the immune gut barrier in that it is acts as a defense for pathogens and is localized within the Golgi

apparatus in epithelial cells(23). The activation of cytokine pathways can lead to the proper immune cell response in response to infiltration by pathogenic organisms(24).

Overexpression of TLR4 has been identified as part of the mechanism involved in the development of IBD and/or CAC(25). TLR4 functions to promote TNF-α and the NF-κB signaling pathway; the overexpression of TNF-α and NF-κB leads to the increased expression of cyclooxygenase-2 (COX-2) among cytokines such as TNF-α. COX-2, acts as a promoter of cell proliferation and inflammation, and is overexpressed in neoplasms(12, 26). Single nucleotide polymorphisms (SNPs) of TLR4 have been known to have varying effects on the downregulation of cytokine expression and the risk for CRC. SNPs in TLR4 can lead to reduced activation of the NF-κB pathway, an increase in the TRIF/TRAM MyD88-independent pathway, and an increase in CRC metastases(27-29). TNF-α promotes colorectal adenoma by the downstream release of NF-κB, which upregulation can lead to loss of control over multiple cellular processes involved in cancer including inflammation, transformation, proliferation, angiogenesis, and metastasis(30, 31). Abnormal activation of the NF-κB signaling pathway, is problematic due to it being a transcription factor that can recruit further inflammatory cytokines (in addition to the increased damage done by ROS)(32). TLR4 receptor expression is also upregulated in tumor cells, which further upregulates the NF-κB signaling pathway preventing apoptosis(31, 33).

TLR4 may also be a mediator in spontaneous colorectal cancer (non-colitis CRC) as well. Chronic inflammation caused by the signaling pathways triggered by TLR4 may increase the risk of DNA damage. Spontaneous CRC is often initiated by the inactivation or modification of certain oncogenes. Inactivation of the adenomatous polyposis coli (APC), the most common inactivated gene in colon cancer, leads to uncontrolled cell growth(34, 35). The KRAS oncogene is also disproportionately inactivated in between 30% to 50% of colorectal cancers and is normally responsible for the inactivation of stimuli from growth factors, cytokines, and

hormones(35, 36). Other common modified oncogenes in spontaneous CRC include SMAD genes (protein complex that regulates transcription of cell differentiation genes) and inactivation of TP53 (responsible for cell cycle arrest and inducing apoptosis)(35, 37, 38).

Calcium and Colorectal Neoplasms

Calcium is an essential nutrient that is obtained solely from the diet. Normal calcium function involves mediating cellular signaling through flow of calcium in or out of cellular membranes. An excess or lack of calcium can cause a break in homeostasis and lead to cell-signaling problems that span across all tissue systems throughout the body. Free fatty acids and bile acids have been shown to potentially be carcinogenic by mechanism of DNA damage leading to inflammation(39). In the gut, calcium binds to free fatty acids and bile acids rendering them inert, reducing colon damage(40). Calcium has also been shown to promote cellular differentiation and apoptosis *via* calcium sensing receptor (CaSR) expressed in the colon. The purpose of these CaSRs is to regulate and maintain homeostasis of Ca^{2+} intra and extracellularly. Calcium in the gut can bind to CaSRs which have been shown to have tumor-suppressive properties and are involved in differentiation of epithelial cells as the cells migrate from the base of the crypt. The CaSR itself can bind to a variety of ligands and can regulate multiple downstream signaling effects involved in inflammation, hormone secretion, gene expression, proliferation, differentiation, and apoptosis(41, 42). Due to strict regulation of calcium pathway, calcium also acts as a secondary messenger through differing calcium-influx channels(43).

Despite being supported by multiple biological mechanisms, there have been conflicting results in both observational studies and randomized control trials (RCTs). In a meta-analysis of 20 prospective observational studies, the authors concluded that there was evidence to suggest

that there was an approximate eight percent decreased risk of CRC with a 300 mg per day increase in calcium intake(44). In another meta-analysis of eight RCTs for colorectal cancer, the researchers determined that calcium did not significantly affect the risk for CRC(45). In a recent RCT, calcium was found to have no effect on APC or β-catenin (involved in activation of the WNT signaling pathway responsible for cell proliferation) while having a modest effect on E-cadherin. Both APC and E-cadherin (an antagonist of β-catenin *via* sequestering it) are downregulated during transition from colorectal adenoma to carcinoma while β-catenin is upregulated(8). A case control study among South Koreans, whose dietary calcium intakes are relatively low, found that both men and women who had the highest quartile calcium intake compared to the lowest quartile calcium intake had an approximate 85% lower risk of CRC(46).

Vitamin D and Colorectal Neoplasms

Vitamin D, a fat-soluble secosteroid, has been well documented in its effects on calcium absorption (and other essential inorganic elements such as magnesium and iron) necessary for normal bodily function(47). Due to the myriad of negative health outcomes associated with chronic inflammation, vitamin D's anti-inflammatory properties have been extensively studied(48).

Vitamin D is normally obtained via two sources. First, vitamin D is produced from its progenitor 7-hydrocholesterol in the form of vitamin D_3 by reacting with ultraviolet radiation from the sun(7). Via biotransformation by cytochrome P450, vitamin D_3 is converted to calcitriol in the kidney and then into its circulating form 25-hydroxylvitamin D_3 (25(OH)D). It is then hydroxylated to its active hormonal form in the kidney and other organs including the colon by the enzyme CYP27B1 to form 1,25-dihydroxylvitamin D [$1,25(OH)_2D$], the active and hormonal

form of vitamin D(7, 49). The other source of vitamin D is the diet, which accounts for a relatively small proportion of vitamin D in majority of individuals and follows the same biochemical pathway conversion to $1,25(OH)_2D$. It is important to note that vitamin D from the diet accounts for only a small portion of available serum vitamin D levels(7). Like the effects of calcium, this active form of vitamin D regulates multiple signaling pathways involved in cell proliferation, apoptosis, differentiation, angiogenesis, and metastasis, signifying its importance in the control and progression of adenocarcinoma(7, 8, 48). Among the myriad of cell processes that vitamin D can modulate is the downregulation of toll-like receptors TLR2 and $TLR4^{27}$ leading to a reduction in the expression of TLR4 and the downstream expression of TNF-α and release of abundant NF-κB(30-32, 40, 50). Low vitamin D levels are a common factor among a myriad of chronic diseases such as type-1 diabetes, colorectal cancer, and cardiovascular disease (10, 40, 51).

There is growing evidence to suggest that the effect of calcium is dependent on serum levels of vitamin D(8, 9, 40, 52). Studies have shown that calcitriol, a metabolite of vitamin D, is necessary for the absorption of dietary calcium into the blood serum(40, 52). In addition, the intracellular calcium gradient within the colon is mediated by vitamin D, and modulates differentiation and apoptosis, two cellular mechanisms that are also altered in cancerous cells(48). Vitamin D also regulates CaSRs, indicating that both work together to ensure Ca^{2+} homeostasis(41, 42).

Vitamin D has been consistently shown to have an inverse relationship with CRC among observational studies. In a meta-analysis of 18 prospective cohort studies (split into nine studies with information on vitamin D intake and nine studies with serum levels of 25(OH)D), there was a decrease in the risk of CRC in both the studies looking at vitamin D intake and serum levels of 25(OH)D. There was a 12% decrease in the risk for CRC among vitamin D intake and a 33%

decrease among serum 25(OH)D(53). Another meta-analysis performed on 42 prospective studies found that increasing dietary vitamin D reduced the risk of CRC by five percent and increasing 25(OH)D reduced the risk of CRC by four percent(54). This strong inverse association does not translate across to RCTs done on vitamin D and CRC however. A small meta-analysis of RCTs consisting of four studies found however that there was no association between vitamin D supplementation and CRC incidence. The study did find that there was a significant inverse association between vitamin D and CRC mortality(55). A recent RCT also found no association between vitamin D supplementation and CRC incidence(56). These RCTs have provided evidence contrary to what is commonly accepted and seen in observational studies, suggesting vitamin D only moderately or slightly decreases the risk of CRC incidence.

Other Risk Factors for Colorectal Cancer

Various environmental factors can contribute to an increased risk of colorectal cancer(57). Smoking has been shown to be a potential risk factor for colorectal cancer, due to its mechanism of causing chronic inflammation and modulation of inflammatory cytokines(58). Obese people and people with high BMI generally express elevated levels of NF-κB, likely due to increased circulating levels of free fatty acids and high levels of cytokines(59). Elevated TLR4 signaling and a weakened adipose tissue response to TLR4 signaling has also has been attributed to obesity(59). Physical activity, a strong preventative risk factor for colorectal cancer, was shown to blunt TLR4 signaling among diet-induced obese rats, leading to downregulation of TNF-α and NF-κB(3, 12, 60). Red and processed meat consumption has been shown to be pro-inflammatory, but the mechanisms of their pro-inflammatory actions are not well understood. It is hypothesized that mutagenic/carcinogenic compounds in the meat such as N-nitroso

compounds, polycyclic aromatic compounds, dietary animal fats, and infectious agents are present in higher amounts in patients with chronic inflammation. These mutagenic compounds are able to cause damage to DNA potentially increasing the risk of CRC(61). Non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin are both medications taken to control inflammation and it is known that the use of NSAIDs or aspirin will have an indirect inverse association with colorectal cancer risk(62). Melatonin has been demonstrated to have an anti-inflammatory effect by regulating TLR4. Melatonin can reduce the expression of the MyD88 and TRIF-dependent signaling pathways, therefore leading to reduced inflammation response(63). Magnesium, has also been suggested to slightly reduce risk of CRC as well, due to it being needed for key cellular functions such as proliferation, differentiation, migration, and apoptosis, genetic stability, and DNA synthesis(64).

Inflammation within the Lamina Propria

The epithelium of the colon and rectum is exposed to many different minerals, metals, compounds, toxicants, and organisms. Primarily, the intestinal epithelium is exposed to many forms of commensal bacteria while maintaining homeostasis(65). This collection of normal gut bacteria is known as the microbiome. Certain risk factors such as type of diet, having type-1 diabetes, and obesity may modify the microbiome, potentially leading to pathogenic organisms causing damage to the colon epithelium and leading to the gut barrier disruption. Studies have shown that obese individuals often have a different composition within their microbiome compared to normal weight individuals, and high gut permeability as indicated by high levels of biomarkers of gut barrier function(66). Switching to high red meat consumption or low fiber diets have also been shown to lead to modified microbiomes compared to the flora in

individuals with low red meat or high fiber consumption(61). It is possible therefore for the gut barrier function to become disrupted, leading to increased permeability to outside organisms. The gut barrier physically prevents infiltration of pathogenic organisms through the epithelial cell layer and tight junctions, a combination of interlinked intra-membrane proteins. In patients with Crohn's disease, these tight junction proteins are downregulated leading to significant leakage. Chronic inflammation and inflammatory cytokines like TNF-α can also lead to abnormal shedding of epithelial cells in which multiple cells shed leaving an open gap that cannot be filled by tight junction proteins(67). Risk factors that increase the inflammation response, such as red meats and obesity can therefore contribute to gut barrier disruption. The immune function of the gut barrier is also sensitive to changes due to chronic inflammation. Changing the microbiome due to dietary changes can cause an increase in pathogenic organisms that can trigger inflammation pathways. Type 1 diabetes has been shown in animal models to lead to an increased number of intraepithelial leukocytes in comparison to normal or other types of diabetes(68, 69). Having chronic inflammatory processes such as Crohn's disease, or irritable bowel syndrome (IBS) are therefore likely linked with increased intestinal permeability(69, 70).

As the integrity of the gut barrier decreases, this allows foreign pathogens to infiltrate the lamina propria easily relative to normal. The lamina propria consists of stromal cells, immune cells (including B cells, T cells, and macrophages), and dendritic cells. Detection of LPS by TLR4 will cause an increase in leukocytes in response. These leukocytes will also have TLR4 receptors present as an immune response to LPS. The dendritic cells also have low levels of receptors such as TLR4 and can also recruit immune cells *via* TLR4 activation. It is therefore possible to be able to detect TLR4 biomarker levels from within the lamina propria due to the recruitment of leukocytes with high levels of TLR4 and the presence of low levels of TLR4 expressed on the dendritic cells(14).

Inflammation is a key component in the development of colorectal cancer, and is involved in many mechanisms involving colorectal adenoma development and transition into carcinoma. In this study, we are interested particularly in the effect of vitamin D and calcium, alone and in combination, on regulation of TLR4 expression within the lamina propria of the colon. Vitamin D has been shown to be a big factor in reducing chronic inflammation, and is also primarily responsible for the uptake of calcium into the cell. Based off the interaction between vitamin D and calcium, this study's hypothesis is that treatment with both vitamin D and calcium will lead to the greatest decrease in TLR4 biomarker expression, followed by the vitamin D treatment group, then the calcium treatment group, as compared to the placebo group.

Methods and Materials

Clinical Trial Protocol and Recruitment

Participants in the parent study were part of a randomized, placebo-controlled, partial 2x2 factorial design chemoprevention trial evaluating the efficacy of both vitamin D and calcium, or individually, for the prevention of adenocarcinomas among participants with colorectal adenomas. The protocols for this base study, including recruitment numbers, have been previously published(8). Participants were eligible if they were between the ages of 45 to 75, was in general good health, within four months of being enrolled had a complete, clean colonoscopy with all polypoid lesions removed (with at least one histologically verified neoplastic polyp greater than two millimeters in diameter), and were scheduled for a follow-up colonoscopy three to five years after their first one. Participants were excluded from the study if they had invasive carcinoma in any of the polyps removed, familial colonic polyposis syndromes, IBS, malabsorption syndromes, history of large bowel resection, narcotic or alcohol dependence, abnormal serum calcium, creatinine greater than 20% above the upper limit of normal levels, abnormal serum 25(OH)D levels, history of kidney stones or hyperparathyroidism, or history of osteoporosis or any medical condition that required supplementation with vitamin D or calcium. Between May 2004 and July 2008, 2,259 participants met final eligibility criteria and were randomized into the RCT(8). Patients were assigned to one of four groups: a placebo arm, a calcium supplementation arm with 1200 mg/d (as calcium carbonate doses twice daily), a vitamin D_3 supplementation arm with 1000 IU/d (500 IU twice daily), and a combined treatment arm with both supplements (1200 mg/d calcium and 1000 IU/d vitamin D). Women who declined calcium supplementation were randomized to only two arms: calcium or calcium and vitamin D together. Patients were randomized by permuted block stratified by sex, clinical

center, scheduled colonoscopy follow-up, and 4-arm *versus* 2 arm participation. The participants all agreed to not take vitamin D or calcium supplements, although 1000 IU/d vitamin D and 400 mg calcium supplements were allowed after April 2008. All participants and study personnel were blinded to treatment. Every six months all study participants were interviewed via telephone regarding their adherence to study, symptoms and illnesses, use of medications and supplements, and colorectal endoscopic or surgical procedures. The investigators also collected blood levels of calcium, creatinine, 25(OH)D, and 1,25(OH_2)D at baseline and one year after randomization.

The adjunct biomarker study was a sub study of the parent study. Specific protocols for this sub study have also been previously published(9). Patients for this sub study were eligible if they visited two of the eleven clinical centers, located in South Carolina and Georgia. Two hundred and thirty-one initial eligible participants were contacted, and 109 patients met final eligibility. In total, 105 final eligible patients gave signed consent, had baseline rectal biopsies taken, and sufficient rectal biopsy tissue for biomarker measurements was obtained at baseline and one year follow up. All 105 participants signed a consent form at enrollment(8). For all participants in the sub study, information on medical history, medication, nutritional supplement use, and diet and lifestyle was recorded. Seventy six percent of participants reported taking 80% of more of their assigned study tablets. Diet was assessed using the Block Brief 2000 food frequency questionnaire (Nutritionquest, Berkeley, CA).

Rectal Biopsy Tissue Collection and TLR4 Quantification

Baseline and one-year biopsy slides were immunohistochemically stained for TLR4. Each patient's baseline and one-year follow up slides were included in the same batch, and each

batch contained four patients from each treatment groups along with a positive and negative control. To quantify the levels of TLR4 present in the lamina propria, a quantitative image method ("scoring") was used. Each patient's slides (five slides for each level with three levels per visit) were scanned using the Scanscope CS digital scanner (Aperio Technologies, Inc., Vista, CA). The images were then reviewed using a custom-designed scoring software, the CellularEyes Image Analysis Suite program (DivEyes LLC, Atlanta, GA) to identify regions acceptable for analysis. A previous technician who was blinded to treatment assignment selected and scored acceptable crypts for TLR4, an acceptable crypt being designated as intact crypts from the muscularis mucosa to the lumen. For this analysis, we, who were also blinded to the treatment assignment, scored the lamina propria region nearest each hemicrypt that was previous scored using a standard protocol.

To score a previously scored hemi-crypt, the technician would locate the crypt(s) previously scored and visually inspect to see whether the width of the borders for the lamina propria region would be wide enough to be suitable for secondary scoring. If suitable, the technician would then trace the outline, being careful to avoid scoring epithelial cells, muscle tissue, staining artifacts, and other non-essential cells. Upon completion of the outline, CellularEyes would then divide the outline into fifty equal bins accounting for slight variations in outline width and then measure the optical density of the biomarker selected (TLR4) across the entire outline. After visual inspection by the technician, CellularEyes would then transfer the data into the MySQL database. The technician would continue this scoring until a minimum of eight lamina propria regions were scored among the five biopsy slides for each patient. Each patient had their baseline visit and one year follow up visit scored. A QC test was given to assess intra-reader scoring reliability, in which four patients that the technician had already scored was re-analyzed. This was done to test the accuracy and reliability of the technician's scoring

methods by ensuring that both the region scored and the biomarker levels were relatively equal between scoring attempts. The QC test results for the intra-class correlation coefficient was from 0.92 to 0.94.

Statistical Analyses

Our primary analyses were done to assess changes in TLR4 biomarker after randomization within the clinical trial arms that received either vitamin D, calcium, or a combination of both. We were also interested in the effect of vitamin D supplementation *versus* no vitamin D supplementation, calcium supplementation *versus* no calcium supplementation, and a combination of vitamin D and calcium supplementation *versus* only calcium supplementation, to accommodate a partial 2x2 study design. In addition to having the optical density (OD) for the entire region, the OD also was divided into the top 40% of the region, surrounding the lumen of the crypt, and the bottom 60% of the region, surrounding the region known as the proliferative area of the crypt, and the ratio between the upper 40% of the region and the whole region (Φ_h). The top 20% of the region was also included in the analyses after visual inspection of the distribution of TLR4 expression in the stroma.

Selected baseline characteristics by treatment groups were assessed to ensure that the groups were comparable using chi-square test for categorical variables and ANOVA/t-test for continuous variables. The treatment effect of vitamin D and/or calcium on TLR4 expression was compared using mixed linear models. All models ran included the intercept, visit time (baseline or one year follow-up), the treatment group, as well as the age of the patient, sex and study center visited. The effect of TLR4 expression by treatment type received was also compared across year one follow-up and baseline between a treatment group of interest and reference

group; the treatments compared were calcium *versus* no calcium, vitamin D *versus no* vitamin D, and combined calcium and vitamin D *versus* only calcium. These models controlled for the same covariates ran in the mixed linear model before, but also included as a covariate the batch in which the biopsy tissue TLR4 OD was measured. Because TLR4 were in OD, relative treatment effects (relative effect = [(treatment group follow-up / treatment group baseline) / (control group follow-up / control group baseline)]) and absolute treatment effects (absolute effect = [(treatment group follow-up - treatment group baseline) - (control group follow-up - control group baseline)]) were calculated. The associations between baseline characteristics and TLR4 expression was also compared across the different regions. For the baseline factors and TLR4 expression, the baseline characteristics of interest were also stratified either dichotomously, or by tertiles (low/medium/high), or by custom division depending on the distribution of that characteristic's data. Baseline characteristics with a p-value of 0.10 or lower across trends was considered as potentially effecting TLR4 expression. P_{trend} was calculated for baseline characteristics that were not dichotomized. For dichotomized variables, we calculated p-values for the difference in the means of the two groups. All OD values were reported across the whole region, the top 20% and 40% of the region, the bottom 60% of the region, and the ratio between the top 40% and the whole region. Proportional differences were calculated by subtracting a (categorized) variable's reference OD value from the category value of interest's OD value and then dividing by the variable's reference OD. This would allow for an interpretation by percentage improvement/reduction based off what level of a categorical predictor an individual had across treatment arms. The effect of treatment type received was also stratified by regular or non-regular NSAID use, regular or non-regular aspirin use, high or low calcium intake, high or low vitamin D intake, high or low serum levels of metabolized vitamin D, and regular or non-regular NSAID or aspirin use together, to look at more detailed

trends based off the effects of calcium, vitamin D, or calcium and vitamin D combined compared to not receiving them. In all analyses patients were kept in their assigned groups, regardless of actual patient adherence to supplement regimens (intention to treat). All statistical analyses were conducted using SAS 9.4 statistical software (SAS Institute Inc., Cary, NC). A p-value of less than or equal to 0.05 was considered statistically significant.

Results

Selected Baseline Characteristics

Selected baseline characteristics for the TLR4 biomarker study are presented in Table 1. Across all treatment arms, the mean age of the participants in the trial was 59 years, 47% of the participants were male, 79% were white, and 63% held a college degree or higher. Fourteen percent of the study population had a previous type 2 diabetes diagnosis, and the average BMI was 29.6% with 79% being classified as overweight by the WHO BMI guidelines. Eight percent of the population were current smokers, and there was a significant difference between treatment groups in the amount of physical activity performed. Among the 56 women participating, 13 (23%) were on hormone replacement therapy at the baseline visit. Regarding dietary intakes, there was a significant difference in the amount of dietary fiber consumed between the treatment groups, but no statistically significant difference was seen in the servings of fruits and vegetables consumed, the servings of red or processed meats in the diet, or the number of alcoholic beverages imbibed per day. The total energy, fat, calcium, and vitamin D intake, as well as serum levels for both calcium and vitamin D were not statistically significant across the treatment groups. Participants across the treatment arms had similar aspirin, NSAIDs, and multivitamin use. Seventy two percent of the participants had only one previously diagnosed adenoma removed, and 19% of the overall study participants had advanced adenomas at baseline. Only nine percent of the study population had a 1st order family member with history of CRC.

TLR4 Biomarker Expression by Treatment Assignment and Agent

The estimated effects of the treatment as per initial assignment on TLR4 expression are presented in Table 2 and the estimated effects by the treatment agent are presented in Table 3. No differences were seen in the biomarker expression when controlling for additional factors that seemed to differ between treatment groups at baseline, so the minimally adjusted results controlling for age, gender, and study center are presented (Unadjusted results for TLR4 expression by treatment effect and treatment agent are presented in Supplementary Tables 1 and 2).

Following one year of treatment, participants in the four-arm calcium treatment group *versus* the placebo treatment group had a 11% decrease in TLR4 expression in the whole lamina propria region (p = 0.789), a 36% decrease in TLR4 expression in the top 20% of the region (p= 0.262), 23% decrease in the upper 40% region (p = 0.528), with no significant change in TLR4 expression in the lower 60% of the region (6% increase, p = 0.911) as well as a 13% decrease in the Φ_h of the region (p = 0.153). Participants in the four-arm vitamin D treatment group *versus* the placebo treatment group had an overall 17% decrease in TLR4 expression (p = 0.657), a decrease of 42% in the top 20% region (p = 0.172), a decrease of 31% in the upper 40% region (p = 0.373), an increase of 13% in the bottom 60% region (p = 0.815) and a decrease of 15% in the Φ_h of the region. Participants within the two-arm vitamin D treatment group had an overall 37% decrease in the entire region (p = 0.282) when compared to placebo, 30% decrease in the top 20% of the region (p = 0.423), 38% decrease in the upper 40% region (p = 0.265), and virtually no change in TLR4 expression was seen in the ratio of the upper region to the entire region (Φ_h). Participants in the two-arm vitamin D treatment group had a 34% decrease in the bottom 60% of the region (p = 0.399) compared to the placebo group. Participants assigned to the four-arm

combined vitamin D and calcium had a 11% decrease in TLR4 in the whole region (p = 0.778) compared to the placebo treatment group, a 32% decrease in the top 20% region (p = 0.329), 22% decrease in the upper 40% region (p = 0.552), 3% increase in the lower 60% region (p = 0.955), and a 11% decrease in the Φ_h of the region (Table 2).

When considering the treatment agent instead of the treatment assignment, participants who underwent calcium supplementation compared to participants who did not undergo calcium supplementation had virtually no change in TLR4 expression across all regions examined including the Φ_h of the region, with a decrease between 0-9% for TLR4 expression. Participants that underwent vitamin D supplementation *versus* participants who did not undergo vitamin D supplementation experienced a 18% decrease in TLR4 expression (p = 0.394), a 21% decrease in the top 20% region (p = 0.314), 22% decrease in the upper 40% region (p = 0.296), and a 9% decrease in the lower 60% region (p = 0.745). There was little difference in TLR4 expression from vitamin D supplementation in the Φ_h of the region. Calcium and vitamin D combined compared to calcium only decreased TLR4 by 21% in the whole region (p = 0.425), 14% in the top 20% region (p = 0.599), 20% in the upper region (p = 0.424), and 18% in the lower 60% region (p = 0.550). There was almost no difference in TLR4 expression by the combined supplementation *versus* calcium alone in the Φ_h of the region (p = 0.910) (Table 3).

TLR4 Biomarker Expression by Baseline Characteristics

The associations between selected baseline characteristics and expression of TLR4 in the lamina propria are presented in Table 4. TLR4 expression was compared based off selected baseline risk factors selected *a priori* based on biological plausibility to determine any potential associations between them. Age, sex, previous type 2 diabetes diagnosis, educational status,

total physical activity, multivitamin use, HRT among women, the number of adenomas removed, whether there were advanced adenomas, 1st order family history of CRC, total energy intake, total fat intake, total calcium intake, fruit and vegetable intake, and alcoholic intake, vitamin D deficiency or vitamin D serum levels were not statistically significantly associated with TLR4 expression at baseline. Non-white participants had a statistically significant higher TLR4 expression compared to white participants within the whole region (64% higher, p = 0.047), the top 20% region (63% higher, p = 0.045), and the upper 40% region (65% higher, p = 0.040). There was a slight association seen in the lower 60% region, but the association was not statistically significant (61% higher, p = 0.105), and there was no association between TLR4 expression and race within the Φ_h of the region (p = 1.000). Compared to participants who had a normal BMI, there was a statistically significant association between participants who were heavily overweight (a BMI between 27.5 and 30.0) and TLR4 expression (108% higher, p = 0.006). This association was observed across all regions analyzed: 84% increase in TLR4 expression within the top 20% region (p = 0.019), 81% increase within the upper 40% region (p = 0.022), a 169% increase within the lower 60% region (p = 0.001). There was a borderline significant association observed between heavily obese BMI participants (a BMI greater than 35) and TLR4 expression (66% increase, p = 0.055). The significant association however was only observed additionally within the lower 60% region (123% increase, p = 0.008). There was a suggestive dose-response association between increasing BMI and higher TLR4 expression in the whole region (p_{trend} = 0.128)

Current smokers had lower TLR4 expression within certain regions in the lamina propria, with a 40% decrease observed overall (p = 0.121), a 51% decrease in the top 20% region (p = 0.028), and a 49% decrease in the upper 40% region (p = 0.034). There was a borderline significant dose-response effect observed for smoking status on TLR4 expression across the

whole region (p_{trend} = 0.128) and a significant dose-response effect observed across the top 20% region (p_{trend} = 0.023) and upper 40% region (p_{trend} = 0.031).

There was a statistically significant inverse association between regular use of aspirin and TLR4 expression, with a 31% decrease observed in the whole region (p = 0.046), a borderline statistically significant association between regular use of aspirin and TLR4 observed within the top 20% region (28% decrease, p = 0.061), the upper 40% region (29% decrease, p = 0.058), and the lower 60% region (32% decrease, p = 0.070).

There was no statistically significant association between regular use of NSAIDs and TLR4 expression across all regions, although the Φ_h was 17% higher compared to non-regular use (p = 0.003). When examining the association between any use of aspirin or NSAIDs compared to no use of either on TLR4 expression, all regions had at least a borderline statistically significant association: a 37% decrease in the whole region (p = 0.084), 40% in the top 20% region and upper 40% region (p = 0.048 and 0.051 respectively), and a 35% decrease in the lower 60% region (p = 0.168).

Medium calcium intake was statistically significantly inversely associated with TLR4 expression in the whole region (44% decrease, p = 0.033), top 20% region (41% decrease, p = 0.044), lower 60% region (50% decrease, p = 0.031) and borderline statistically significant within the upper 40% region (40% decrease, p = 0.056). There did not appear to be a dose response relationship between increasing calcium intake and TLR4 expression.

There was a statistically significant association between high vitamin D intake and TLR4 expression compared to participants with a low vitamin D intake. Within the whole region, there was a 52% decrease (p = 0.003), a 47% decrease within the top 20% region (p = 0.011), a 48% decrease within the upper 40% region (p = 0.007), and a 57% decrease within the lower

60% region (p = 0.003). There was a statistically significant inverse dose-response where increasing levels of vitamin D would correspond with lower TLR4 expression.

Compared to participants who did not consume a low amount of red or processed meats, there was a borderline statistically significant inverse association observed for participants who ate a medium or large amount of red or processed meats (relative to the entire participant cohort) on TLR4 expression. High levels of red or processed meat consumption on TLR4 expression compared to low levels was statistically significant in the whole region (51% decrease, p = 0.008), and within the top 20% region (48% decrease, p = 0.011). There was a borderline significant dose-response for the association observed in the whole region (p_{trend} = 0.063) and a significant dose-response for the association observed in the 20% region (p_{trend} = 0.031).

Serum calcium levels did not show any statistically significant association on TLR4 expression, when comparing high serum calcium to low serum calcium within the lower 60% region, there was a 18% decrease in TLR4 expression (p = 0.069). The Φ_h of the region was also statistically significantly associated for both the 16% decrease for TLR4 expression observed within high serum calcium participants and 13% decrease observed within medium serum calcium participants compared to low serum calcium participants (p = 0.003 and p = 0.015 respectively).

Stratified Analyses

Secondary analyses were conducted to assess whether treatment effects on TLR4 expression differ by regular NSAID and/or aspirin use, high or low vitamin D intake, high or low

calcium intake, and high or low serum vitamin D levels. The results of these analyses are presented in the appendix. (Supplementary Tables 4-9).

Most the participants were not regular NSAID users, therefore only results among non-regular users are discussed here. Among non-regular users of NSAIDs, vitamin D supplementation decreased TLR4 expression in the whole region by 27% ($p = 0.225$), and a combined supplementation of vitamin D and calcium compared to calcium alone showed a 24% decrease ($p = 0.381$). This trend is mirrored across the top 20% region and upper 40% region. The combined treatment *versus* calcium alone had a 23% decrease in the bottom 60% region ($p = 0.487$). For the vitamin D treatment *versus* no vitamin D, there was a 21% decrease in the bottom 60% region ($p = 0.440$) (Supplementary Table 4).

The effects of treatment agents on TLR4 expression did not differ by regular aspirin use status. Among non-regular aspirin users, calcium treatment compared to no calcium treatment did not affect TLR4 expression. Compared to no vitamin D, participants with vitamin D supplementation had a 21% reduction in the whole region ($p = 0.414$), a 14% decrease in the top 20% region ($p = 0.601$), a 20% decrease in the upper 40% region ($p = 0.396$), and a 16% decrease in the lower 60% region ($p = 0.636$). The combined treatment had a 25% decrease in both the whole region ($p = 0.425$) and the lower 60% region ($p = 0.527$), a 12% decrease in the top 20% ($p = 0.396$), and a 21% decrease in the upper 40% region ($p = 0.482$). There was no effect based off the Φ_h of the region (Supplementary Table 5).

When combining either NSAIDs or aspirin together, there was a 20% reduction in TLR4 expression for the effect of calcium *versus* no calcium among the whole region for regular users of either aspirin or NSAIDs ($p = 0.570$), and a 13% reduction in the top 20% region ($p = 0.738$). Vitamin D treatment had no effect within the whole region, and a 16% reduction in the top 20%

region (p = 0.612). For the combined treatment of vitamin D and calcium, there was no relative effect in the whole region, and a slight 11% reduction in TLR4 expression for the top 20% region (p = 0.769). For non-regular users, calcium treatment compared to no calcium had no inverse effect on TLR4 expression within the whole region, and a 9% reduction in the top 20% region (p = 0.793). There was a 30% reduction in TLR4 expression among non-regular users of either NSAIDs or aspirin who were given vitamin D compared to no vitamin D within the whole region (p = 0.307), and a 19% reduction in the top 20% region. Combined treatment *versus* only calcium had a 25% reduction in TLR4 for the whole region (p = 0.501), and an eight percent reduction in the top 20% region (p = 0.849) (Supplementary Table 6).

Participants who had a lower calcium intake generally had no benefit from either vitamin D or calcium treatment. Among participants who had a higher calcium intake among all study participants, there was a significant effect due to the vitamin D treatment, but not from calcium treatment alone. In the high calcium intake group, vitamin D supplementation compared to no vitamin D led to a 51% decrease in TLR4 expression overall (p = 0.072), a 55% decrease seen in the top 20% region (p = 0.039) and the upper 40% region (p = 0.042), and a 45% decrease seen in the lower 60% region (p = 0.193). The combined treatment of vitamin D and calcium compared to calcium alone followed the same trend. There was a 51% decrease in TLR4 expression in the whole region (p = 0.108), 47% decrease in the top 20% region (p = 0.138), a 52% decrease in the upper 40% region (p = 0.101), and a 53% decrease in the lower 60% region (p = 0.136). There seemed to be no effect based off the Φ_h of the region (Supplementary Table 7).

Regardless of high or low vitamin D intake, all participants showed a reduction in TLR4 expression when they had vitamin D supplementation, regardless of the presence or absence of calcium supplementation concurrently. Calcium treatment alone did not seem to influence TLR4

expression. Among high vitamin D intake participants, there was a 37% decrease ($p = 0.152$) in the whole region, a 42% decrease ($p = 0.121$) in the top 20% region, a 41% decrease ($p = 0.114$), and a 30% decrease ($p = 0.294$) in the lower 60% region when comparing vitamin D supplementation to no vitamin D supplementation. Among participants with a lower median vitamin D intake, the effects of vitamin D supplementation were subtler, with reductions in TLR4 expression ranging from no change in the lower 60% region ($p = 0.994$) to a 9% reduction seen in the top 20% region ($p = 0.740$). The combined treatment followed the same trends in both the high median vitamin D intake and low median vitamin D intake. In the high vitamin D intake group, both vitamin D and calcium led to a 29% decrease in TLR4 expression in the whole region ($p = 0.328$), a 26% decrease in the top 20% region ($p = 0.406$), a 29% decrease in the top 40% region ($p = 0.332$), and a 28% decrease in the lower 60% region ($p = 0.391$). In the low vitamin D intake group, the combined treatment led to a 13% decrease in TLR4 expression in the whole region ($p = 0.751$), 17% decrease in the top 20% region ($p = 0.636$), and an 8% decrease in both the upper 40% and lower 60% regions ($p = 0.841$ and $p= 0.886$ respectively). There seemed to be no effect based off the Φ_h of the region for all treatment agents (Supplementary Table 8).

Like vitamin D intake, calcium supplementation did not differentially affect TLR4 expression regardless of high or low serum levels of vitamin D for almost all regions examined. Among participants with high median serum vitamin D, there was a 25% reduction ($p = 0.454$) for vitamin D *versus* no vitamin D and a 20% reduction ($p = 626$) for combined treatment *versus* only calcium when looking at the entire region. For the top 20% region, there was a 30% reduction for participants taking vitamin D supplementation alone ($p = 0.327$), and a 19% reduction for participants taking both vitamin D and calcium ($p = 0.614$). Within the upper 40% region, there was a 27% reduction for vitamin D only ($p = 0.393$), and a 22% decrease for combined vitamin D and calcium ($p = 0.590$). Finally, for the lower 60% region, there was a 17%

decrease for vitamin D only participants (p = 0.680) and a 11% decrease for combined vitamin D and calcium participants (p = 0.818). Among participants with low median serum vitamin D, there was a 7% decrease (p = 0.796) and a 19% decrease (p = 0.574) seen for vitamin D alone and combined treatment respectively. There was virtually no change seen across treatment types in the top 20% region. In the upper 40% region, there was a 11% reduction in TLR4 expression in the vitamin D only group (p = 0.687), and a 15% reduction seen in the combined treatment group (p = 0.648). Calcium did seem to have an effect at the lower 60% region level; there was a 23% reduction of TLR4 expression (p = 0.467). Vitamin D alone did have an effect at the lower 60% region, but the combined treatment had a 25% reduction in TLR4 expression (p = 0.517). Φ_h did not cause any variation in TLR4 expression across all treatment agents (Supplementary Table 9).

Discussion

Primary Findings

Our results demonstrated that treatment with vitamin D and calcium, alone or in combination, did not have a statistically significant effect on TLR4 biomarker expression in the stroma of normal-appearing rectal mucosa of colorectal adenoma patients. However, there was a statistically non-significant moderate reduction in TLR4 across all treatments in the whole region, and there was generally a larger relative treatment effect within the top 20% region and the upper 40% region. The highest proportion of TLR4 was generally concentrated within the top 20% region of the lamina propria. Treatment with vitamin D alone had a stronger relative treatment effect compared to both calcium and the combined calcium and vitamin D treatment. Several *a priori* identified biological plausible factors were found to be associated with baseline expression with TLR4 including race, being overweight, smoking status, regular use of aspirin, consumption of red and processed meats, total levels of calcium, and total levels of vitamin D.

TLR4 and Gut Barrier Health

Chronic Inflammation has been shown to be a strong risk factor for both spontaneous CRC and CAC(12). Activation of the inflammation pathways within the colon is an integral and normal activity of the colon as it serves a dual function of interacting with commensal bacteria and preventing infection at the same time. The recruitment of pro-inflammatory cytokines is normally advantageous for the host, as it prevents pathogenic bacteria from colonizing the lumen or infiltrating into the lamina propria. It is when the system is chronically active where it becomes deleterious to the host. In colorectal neoplasms, inflammation plays an important role

in its development (preceding CAC development and concurrently in CRC), which in a chronic state allows the buildup of conditions that promote DNA damage through the activation of pro-inflammatory cytokine pathways such as TNF-α, IL-1, NF-κB, and other pro-inflammatory responses(15, 19). TLR4 is a key toll-like receptor responsible for initiating immune response toward LPS, a component of the gram-negative bacteria membrane(28). Normally, TLR4 functions in the recruitment of immune cells to combat and remove LPS(14, 17). Its overexpression in the immune cells within the lamina propria suggests a higher exposure to LPS likely in part due to weakening in the gut barrier responsible for normal protection against pathogens. The weakening of the gut barrier can involve the downregulation of tight junction proteins, responsible for maintaining the physical barrier between the single-layer epithelial cell line; an increase in the rate of epithelial cell shedding can also occur when epithelial cell shedding outpaces the proliferation of new cells(67). Chronic inflammation has been linked to the downregulation of the epithelial cell line and upregulation of epithelial cell shedding. Infiltration of gram negative bacteria will cause an immune reaction that involves the recruitment of leukocytes to the site of bacterial entry as well as triggering of the expected inflammation response(27). Because of the anatomy of the intestine, it is expected that the upper regions of the lamina propria will have a higher probability of TLR4 expression, due to a higher surface area of exposure among the upper region compared to the lower region. In our analyses, the top 20% region typically had the highest proportion of TLR4 expression, indicating that this expectation was met within this study. Because of the importance of TLR4 in the regulation and maintenance in gut barrier function and immune response, any chronic increase in TLR4 levels are potentially modifiable risk factors for the development of CRC.

Previous Studies

Our results are similar to previous studies on calcium and vitamin D supplementation on potential biomarkers linked with CRC. C-reactive protein (CRP) is another inflammation biomarker that has been studied extensively. Observational studies have seen an inverse association between serum 25(OH)D and CRP levels(71). Small RCTs have also seen a reduction in CRP after treatment with vitamin D or calcium(40). Another biomarker sub-study of the parent trial looking at APC, APC/β-catenin, and E-cadherin found that there was a modest increased expression of these biomarkers after supplementation of vitamin D and increased expression of E-cadherin after supplementation of calcium(8). Like TLR4, those biomarkers are all potential modifiable risk factors, with APC and E-cadherin being downregulated in adenomas and carcinomas and β-catenin being upregulated. Vitamin D has been shown to be inversely associated with NF-κB activity by physically blocking its activation, leading to a reduced inflammation response(72). Small RCTs have also seen a modest reduction in TNF-α levels, an important pro-inflammatory cytokine, after supplementation with vitamin D, calcium, or a combination of both(40, 73). Vitamin D and calcium were also suggested to reduce IL-1 and IL-6 proinflammatory cytokine expression(40). In our study, we considered TLR4 expression as a potential modifiable risk factor for the development of CRC by inflammation pathways. Vitamin D (and calcium to a lesser extent) had a modest effect of reducing TLR4 expression which was concentrated primarily in the top 20% of the lamina propria. Because previous RCTs have indicated a potential effect of vitamin D and/or calcium on inflammation biomarkers (such as CRP, TNF-α, IL-1, IL-6 NF-κB) which are also present and produced within the colon, it is plausible that treatment with vitamin D and/or calcium may reduce inflammation in the colon in part through the TLR4 pathway.

In our stratified analysis, we found that lower than normal NSAID usage led to a stronger relative treatment effect for vitamin D compared to regular NSAID usage. This same trend was not seen within calcium. Among regular aspirin users, the relative treatment effect was lower than among those who did not take aspirin regularly. A stronger relative treatment effect for NSAID and aspirin non-users is possible, as both aspirin and NSAID usage is meant to reduce the effects of inflammation which may interact with the effects of vitamin D and calcium. Biologically this is plausible, as NSAID usage has been linked with reduction of COX-2 expression, which reduces the number of pro-inflammatory cytokines and signals being produced, therefore working in tandem with TLR4 to reduce inflammation and the recruitment of immune cells to the lamina propria(12, 26). This reduction is also seen in our analysis of baseline characteristics, in which participants who had regular NSAID usage, regular aspirin usage, or any use of either was generally found to have lower TLR4 levels at baseline.

Within our analyses of high calcium intake *versus* low calcium intake, we found that participants who had a high intake responded better to vitamin D supplementation than those with a low calcium intake. There was also no effect observed by calcium treatment alone. The same trends were seen across both vitamin D intake and to a lesser extent 25(OH)D serum levels; the reduced treatment effect among participants with low vitamin D or serum 25(OH)D levels are also expected. This is expected, as the synergistic relationship between calcium and vitamin D and the effect of vitamin D on inflammation has been well documented(8, 9, 40, 52).

Among the baseline characteristics examined, one potential significant factor associated with TLR4 expression was race. Compared to white participants, non-white participants on average had a relatively higher TLR4 expression regardless of treatment type(74). This is likely due to racial differences in health and comorbidities. It is possible that the non-white

participants who were recruited into the sub-study were generally less healthy than their white counterparts and had worse health conditions on average.

Being overweight was also associated with higher TLR4 expression compared to normal BMI. BMI biologically has been linked with inflammation as it may release more pro-inflammatory cytokines such as IL-6 and TNF-α compared to patients with normal BMI(59). Overweight individuals were also shown to have a higher abundance of gram negative bacteria, leading to increased exposure to LPS(75). It is also well established that there is a difference in the diet of overweight individuals compared to normal weight individuals; overweight individuals are more likely to consume an unhealthier diet that can also contribute to promoting inflammation *via* dietary-based risk factors(75).

Current cigarette smoking was found to have a negative association with TLR4 expression. Past research has clearly defined how smoking status will increase inflammation processes throughout the body(58, 76). It is possible that this reduction can be possibly explained by nicotine diminishing the immune response, causing a reduction in proinflammatory cytokines TNF and IL-1(77). It is likely that this observation is due to chance as there were only a few participants who reported that they regularly smoked. Additional analyses that also controlled for BMI did not explain the inverse association that we observed for current smokers and former smokers.

High consumption of red or processed meats was associated with lower TLR4 expression, despite previous data supporting a positive association between red meats and inflammation(60, 61). When stratifying by relative consumption, we do see that there is an increase in TLR4 expression among medium and high consumers of red meat compared to low consumers of red meat, which does not match the literature on red meat consumption and TLR4

expression. This is likely due to the low number of participants who reported having no red meat in their diet. Another possible explanation is the small range between the categories: a man, for example, would be considered high if he consumed 1.4 servings of red or processed meat, but would be considered low if he consumed less than 0.8 servings of red meat. This small range of red meat intake in this population provided low power to detect differences associated with extreme intakes of red meat.

We observed lower TLR4 expression among regular users of aspirin in our study, a finding that is expected due to aspirin being an anti-inflammatory drug that targets the COX pathway, inhibiting pro-inflammatory cytokines that are downregulated by COX-2(26).

Strengths and Limitations

Our sub-study had several strengths and limitations. One strength was in the study design, because our study was based off a RCT we could reduce the amount of bias that is typical in more standard observational studies. Another strength was that there was a high adherence in the study, with 76% of the participants taking 80% or more of the assigned treatment tablets. We also explored the colon tissue directly, instead of using peripheral markers to measure TLR4. Because we looked at the expression of TLR4 instead of CRC, we did not have to wait for CRC to develop within our patients as well as being able to work with a smaller sample. There is biological support for our hypothesis as well, as vitamin D and calcium's effect on inflammation have been well studied in the past. One of the limitations included the small sample size (n = 105), which could potentially limit our stratified analyses. Despite this sample size issue, the associations found followed typical trends across our analyses. Another limitation was the inability to explore how vitamin D and calcium affects the

risk for developing CRC. However, because of the latency period for CRC being around five to 10 years, and this study only examining one year follow-up, it would be biologically implausible and erroneous to make that association. Another possible limitation was the relatively low dose of vitamin D given during the study, although we observed substantial changes in circulating vitamin D levels at follow-up despite the low dose.

Conclusion

In summary, there were no statistically significant reductions in TLR4 due to vitamin D, calcium, or combined supplementation. The study results however do indicate a relatively moderate but not statistically significant effect of vitamin D, alone or in combination with calcium, on reducing the expression of TLR4 among colorectal adenoma patients. Calcium supplementation by itself either showed an attenuated, with respect to vitamin D, inverse relationship with TLR4 expression. Several baseline factors were associated with TLR4 expression, including race, smoking status, being overweight, regular use of aspirin, total dietary consumption of either vitamin D or calcium, and red meat consumption. Other modifiable factors could be used to modulate TLR4 expression, but future studies are needed. Our study provides some support for the hypothesis that vitamin D and calcium have an inverse relationship with CRC, primarily through a reduction in the TLR4 biomarker. Due to the limitations within our study as well as the results seen, we cannot say that this relationship is for certain based from our study. Further studies on the effect of TLR4 biomarker on colorectal neoplasms, on adenoma-adenocarcinoma transition, as well as potential treatment options for TLR4 will be useful in future care options and prevention of CRC.

Public Health Impact and Future Directions

Summary

The relationship between vitamin D and calcium and their effect on CRC has been well studied due to the public health impact that CRC poses. The TLR4 biomarker has also been linked with the inflammation pathway in the colon, responsible for the defense against gram-negative bacteria. CRC typically has a latency period of five to 10 years, meaning most RCTs on the effect of any treatment on CRC will not adequately assess intervention due to time limitations. Measuring TLR4 has the potential to be an innovative approach to be a real-time gauge of potential CRC risk, due to the link between TLR4, inflammation and colorectal neoplasms. TLR4 biomarker could potentially be used for colorectal neoplasms risk assessment, as chronic inflammation is a well-known risk factor for DNA damage and progression from a colon adenoma to carcinoma. This can be a potential early detection tool for CRC risk assessment or used in tandem with rectal biopsies to help guide treatment options for patients.

Possible Future Directions

Through our study, we were able to explore the effects of several well-known preventative treatments for CRC: vitamin D and calcium. Based off our cross-sectional analysis, several modifiable factors with a strong association to TLR4 expression were identified including BMI, smoking status, aspirin use, total dietary intake of either vitamin D or calcium, and red meat consumption. Future studies can explore other well-known preventative risk factors such as high physical activity or high fiber intake and their effect on TLR4 expression. While our study did not see a statistically significant preventative effect from regular NSAID usage, our study

population for this stratified analysis was small and the moderate reduction in TLR4 expression across treatment agents for non-regular NSAID usage is similar to what has been observed in previous studies. Repeating the study on a larger scale is a potential approach, as a larger sample size would help with reducing any errant associations due to random chance alone. Many of the associations and effects were borderline statistically significant or close to statistically significant, something that a large sample size may help in uncovering a more accurate estimate of effect. A future study could also look at other risk factors not explored in this study such as melatonin levels, as well as other preventative measures such as high magnesium. More studies on TLR4 and CRC are warranted, as TLR4 is linked with the pro-inflammatory signaling pathways and can be a potential driving force in colorectal cancer development.

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Table 1: Baseline Characteristics of Clinical Trial Participants by Treatment Assignment to Vitamin D, Calcium, Both Vitamin D and Calcium, or Placebo (N=105)[a]

	Treatment Assignment							
	Randomization to Vitamin D and Calcium (4-Arm)					*Randomization to Vitamin D Only (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]*
Demographic Information								
Age, Years	59.92 (7.23)	59.88 (6.47)	59.18 (7.76)	58.00 (7.06)	0.857	58.17 (5.34)	59.21 (7.28)	0.598
Male, %[d]	75.00	81.25	70.59	83.33	0.833	--	--	--
White, %	83.33	75.00	70.59	94.44	0.396	69.57	84.21	0.575
BMI, kg/m^2	29.39 (4.89)	32.32 (7.62)	28.66 (5.49)	30.15 (4.41)	0.313	29.74 (5.64)	27.51 (4.74)	0.178
Diabetes, %	8.33	18.75	11.76	11.11	0.878	17.39	15.79	1.000
College graduate or higher, %[e]	75.00	50.00	76.47	72.22	0.382	56.52	52.63	0.801
Physical activity, MET-min/wk[f•]	1620.21 (1194.82)	2127.66 (2377.91)	2781.71 (2763.96)	4042.08 (2455.78)	0.033	1458.2 (1234.7)	3020.8 (3468.7)	0.051
Current smoker, %	25.00	6.25	0.00	5.56	0.150	0.00	15.79	0.158
Regular non-aspirin NSAID use, %[g]	8.33	18.75	17.65	5.56	0.630	8.70	10.53	0.841
Regular aspirin use, %[g]	41.67	68.75	29.41	33.33	0.098	21.74	31.58	0.470
Multivitamin Use, %	41.67	81.25	47.06	66.67	0.102	69.57	89.47	0.149
Among women, HRT users (n=56), %[•••]	100.00	0.00	20.00	33.33	0.125	14.29	31.58	0.265
Number of adenomas removed	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 adenomas, %[•••]	36.36	6.67	23.53	27.78	0.295	9.09	15.79	0.649
1° family history of CRC, %[•••]	0.00	12.50	20.00	5.56	0.367	4.35	11.11	0.573
Dietary Intakes								
Total energy intake, kcal/d[•••]	1314.42 (380.92)	1736.93 (555.60)	1436.55 (527.11)	1569.01 (565.12)	0.213	1253.7 (548.9)	1429.1 (594.9)	0.327
Total fat, gm/d[•••]	57.06 (22.28)	68.92 (25.64)	60.46 (27.26)	61.55 (26.80)	0.689	50.27 (25.87)	61.46 (36.09)	0.249
Total calcium, mg/d[h]	715.34 (455.40)	894.52 (263.94)	671.29 (278.28)	667.13 (254.66)	0.143	995.6 (497.6)	1232.3 (562.9)	0.198
Total vitamin D, IU/d[i]	354.00 (306.11)	457.45 (189.17)	312.99 (278.37)	420.55 (295.63)	0.485	521.4 (354.2)	633.6 (275.6)	0.341
Dietary fiber, gm/d[•••]	9.53 (4.06)	15.81 (5.56)	13.73 (6.24)	15.10 (5.70)	0.043	13.83 (5.40)	17.21 (4.96)	0.043
Red/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	3.01 (1.68)	4.37 (1.99)	4.51 (2.48)	4.28 (1.73)	0.278	4.70 (1.67)	5.99 (2.37)	0.045
Alcohol intake, drinks/day	0.678 (0.74)	0.81 (1.04)	0.85 (0.95)	0.86 (0.90)	0.950	0.54 (0.98)	0.34 (0.50)	0.422
Serum Levels								
25-OH-vitamin D, ng/mL	22.43 (8.24)	24.50 (13.38)	23.11 (8.68)	22.71 (6.38)	0.934	24.79 (8.87)	26.54 (9.61)	0.543
Ca^{2+}, mg/dL	9.19 (0.19)	9.33 (0.32)	9.35 (0.34)	9.42 (0.29)	0.241	9.49 (0.33)	9.42 (0.32)	0.516

Abbreviations: BMI = Body Mass Index, NSAID = Non-steroidal anti-inflammatory drug, CRC = Colorectal cancer, HRT = Hormone replacement therapy, IU = International units, kcal = Kilocalories, d = Day, wk = Week

● denotes a missing patient per symbol

[a]Data is presented as means (SD) unless indicated otherwise

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

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

[d]Only women in the 2-arm randomization

[e]Received an associates degree or higher

[f]MET: Metabolic equivalent of task

[g]Regular use of non-aspirin NSAID or aspirin was considered usage of ≥ four or more times a week

[h]Dietary calcium and supplemental calcium, missing information from 2 placebo-arm, 1 calcium-arm, 1 vitamin D-arm, 1 combined-arm, 6 placebo-arm (2-Arm), and 1 vitamin D-arm (2-Arm) patient (Total: ● x12)

[i]Dietary vitamin D and supplemental vitamin D, missing information from 3 placebo-arm, 2 calcium-arm, 2 vitamin D-arm, 1 combined-arm, 6 placebo-arm (2-Arm), and 5 vitamin D-arm (2-Arm) patient (Total: ● x 19)

Table 2: TLR4 Expression in the Lamina Propria Region by Treatment Assignment to Vitamin D, Calcium, Both Vitamin D and Calcium, or Placebo[a]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Treatment Group	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Whole Region - 4 Arm														
Placebo	12	93.85	53.57	164.42	--	90.86	51.86	159.19	--	Ref.	--	--	--	--
Calcium	16	151.50	92.39	248.41	0.191	130.43	79.54	213.88	0.322	0.89	0.37	2.13	0.789	-18.08
Vitamin D	17	132.00	83.71	208.16	0.337	105.72	67.04	166.72	0.669	0.83	0.35	1.94	0.657	-23.29
Calcium + Vitamin D	18	110.59	70.11	174.41	0.642	95.01	60.24	149.86	0.899	0.89	0.38	2.06	0.778	-12.58
Whole Region - 2 Arm														
Placebo	23	99.89	62.22	160.39	--	79.86	49.73	128.23	--	Ref.	--	--	--	--
Vitamin D	19	88.97	53.26	148.64	0.740	44.88	26.86	74.97	0.104	0.63	0.27	1.48	0.282	-24.06
Top 20% of Region - 4 Arm														
Placebo	12	28.72	16.54	49.86	--	33.64	19.38	58.40	--	Ref.	--	--	--	--
Calcium	16	56.11	34.24	91.98	0.064	41.93	25.58	68.72	0.537	0.64	0.29	1.41	0.262	-19.11
Vitamin D	17	44.40	27.87	70.74	0.219	30.23	18.97	48.16	0.762	0.58	0.27	1.27	0.172	-19.09
Calcium + Vitamin D	18	32.61	20.46	51.97	0.929	26.09	16.38	41.58	0.469	0.68	0.31	1.48	0.329	-11.43
Top 20% of Region - 2 Arm														
Placebo	23	32.31	19.27	54.18	--	24.02	14.32	40.28	--	Ref.	--	--	--	--
Vitamin D	19	27.07	15.46	47.39	0.642	13.98	7.99	24.49	0.160	0.70	0.28	1.72	0.423	-4.79
Upper 40% of Region - 4 Arm														
Placebo	12	46.89	26.70	82.34	--	51.14	29.12	89.81	--	Ref.	--	--	--	--
Calcium	16	94.73	57.61	155.74	0.058	79.09	48.11	130.03	0.234	0.77	0.33	1.78	0.528	-19.89
Vitamin D	17	79.58	50.36	125.75	0.141	60.03	37.99	94.87	0.652	0.69	0.30	1.57	0.373	-23.79
Calcium + Vitamin D	18	62.48	39.52	98.81	0.419	53.45	33.80	84.53	0.901	0.78	0.35	1.77	0.552	-13.28
Upper 40% of Region - 2 Arm														
Placebo	23	56.98	35.05	92.64	--	45.41	27.93	73.83	--	Ref.	--	--	--	--
Vitamin D	19	48.76	28.81	82.55	0.664	24.25	14.32	41.05	0.085	0.62	0.27	1.45	0.265	-12.95
Lower 60% of Region - 4 Arm														
Placebo	12	39.88	20.99	75.78	--	34.01	17.90	64.62	--	Ref.	--	--	--	--
Calcium	16	45.97	26.13	80.87	0.733	41.62	23.66	73.23	0.628	1.06	0.37	3.08	0.911	1.53
Vitamin D	17	39.41	23.41	66.35	0.977	37.97	22.55	63.93	0.786	1.13	0.40	3.19	0.815	4.43
Calcium + Vitamin D	18	40.11	23.84	67.48	0.989	35.22	20.93	59.25	0.931	1.03	0.37	2.88	0.955	0.98
Lower 60% of Region - 2 Arm														
Placebo	23	37.63	22.71	62.34	--	26.48	15.99	43.87	--	Ref.	--	--	--	--
Vitamin D	19	34.04	19.68	58.88	0.788	15.90	9.19	27.50	0.175	0.66	0.25	1.76	0.399	-7.00

		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Treatment Group	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
φh - 4 Arm[e]														
Placebo	12	51.26	44.96	57.56	--	56.84	50.54	63.14	--	Ref.	--	--	--	--
Calcium	16	63.23	57.69	68.77	0.005	61.10	55.56	66.64	0.300	-7.72	-18.38	2.94	0.153	0.87
Vitamin D	17	61.89	56.78	67.00	0.010	58.45	53.34	63.57	0.687	-9.02	-19.41	1.37	0.088	0.85
Calcium + Vitamin D	18	57.52	52.42	62.62	0.118	57.01	51.91	62.11	0.967	-6.10	-16.38	4.18	0.240	0.89
φh - 2 Arm														
Placebo	23	57.60	53.24	61.96	--	58.12	53.76	62.49	--	Ref.	--	--	--	--
Vitamin D	19	55.20	50.47	59.93	0.456	55.31	50.58	60.03	0.382	-0.41	-8.24	7.41	0.915	0.99

[a]TLR4 by treatment assignment was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (4-arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

Table 3: Comparison of TLR4 Expression in the Lamina Propria Region by Treatment Type[a]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Treatment Group	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Whole Region														
No calcium	29	115.05	80.29	164.89	--	99.29	69.28	142.28	--	Ref.	--	--	--	--
Calcium[e]	34	127.65	90.14	180.77	0.659	109.78	77.52	155.46	0.669	1.00	0.57	1.74	0.991	-2.10
No vitamin D	51	118.81	87.97	160.45	--	101.43	75.11	136.98	--	Ref.	--	--	--	--
Vitamin D[f]	54	108.76	82.13	144.03	0.655	75.78	57.23	100.35	0.142	0.82	0.51	1.31	0.394	-15.60
Calcium only	39	140.79	96.45	205.53	--	115.90	79.39	169.20	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	37	110.53	76.47	159.76	0.304	72.25	49.98	104.43	0.047	0.79	0.45	1.41	0.425	-13.39
Top 20% of Region														
No calcium	29	36.83	25.44	53.32	--	31.39	21.68	45.44	--	Ref.	--	--	--	--
Calcium	34	42.05	29.35	60.24	0.581	32.58	22.74	46.67	0.877	0.91	0.54	1.54	0.718	-4.03
No vitamin D	51	39.27	28.74	53.65	--	32.54	23.82	44.46	--	Ref.	--	--	--	--
Vitamin D	54	33.87	25.20	45.51	0.471	22.08	16.43	29.68	0.061	0.79	0.49	1.26	0.314	-5.05
Calcium only	39	48.03	32.35	71.32	--	35.78	24.10	53.13	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	33.61	22.80	49.54	0.146	21.48	14.58	31.66	0.039	0.86	0.48	1.53	0.599	0.12
Upper 40% of Region														
No calcium	29	64.37	44.64	92.80	--	56.13	38.93	80.92	--	Ref.	--	--	--	--
Calcium	34	75.54	53.00	107.67	0.503	63.91	44.84	91.09	0.587	0.97	0.57	1.66	0.911	-3.39
No vitamin D	51	67.65	49.78	91.93	--	58.68	43.18	79.74	--	Ref.	--	--	--	--
Vitamin D	54	61.92	46.50	82.46	0.660	42.07	31.59	56.03	0.100	0.78	0.49	1.24	0.296	-10.88
Calcium only	39	83.92	57.13	123.29	--	68.13	46.37	100.08	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	62.25	42.83	90.50	0.210	40.31	27.73	58.59	0.030	0.80	0.46	1.40	0.424	-6.15
Lower 60% of Region														
No calcium	29	39.53	26.34	59.33	--	36.30	24.19	54.48	--	Ref.	--	--	--	--
Calcium	34	42.68	28.87	63.11	0.773	38.00	25.71	56.19	0.863	0.97	0.49	1.91	0.928	-1.45
No vitamin D	51	43.12	30.96	60.06	--	34.22	24.57	47.67	--	Ref.	--	--	--	--
Vitamin D	54	37.83	27.74	51.58	0.549	27.39	20.08	37.35	0.309	0.91	0.52	1.59	0.745	-1.54
Calcium only	39	49.09	32.42	74.34	--	38.16	25.20	57.78	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	41.08	27.40	61.58	0.493	26.08	17.40	39.10	0.145	0.82	0.42	1.60	0.550	-4.07
		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Treatment Group	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
φh[h]														
No calcium	29	57.66	53.48	61.84	--	57.77	53.59	61.95	--	Ref.	--	--	--	--
Calcium	34	60.13	56.10	64.15	0.370	58.87	54.85	62.90	0.687	-1.36	-8.32	5.60	0.697	0.98
No vitamin D	51	57.94	54.71	61.17	--	58.78	55.55	62.02	--	Ref.	--	--	--	--
Vitamin D	54	57.97	54.95	60.99	0.988	56.76	53.74	59.78	0.340	-2.06	-7.14	3.02	0.423	0.96
Calcium only	39	60.23	56.44	64.02	--	59.70	55.91	63.50	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	56.95	53.25	60.64	0.166	56.75	53.06	60.45	0.213	0.33	-5.47	6.14	0.910	1.02

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[f]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[g]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the

Table 4: TLR4 Expression in the Lamina Propria Region by Baseline Categorical Predictors Within the Whole Region and Top 20% Region[a]

		Whole Region						*Top 20% of Region*					
		Geometric			*p-*	*Proportional*		*Geometric*			*p-*	*Proportional*	
Covariate	*n*	*Mean*[c]	*95% CI*		*value*[d]	*Difference*[e]	*p-trend*[d]	*Mean*	*95% CI*		*value*	*Difference*	*p-trend*
Age													
≤ 55 years	39	102.61	72.48	145.27	Ref.	Ref.		33.49	24.06	46.63	Ref.	Ref.	
> 55 years, ≤ 63 years	38	99.66	72.76	136.50	0.882	-2.88%	0.472	30.08	22.09	40.96	0.571	-10.18%	0.669
≥ 63 years	28	117.50	82.62	167.10	0.564	14.51%		35.66	25.25	50.37	0.780	6.47%	
Gender													
Male	49	115.93	83.64	160.67	Ref.	Ref.		34.85	25.30	48.02	Ref.	Ref.	
Female	56	100.47	76.29	132.31	0.567	-13.33%		32.16	24.54	42.14	0.744	-7.73%	
Race													
White	83	94.92	73.84	122.02	Ref.	Ref.		29.66	23.24	37.86	Ref.	Ref.	
Non-White	22	156.03	99.90	243.67	0.047	64.38%		48.27	31.21	74.65	0.045	62.72%	
BMI													
Normal (< 25.00)	22	69.10	45.55	104.83	Ref.	Ref.		24.33	16.09	36.78	Ref.	Ref.	
Overweight I (25.00 - 27.49)	19	94.51	57.33	155.81	0.258	36.77%		33.47	21.18	52.89	0.228	37.57%	
Overweight II (27.50 - 29.99)	24	143.38	100.97	203.61	0.006	107.50%	0.128	44.77	31.54	63.54	0.019	84.02%	0.543
Obese I (30.00 - 32.49)	15	97.61	61.24	155.57	0.272	41.26%		28.41	17.85	45.23	0.618	16.80%	
Obese II (≥ 35.00)	25	114.37	79.92	163.67	0.055	65.52%		32.49	22.74	46.41	0.264	33.54%	
Diabetes													
No	90	102.13	80.34	129.82	Ref.	Ref.		31.98	25.26	40.49	Ref.	Ref.	
Yes	15	137.80	81.47	233.08	0.268	34.93%		40.30	24.02	67.63	0.386	26.01%	
College graduate													
Less than an associate's degree	39	119.39	86.89	164.05	Ref.	Ref.		37.12	27.11	50.81	Ref.	Ref.	
Associate's degree or higher	66	96.66	72.82	128.30	0.273	-19.04%		30.21	22.99	39.69	0.271	-18.61%	
Total physical activity (MET-min/wk)[fh•]													
Low	37	98.37	70.35	137.56	Ref.	Ref.		32.33	23.51	44.45	Ref.	Ref.	
Medium	34	109.03	76.35	155.70	0.639	10.83%	0.511	33.10	23.27	47.09	0.910	2.40%	0.841
High	33	113.32	80.83	158.86	0.513	15.19%		33.69	24.10	47.08	0.843	4.20%	
Smoking status													
Never	61	121.09	90.82	161.46	Ref.	Ref.		38.50	29.28	50.62	Ref.	Ref.	
Former or occasional	36	95.57	68.19	133.94	0.243	-21.08%	0.082	29.86	21.57	41.33	0.186	-22.44%	0.023
Current	8	72.77	39.60	133.71	0.121	-39.91%		19.06	10.58	34.33	0.028	-50.50%	
Regular use of NSAIDs (4/wk or more)													
No	93	110.36	84.96	143.36	Ref.	Ref.		31.68	24.51	40.94	Ref.	Ref.	
Yes	12	89.80	53.34	151.17	0.485	-18.63%		38.03	22.78	63.51	0.530	20.07%	
Regular use of aspirin (4/wk or more)													
No	63	118.85	92.19	153.23	Ref.	Ref.		36.39	28.43	46.58	Ref.	Ref.	
Yes	38	82.60	59.22	115.21	0.046	-30.50%		26.09	18.78	36.26	0.061	-28.29%	
Any use of NSAIDs or aspirin													
No	11	160.41	94.82	271.36	Ref.	Ref.		52.52	31.42	87.80	Ref.	Ref.	
Yes	94	101.31	80.18	128.00	0.084	-36.84%		31.37	24.99	39.37	0.048	-40.28%	
Multivitamin user													
No	34	120.26	88.09	164.17	Ref.	Ref.		35.58	26.42	47.90	Ref.	Ref.	
Yes	71	94.33	70.06	127.00	0.225	-21.56%		30.46	22.69	40.87	0.416	-14.39%	
Among women (n=56), currently on HRT[•••]													
No	40	104.39	72.73	149.82	Ref.	Ref.		33.34	23.17	47.96	Ref.	Ref.	
Yes	13	117.72	58.95	235.09	0.775	12.78%		40.88	20.38	82.00	0.630	22.61%	
Number of adenomas removed													
One	76	107.70	82.25	141.01	Ref.	Ref.		33.26	25.58	43.25	Ref.	Ref.	
More than one	29	102.03	70.95	146.71	0.795	-5.27%		32.08	22.42	45.90	0.859	-3.55%	
Had advanced adenomas[•••]													
No	83	104.60	80.28	136.28	Ref.	Ref.		33.97	26.27	43.92	Ref.	Ref.	
Yes	19	115.53	74.98	178.00	0.685	10.45%		31.26	20.47	47.73	0.726	-7.98%	
1° family history of CRC[•••]													
No	93	111.49	87.59	141.92	Ref.	Ref.		33.93	26.68	43.16	Ref.	Ref.	
Yes	9	120.67	69.12	210.66	0.787	8.23%		36.91	21.11	64.56	0.774	8.79%	
Total energy intake (kcal/d)[h•••]													
Low	35	121.06	86.61	169.21	Ref.	Ref.		37.67	27.23	52.12	Ref.	Ref.	
Medium	35	91.54	63.06	132.88	0.206	-24.38%	0.320	30.20	20.93	43.58	0.304	-19.83%	0.273
High	32	98.93	70.53	138.78	0.342	-18.28%		30.28	21.73	42.18	0.291	-19.64%	

Table 4 (Continued)

		Whole Region						Top 20% of Region					
		Geometric			p-	Proportional		Geometric			p-	Proportional	
Covariate	n	Mean[c]	95% CI		value[d]	Difference[e]	p-trend[d]	Mean	95% CI		value	Difference	p-trend
Total fat (gm/d)[h●●●]													
Low	35	131.74	81.30	213.47	Ref.	Ref.		38.77	24.14	62.25	Ref.	Ref.	
Medium	35	91.60	62.42	134.41	0.126	-30.47%	0.208	27.95	19.19	40.73	0.159	-27.89%	0.320
High	32	92.16	53.80	157.88	0.418	-30.04%		31.39	18.55	53.13	0.625	-19.03%	
Total calcium (mg/d)[g●i]													
Low	32	124.81	86.32	180.46	Ref.	Ref.		37.91	26.54	54.15	Ref.	Ref.	
Medium	32	69.89	46.71	104.58	0.033	-44.00%	0.661	22.27	14.98	33.09	0.044	-41.26%	0.902
High	29	101.41	70.33	146.23	0.412	-18.75%		33.63	23.43	48.26	0.629	-11.29%	
Total vitamin D (IU/d)[hi]													
Low	30	141.33	101.89	196.03	Ref.	Ref.		39.08	28.22	54.11	Ref.	Ref.	
Medium	29	109.56	78.90	152.14	0.232	-22.48%	0.004	36.79	26.22	51.62	0.777	-5.85%	0.016
High	27	68.23	45.80	101.65	0.003	-51.72%		20.83	13.82	31.40	0.011	-46.69%	
Dietary fiber (gm/d)[i●●●]													
Low	36	84.45	59.71	119.45	Ref.	Ref.		29.88	21.17	42.18	Ref.	Ref.	
Medium	34	120.54	83.87	173.26	0.102	42.74%	0.151	35.04	24.44	50.24	0.454	17.26%	0.536
High	32	117.13	77.31	177.46	0.234	38.69%		34.28	22.91	51.29	0.611	14.71%	
Red/processed meats (servings/d)[i]													
Low	28	127.89	83.31	196.33	Ref.	Ref.		41.99	27.17	64.90	Ref.	Ref.	
Medium	38	86.12	63.92	116.03	0.081	-32.66%	0.063	29.20	21.67	39.34	0.113	-30.45%	0.031
High	30	84.79	61.10	117.67	0.141	-33.70%		25.12	18.00	35.06	0.072	-40.17%	
Fruits and vegetables (servings/d)[i●●●]													
Low	35	94.83	66.22	135.79	Ref.	Ref.		30.49	21.54	43.15	Ref.	Ref.	
Medium	34	97.20	68.54	137.84	0.913	2.50%	0.280	30.44	21.60	42.89	0.994	-0.15%	0.368
High	33	123.41	84.98	179.23	0.269	30.14%		37.92	26.35	54.55	0.350	24.37%	
Alcohol intake (drinks/d)													
≤ 0.30 drinks	54	98.96	73.45	133.33	Ref.	Ref.		31.46	23.47	42.15	Ref.	Ref.	
> 0.30 drinks	51	111.22	79.19	156.20	0.575	12.39%		34.31	24.79	47.47	0.665	9.07%	
Serum level 25-OH deficiency													
Deficient (< 20 ng/mL)	46	111.66	83.02	150.19	Ref.	Ref.		32.71	24.47	43.71	Ref.	Ref.	
Sufficient (≥ 20 ng/mL)	59	99.42	72.54	136.26	0.560	-10.97%		33.08	24.41	44.83	0.953	1.14%	
Serum 25-OH[i]													
Low	36	100.67	72.31	140.13	Ref.	Ref.		31.06	22.41	43.03	Ref.	Ref.	
Medium	36	115.12	83.01	159.65	0.537	14.35%	0.911	33.53	24.42	46.02	0.716	7.96%	0.452
High	33	100.08	69.50	144.12	0.979	-0.58%		34.92	24.40	49.96	0.582	12.43%	
Serum Ca^{2+}[i]													
Low	42	109.45	79.27	151.13	Ref.	Ref.		30.12	21.98	41.29	Ref.	Ref.	
Medium	37	117.09	85.24	160.85	0.750	6.98%	0.186	39.36	29.00	53.40	0.192	30.64%	0.518
High	36	79.97	54.49	117.36	0.154	-26.93%		26.26	18.01	38.27	0.521	-12.84%	

Abbreviations: BMI = Body Mass Index, NSAID = Non-steroidal anti-inflammatory drug, CRC = Colorectal cancer, HRT = Hormone replacement therapy, IU = International units, kcal = Kilocalories, d = Day, wk = Week

● denotes a missing patient per symbol

[a]TLR4 expression was modeled using a generalized linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), batch, and study center. All dietary intakes are also controlled for total calorie intake

[b]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

[c]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[d]p-values are comparing selected categorical value to its reference value. P-trend is comparing dose-response effect by increasing categorization level for variables with more than two categories

[e]Calculated as follows: [(Category OD - Reference OD) / Reference OD x 100%]

[f]MET: Metabolic equivalent of task

[g]Dietary calcium and supplemental calcium, missing information from 12 patients (● x 12)

[h]Dietary vitamin D and supplemental vitamin D, missing information from 19 patients (● x 19)

[i]Categorized by tertiles, including sex-specific and study arm-specific considerations when applicable.

Total Energy Intake, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 1328.4, ≤ 899.1, ≤ 946.8 kcal/d, Medium: ≤ 1701, ≤ 1384.8, ≤ 1570.6 kcal/d, High: > 1701, > 1384.8, > 1570.6 kcal/d

Total Fat, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 46.4, ≤ 36.9, ≤ 36.2 gm/d, Medium: ≤ 79.5, ≤ 58.9, ≤ 62.5 gm/d, High: > 79.5, > 58.9, > 62.5 gm/d

Total Calcium, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 607.0, ≤ 482.5, ≤ 826.3 mg/d, Medium: ≤ 823.1, ≤ 644.7, ≤ 1270.8 mg/d High: > 823.1, > 644.7, > 1270.8 mg/d

Total Vitamin D, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 451.21, ≤ 86.60, ≤ 480.28 IU/d, Medium: ≤ 556.87, ≤ 171.69, ≤ 661.57 IU/d, High: > 556.87, > 171.69, > 661.57 IU/d

Dietary Fiber, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 11.1, ≤ 10.7, ≤ 12.1 gm/d, Medium: ≤ 16.5, ≤ 14.4, ≤ 16.6 gm/d, High: > 16.5, > 14.4, > 16.6 gm/d

Red/processed meats, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 0.8, ≤ 0.3, ≤ 0.3 servings/d, Medium: ≤ 1.4, ≤ 0.6, ≤ 0.8 servings/d, High: > 1.4, > 0.6, > 0.8 servings/d

Fruits and vegetables, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 3.2, ≤ 3.5, ≤ 3.9 servings/d, Medium: ≤ 4.5, ≤ 4.9, ≤ 6.3 servings/d, High: > 4.5, > 4.9, > 6.3 servings/d

Serum 25-OH, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 18.45, ≤ 14.97, ≤ 19.09 ng/mL, Medium: ≤ 26.11, ≤ 23.81, ≤ 30.86 ng/mL, High: > 26.11, > 23.81, > 30.86 ng/mL

Serum Ca^{2+}, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 9.2, ≤ 9.0, ≤ 9.3 mg/dL, Medium: ≤ 9.5, ≤ 9.4, ≤ 9.6 mg/dL, High: > 9.5, > 9.4, > 9.6 mg/dL

Total Physical Activity (MET-min/wk)), Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 1440, ≤ 666, ≤ 720, Medium: ≤ 3306, ≤ 1862.5, ≤ 2364, High: > 3306, > 1862.5, > 2364

Appendices

Supplementary Table 1: TLR4 Expression in the Lamina Propria Region by Treatment Assignment - Unadjusted[a]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Treatment Group	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Whole Region - 4 Arm														
Placebo	12	91.66	52.86	158.92	--	88.75	51.18	153.87	--	Ref.	--	--	--	--
Calcium	16	144.92	90.47	232.18	0.211	124.77	77.88	199.90	0.350	0.89	0.37	2.13	0.789	-17.24
Vitamin D	17	126.19	81.05	196.47	0.369	101.07	64.92	157.35	0.714	0.83	0.35	1.94	0.657	-22.21
Calcium + Vitamin D	18	99.08	64.44	152.35	0.824	85.13	55.37	130.91	0.906	0.89	0.38	2.06	0.778	-11.03
Whole Region - 2 Arm														
Placebo	23	97.79	61.75	154.84	--	78.17	49.37	123.79	--	Ref.	--	--	--	--
Vitamin D	19	89.09	53.73	147.73	0.784	44.93	27.10	74.51	0.109	0.63	0.27	1.48	0.282	-24.54
Top 20% of Region - 4 Arm														
Placebo	12	26.78	15.71	45.66	--	31.37	18.40	53.48	--	Ref.	--	--	--	--
Calcium	16	51.14	32.22	81.17	0.072	38.21	24.07	60.65	0.578	0.64	0.29	1.41	0.262	-17.51
Vitamin D	17	41.44	26.47	64.88	0.215	28.21	18.02	44.17	0.762	0.58	0.27	1.27	0.172	-17.81
Calcium + Vitamin D	18	29.33	18.97	45.34	0.793	23.47	15.18	36.28	0.403	0.68	0.31	1.48	0.329	-10.44
Top 20% of Region - 2 Arm														
Placebo	23	31.90	19.33	52.63	--	23.71	14.37	39.13	--	Ref.	--	--	--	--
Vitamin D	19	27.19	15.68	47.18	0.667	14.05	8.10	24.38	0.163	0.70	0.28	1.72	0.423	-4.96
Upper 40% of Region - 4 Arm														
Placebo	12	44.30	25.57	76.77	--	48.32	27.89	83.73	--	Ref.	--	--	--	--
Calcium	16	86.74	54.17	138.89	0.068	72.43	45.23	115.98	0.268	0.77	0.33	1.78	0.528	-18.33
Vitamin D	17	73.93	47.50	115.04	0.152	55.77	35.84	86.79	0.686	0.69	0.30	1.57	0.373	-22.18
Calcium + Vitamin D	18	55.40	36.05	85.14	0.524	47.39	30.83	72.84	0.956	0.78	0.35	1.77	0.552	-12.03
Upper 40% of Region - 2 Arm														
Placebo	23	56.10	35.04	89.80	--	44.71	27.93	71.56	--	Ref.	--	--	--	--
Vitamin D	19	48.85	29.11	81.98	0.692	24.29	14.48	40.77	0.086	0.62	0.27	1.45	0.265	-13.17
Lower 60% of Region - 4 Arm														
Placebo	12	40.98	21.65	77.56	--	34.94	18.46	66.13	--	Ref.	--	--	--	--
Calcium	16	46.80	27.10	80.82	0.753	42.38	24.54	73.19	0.647	1.06	0.37	3.08	0.911	1.61
Vitamin D	17	39.08	23.39	65.29	0.908	37.66	22.54	62.91	0.855	1.13	0.40	3.19	0.815	4.61
Calcium + Vitamin D	18	36.55	22.19	60.18	0.778	32.09	19.49	52.84	0.834	1.03	0.37	2.88	0.955	1.58
Lower 60% of Region - 2 Arm														
Placebo	23	36.44	22.26	59.66	--	25.65	15.67	41.98	--	Ref.	--	--	--	--
Vitamin D	19	34.07	19.81	58.59	0.854	15.91	9.25	27.37	0.195	0.66	0.25	1.76	0.399	-7.36
		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Treatment Group	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
φh - 4 Arm[e]														
Placebo	12	49.38	42.76	56.00	--	54.96	48.34	61.59	--	Ref.	--	--	--	--
Calcium	16	60.76	55.09	66.43	0.011	58.63	52.96	64.30	0.404	-7.72	-18.38	2.94	0.153	0.87
Vitamin D	17	60.27	54.95	65.60	0.013	56.84	51.51	62.16	0.661	-9.02	-19.41	1.37	0.088	0.85
Calcium + Vitamin D	18	56.90	51.72	62.07	0.079	56.38	51.20	61.56	0.737	-6.10	-16.38	4.18	0.240	0.89
φh - 2 Arm														
Placebo	23	57.95	53.68	62.22	--	58.47	54.21	62.74	--	Ref.	--	--	--	--
Vitamin D	19	55.21	50.51	59.90	0.388	55.32	50.62	60.01	0.321	-0.41	-8.24	7.41	0.915	0.99

[a]TLR4 by treatment assignment was modeled using a mixed linear model in SAS 9.4 (Cary, NC), not adjusting for any covariates

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

Supplementary Table 2: Comparison of TLR4 Expression in the Lamina Propria Region by Treatment Type - Unadjusted[a]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Treatment Group	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Whole Region														
No calcium	29	111.30	78.87	157.06	--	96.03	68.05	135.53	--	Ref.	--	--	--	--
Calcium[e]	34	117.78	85.76	161.76	0.810	101.29	73.75	139.11	0.821	1.00	0.57	1.74	0.991	-1.22
No vitamin D	51	108.71	81.91	144.29	--	92.80	69.93	123.17	--	Ref.	--	--	--	--
Vitamin D[f]	54	103.00	78.65	134.87	0.785	71.77	54.81	93.98	0.195	0.82	0.51	1.31	0.394	-15.32
Calcium only	39	114.22	82.40	158.32	--	94.02	67.83	130.32	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	37	93.82	67.39	130.61	0.402	61.32	44.04	85.37	0.071	0.79	0.45	1.41	0.425	-12.30
Top 20% of Region														
No calcium	29	34.59	24.44	48.95	--	29.48	20.83	41.72	--	Ref.	--	--	--	--
Calcium	34	38.10	27.65	52.50	0.684	29.52	21.42	40.68	0.996	0.91	0.54	1.54	0.718	-3.47
No vitamin D	51	35.50	26.54	47.47	--	29.41	22.00	39.34	--	Ref.	--	--	--	--
Vitamin D	54	31.84	24.01	42.24	0.596	20.76	15.65	27.54	0.091	0.79	0.49	1.26	0.314	-5.00
Calcium only	39	38.71	27.63	54.25	--	28.84	20.58	40.41	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	28.21	19.95	39.89	0.196	18.03	12.75	25.50	0.057	0.86	0.48	1.53	0.599	-0.30
Upper 40% of Region														
No calcium	29	60.46	42.72	85.56	--	52.71	37.25	74.60	--	Ref.	--	--	--	--
Calcium	34	67.92	49.32	93.53	0.624	57.47	41.73	79.13	0.716	0.97	0.57	1.66	0.911	-2.71
No vitamin D	51	60.79	45.59	81.07	--	52.74	39.55	70.32	--	Ref.	--	--	--	--
Vitamin D	54	58.04	44.12	76.35	0.817	39.43	29.98	51.87	0.150	0.78	0.49	1.24	0.296	-10.55
Calcium only	39	66.63	47.85	92.77	--	54.09	38.85	75.31	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	51.94	37.13	72.63	0.296	33.63	24.04	47.03	0.048	0.80	0.46	1.40	0.424	-5.77
Lower 60% of Region														
No calcium	29	39.82	26.83	59.08	--	36.57	24.64	54.26	--	Ref.	--	--	--	--
Calcium	34	40.89	28.43	58.82	0.921	36.41	25.32	52.37	0.988	0.97	0.49	1.91	0.928	-1.23
No vitamin D	51	40.39	29.52	55.28	--	32.06	23.43	43.87	--	Ref.	--	--	--	--
Vitamin D	54	36.42	27.01	49.10	0.636	26.37	19.55	35.55	0.373	0.91	0.52	1.59	0.745	-1.71
Calcium only	39	40.23	28.07	57.64	--	31.27	21.82	44.81	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	35.25	24.48	50.76	0.609	22.38	15.54	32.23	0.197	0.82	0.42	1.60	0.550	-3.91

		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Treatment Group	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
φh[h]														
No calcium	29	55.99	51.77	60.21	--	56.10	51.88	60.32	--	Ref.	--	--	--	--
Calcium	34	58.65	54.77	62.54	0.357	57.40	53.51	61.29	0.652	-1.36	-8.32	5.60	0.697	0.98
No vitamin D	51	56.89	53.85	59.92	--	57.73	54.70	60.77	--	Ref.	--	--	--	--
Vitamin D	54	57.37	54.48	60.26	0.821	56.15	53.26	59.04	0.456	-2.06	-7.14	3.02	0.423	0.96
Calcium only	39	59.06	55.85	62.27	--	58.53	55.32	61.74	--	Ref.	--	--	--	--
Vitamin D and Calcium	37	56.03	52.78	59.28	0.190	55.83	52.58	59.09	0.243	0.33	-5.47	6.14	0.910	1.02

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), not adjjusting for any covariates

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[f]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[g]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

Supplementary Table 3: TLR4 Expression in the Lamina Propria Region by Baseline Categorical Predictors Within the Upper 40% Region, Lower 60% Region, and φh of the Region[a]

		Upper 40% of Region						*Lower 60% of Region*						*φh[b]*					
		Geometric			*p-*	*Proportional*		*Geometric*			*p-*	*Proportional*					*p-*	*Proportional*	
Covariate	*n*	*Mean[c]*	*95% CI*		*value[d]*	*Difference[e]*	*p-trend[d]*	*Mean*	*95% CI*		*value*	*Difference*	*p-trend*	*Mean*	*95% CI*		*value*	*Difference*	*p-trend*
Age																			
≤ 55 years	39	59.91	42.70	84.07	Ref.	Ref.		34.61	23.04	52.00	Ref.	Ref.		59.54	54.98	64.09	Ref.	Ref.	
> 55 years, ≤ 63 years	38	55.91	41.15	75.96	0.718	-6.68%	0.615	37.30	25.81	53.91	0.745	7.77%	0.423	57.11	52.99	61.23	0.349	-4.07%	0.337
≥ 63 years	28	63.25	44.88	89.14	0.813	5.57%		46.62	30.87	70.41	0.280	34.69%		54.52	49.91	59.14	0.106	-8.42%	
Gender																			
Male	49	62.28	45.33	85.57	Ref.	Ref.		46.82	31.94	68.65	Ref.	Ref.		54.20	49.92	58.49	Ref.	Ref.	
Female	56	57.64	44.10	75.35	0.750	-7.46%		33.84	24.51	46.72	0.270	-27.73%		58.63	55.02	62.25	0.180	8.17%	
Race																			
White	83	53.33	41.79	68.06	Ref.	Ref.		34.91	25.94	46.98	Ref.	Ref.		57.24	53.85	60.63	Ref.	Ref.	
Non-White	22	88.01	57.09	135.69	0.040	65.03%		56.28	33.23	95.34	0.105	61.21%		57.24	51.22	63.26	1.000	0.00%	
BMI																			
Normal (< 25.00)	22	43.06	28.48	65.11	Ref.	Ref.		20.27	12.58	32.66	Ref.	Ref.		63.37	57.90	68.84	Ref.	Ref.	
Overweight I (25.00 - 27.49)	19	56.72	34.54	93.13	0.315	31.71%		31.23	17.62	55.33	0.173	54.07%		60.37	53.81	66.94	0.408	-4.73%	
Overweight II (27.50 - 29.99)	24	77.87	54.99	110.27	0.022	80.85%	0.334	54.57	36.53	81.51	0.001	169.23%	0.021	55.44	50.83	60.04	0.020	-12.52%	0.009
Obese I (30.00 - 32.49)	15	54.63	34.40	86.74	0.445	26.87%		40.16	23.56	68.46	0.060	98.13%		56.56	50.44	62.68	0.101	-10.74%	
Obese II (≥ 35.00)	25	60.99	42.74	87.02	0.178	41.63%		45.26	30.03	68.20	0.008	123.28%		54.20	49.50	58.91	0.009	-14.46%	
Diabetes																			
No	90	57.76	45.71	73.00	Ref.	Ref.		36.85	27.85	48.75	Ref.	Ref.		57.66	54.51	60.82	Ref.	Ref.	
Yes	15	74.21	44.45	123.91	0.342	28.48%		56.44	30.57	104.18	0.178	53.16%		54.08	47.18	60.99	0.314	-6.21%	
College graduate																			
Less than an associate's degree	39	67.41	49.50	91.80	Ref.	Ref.		43.74	30.12	63.52	Ref.	Ref.		57.56	53.35	61.76	Ref.	Ref.	
Associate's degree or higher	66	54.19	41.15	71.37	0.244	-19.61%		35.40	25.39	49.36	0.349	-19.07%		57.00	53.25	60.75	0.827	-0.96%	
Total physical activity (MET-min/wk)[f•]																			
Low	37	56.19	40.52	77.91	Ref.	Ref.		35.06	23.68	51.89	Ref.	Ref.		58.04	53.64	62.45	Ref.	Ref.	
Medium	34	59.85	42.28	84.71	0.768	6.52%	0.546	42.36	27.92	64.26	0.462	20.83%	0.551	55.99	51.30	60.67	0.477	-3.54%	0.793
High	33	63.74	45.85	88.61	0.549	13.45%		40.80	27.48	60.58	0.548	16.39%		57.27	52.83	61.71	0.785	-1.33%	
Smoking status																			
Never	61	68.98	52.33	90.93	Ref.	Ref.		44.23	31.48	62.16	Ref.	Ref.		57.73	54.00	61.47	Ref.	Ref.	
Former or occasional	36	54.90	39.70	75.91	0.241	-20.42%	0.031	32.76	21.98	48.83	0.211	-25.94%	0.268	58.59	54.21	62.98	0.744	1.49%	0.212
Current	8	35.18	19.62	63.11	0.034	-49.00%		34.57	16.84	70.99	0.523	-21.84%		49.47	41.57	57.37	0.054	-14.32%	
Regular use of NSAIDs (4/wk or more)																			
No	93	59.81	46.33	77.22	Ref.	Ref.		43.47	32.15	58.78	Ref.	Ref.		55.27	51.98	58.56	Ref.	Ref.	
Yes	12	58.30	35.06	96.95	0.929	-2.53%		24.78	13.59	45.18	0.101	-43.00%		64.93	58.37	71.48	0.003	17.48%	
Regular use of aspirin (4/wk or more)																			
No	63	66.27	51.72	84.91	Ref.	Ref.		43.87	32.53	59.16	Ref.	Ref.		56.75	53.33	60.17	Ref.	Ref.	
Yes	38	47.30	34.19	65.44	0.058	-28.62%		29.77	20.12	44.03	0.070	-32.15%		58.28	53.80	62.76	0.528	2.70%	
Any use of NSAIDs or aspirin																			
No	11	93.96	56.50	156.24	Ref.	Ref.		57.24	30.78	106.47	Ref.	Ref.		59.47	52.45	66.49	Ref.	Ref.	
Yes	94	56.73	45.24	71.13	0.051	-39.62%		37.21	28.23	49.04	0.168	-35.00%		57.01	53.88	60.13	0.484	-4.14%	
Multivitamin user																			
No	34	65.57	48.38	88.88	Ref.	Ref.		46.49	32.35	66.82	Ref.	Ref.		55.71	51.62	59.81	Ref.	Ref.	
Yes	71	54.53	40.78	72.92	0.345	-16.83%		32.91	23.27	46.55	0.140	-29.21%		58.61	54.70	62.52	0.271	5.20%	
Among women (n=56), currently on HRT[•••]																			
No	40	59.15	41.16	85.01	Ref.	Ref.		36.45	24.99	53.15	Ref.	Ref.		58.15	54.71	61.60	Ref.	Ref.	
Yes	13	72.42	36.18	144.97	0.632	22.42%		32.46	15.77	66.82	0.792	-10.94%		62.34	55.75	68.93	0.300	7.20%	
Number of adenomas removed																			
One	76	60.35	46.43	78.45	Ref.	Ref.		39.17	28.56	53.71	Ref.	Ref.		57.04	53.50	60.58	Ref.	Ref.	
More than one	29	57.79	40.58	82.30	0.831	-4.24%		37.92	24.77	58.03	0.894	-3.20%		57.65	52.88	62.42	0.822	1.08%	
Had advanced adenomas[•••]																			
No	83	59.92	46.30	77.56	Ref.	Ref.		37.47	27.47	51.11	Ref.	Ref.		58.10	54.58	61.62	Ref.	Ref.	
Yes	19	61.65	40.44	93.96	0.905	2.88%		44.98	27.08	74.70	0.525	20.05%		54.90	49.14	60.65	0.327	-5.51%	
1° family history of CRC[•••]																			
No	93	61.48	48.43	78.05	Ref.	Ref.		42.63	32.49	55.93	Ref.	Ref.		55.88	52.76	59.01	Ref.	Ref.	
Yes	9	69.42	40.01	120.43	0.676	12.90%		42.76	22.84	80.04	0.993	0.31%		59.05	51.84	66.26	0.405	5.67%	
Total energy intake (kcal/d)[hi•••]																			
Low	35	67.96	49.04	94.19	Ref.	Ref.		43.73	29.64	64.51	Ref.	Ref.		57.34	53.08	61.59	Ref.	Ref.	
Medium	35	54.77	38.09	78.76	0.316	-19.41%	0.252	29.70	19.27	45.78	0.133	-32.08%	0.607	60.54	55.81	65.28	0.253	5.59%	0.511
High	32	53.95	38.79	75.03	0.265	-20.62%		39.14	26.42	57.99	0.653	-10.48%		55.33	51.03	59.63	0.457	-3.50%	

Supplementary Table 3 (Continued)

		Upper 40% of Region						Lower 60% of Region						φh[b]					
Covariate	n	Geometric Mean[c]	95% CI		p-value[d]	Proportional Difference[e]	p-trend[d]	Geometric Mean	95% CI		p-value	Proportional Difference	p-trend	Mean	95% CI		p-value	Proportional Difference	p-trend
Total fat (gm/d)[h•••]																			
Low	35	72.92	45.61	116.60	Ref.	Ref.		50.53	28.72	88.91	Ref.	Ref.		56.33	50.05	62.61	Ref.	Ref.	
Medium	35	52.24	35.98	75.85	0.450	-28.36%	0.236	33.21	21.20	52.03	0.131	-34.27%	0.192	57.70	52.71	62.69	0.655	2.43%	0.660
High	32	52.69	31.22	88.92	0.394	-27.75%		31.84	16.96	59.80	0.371	-36.99%		58.30	51.30	65.31	0.730	3.51%	
Total calcium (mg/d)[gi]																			
Low	32	67.93	47.44	97.27	Ref.	Ref.		46.85	30.43	72.13	Ref.	Ref.		55.53	50.70	60.36	Ref.	Ref.	
Medium	32	41.06	27.73	60.79	0.056	-39.55%	0.863	23.57	14.71	37.78	0.031	-49.68%	0.616	59.68	54.40	64.96	0.236	7.49%	0.290
High	29	59.62	41.75	85.14	0.596	-12.23%		36.06	23.50	55.34	0.377	-23.03%		59.42	54.63	64.22	0.242	7.02%	
Total vitamin D (IU/d)[hi]																			
Low	30	76.01	54.77	105.47	Ref.	Ref.		58.24	40.08	84.63	Ref.	Ref.		54.83	50.16	59.49	Ref.	Ref.	
Medium	29	62.81	45.21	87.26	0.370	-17.36%	0.007	37.64	25.87	54.76	0.075	-35.38%	0.003	57.80	53.12	62.48	0.327	5.42%	0.395
High	27	38.86	26.07	57.93	0.007	-48.87%		25.02	15.87	39.45	0.003	-57.04%		57.58	51.89	63.26	0.419	5.02%	
Dietary fiber (gm/d)[i•••]																			
Low	36	50.25	35.79	70.55	Ref.	Ref.		28.24	18.88	42.25	Ref.	Ref.		60.31	55.88	64.75	Ref.	Ref.	
Medium	34	66.70	46.77	95.12	0.182	32.74%	0.283	43.66	28.65	66.55	0.085	54.61%	0.069	56.48	51.84	61.12	0.167	-6.36%	0.088
High	32	63.26	42.13	94.99	0.390	25.89%		47.29	29.18	76.63	0.108	67.45%		54.79	49.47	60.10	0.117	-9.16%	
Red/processed meats (servings/d)[i]																			
Low	28	73.26	48.11	111.56	Ref.	Ref.		47.37	28.26	79.42	Ref.	Ref.		57.87	51.56	64.19	Ref.	Ref.	
Medium	38	50.29	37.54	67.38	0.090	-31.35%	0.044	30.36	21.20	43.49	0.103	-35.91%	0.135	59.30	54.91	63.70	0.663	2.48%	0.686
High	30	46.73	33.88	64.45	0.101	-36.21%		31.42	21.16	46.63	0.221	-33.68%		55.99	51.17	60.82	0.645	-3.24%	
Fruits and vegetables (servings/d)[i•••]																			
Low	35	52.99	37.40	75.07	Ref.	Ref.		35.25	23.16	53.67	Ref.	Ref.		56.73	52.10	61.37	Ref.	Ref.	
Medium	34	55.83	39.78	78.36	0.811	5.36%	0.255	33.79	22.45	50.85	0.872	-4.17%	0.363	58.31	53.80	62.82	0.587	2.78%	0.846
High	33	69.22	48.19	99.43	0.248	30.64%		45.96	29.69	71.12	0.341	30.36%		57.22	52.40	62.04	0.873	0.87%	
Alcohol intake (drinks/d)																			
≤ 0.30 drinks	54	56.31	42.16	75.21	Ref.	Ref.		35.64	25.14	50.51	Ref.	Ref.		57.99	54.17	61.81	Ref.	Ref.	
> 0.30 drinks	51	62.24	44.76	86.56	0.620	10.55%		40.83	27.44	60.75	0.576	14.58%		56.72	52.37	61.07	0.635	-2.18%	
Serum level 25-OH deficiency																			
Deficient (< 20 ng/mL)	46	60.63	45.41	80.94	Ref.	Ref.		42.74	30.24	60.41	Ref.	Ref.		55.56	51.71	59.40	Ref.	Ref.	
Sufficient (≥ 20 ng/mL)	59	58.23	42.82	79.18	0.835	-3.95%		34.60	23.95	49.99	0.365	-19.05%		59.19	55.10	63.28	0.163	6.53%	
Serum 25-OH[i]																			
Low	36	55.04	39.89	75.94	Ref.	Ref.		37.90	25.75	55.78	Ref.	Ref.		55.97	51.69	60.24	Ref.	Ref.	
Medium	36	64.19	46.69	88.25	0.467	16.63%	0.653	42.81	29.21	62.74	0.631	12.97%	0.495	56.76	52.53	60.99	0.776	1.42%	0.034
High	33	59.86	41.97	85.36	0.691	8.76%		34.12	22.28	52.24	0.679	-9.98%		60.18	55.47	64.90	0.136	7.53%	
Serum Ca^{2+}[i]																			
Low	42	56.74	41.48	77.63	Ref.	Ref.		44.77	30.70	65.30	Ref.	Ref.		52.51	48.48	56.54	Ref.	Ref.	
Medium	37	69.98	51.41	95.26	0.309	23.33%	0.383	39.50	27.25	57.26	0.612	-11.78%	0.095	60.76	56.80	64.72	0.003	15.71%	0.027
High	36	46.52	32.05	67.52	0.351	-18.02%		27.94	17.84	43.76	0.069	-37.59%		59.27	54.48	64.06	0.015	12.87%	

Abbreviations: BMI = Body Mass Index, NSAID = Non-steroidal anti-inflammatory drug, CRC = Colorectal cancer, HRT = Hormone replacement therapy, IU = International units, kcal = Kilocalories, d = Day, wk = Week

● denotes a missing patient per symbol

[a]TLR4 expression was modeled using a generalized linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), batch, and study center. All dietary intakes are also controlled for total calorie intake

[b]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

[c]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[d]p-values are comparing selected categorical value to its reference value. P-trend is comparing dose-response effect by increasing categorization level for variables with more than two categories

[e]Calculated as follows: [(Category OD - Reference OD) / Reference OD x 100%]

[f]MET: Metabolic equivalent of task

[g]Dietary calcium and supplemental calcium, missing information from 12 patients (● x 12)

[h]Dietary vitamin D and supplemental vitamin D, missing information from 19 patients (● x 19)

[i]Categorized by tertiles, including sex-specific and study arm-specific considerations when applicable

Total Energy Intake, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 1328.4, ≤ 899.1, ≤ 946.8 kcal/d, Medium: ≤ 1701, ≤ 1384.8, ≤ 1570.6 kcal/d, High: > 1701, > 1384.8, > 1570.6 kcal/d

Total Fat, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 46.4, ≤ 36.9, ≤ 36.2 gm/d, Medium: ≤ 79.5, ≤ 58.9, ≤ 62.5 gm/d, High: > 79.5, > 58.9, > 62.5 gm/d

Total Calcium, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 607.0, ≤ 482.5, ≤ 826.3 mg/d, Medium: ≤ 823.1, ≤ 644.7, ≤ 1270.8 mg/d High: > 823.1, > 644.7, > 1270.8 mg/d

Total Vitamin D, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 451.21, ≤ 86.60, ≤ 480.28 IU/d, Medium: ≤ 556.87, ≤ 171.69, ≤ 661.57 IU/d, High: > 556.87, > 171.69, > 661.57 IU/d

Dietary Fiber, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 11.1, ≤ 10.7, ≤ 12.1 gm/d, Medium: ≤ 16.5, ≤ 14.4, ≤ 16.6 gm/d, High: > 16.5, > 14.4, > 16.6 gm/d

Red/processed meats, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 0.8, ≤ 0.3, ≤ 0.3 servings/d, Medium: ≤ 1.4, ≤ 0.6, ≤ 0.8 servings/d, High: > 1.4, > 0.6, > 0.8 servings/d

Fruits and vegetables, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 3.2, ≤ 3.5, ≤ 3.9 servings/d, Medium: ≤ 4.5, ≤ 4.9, ≤ 6.3 servings/d, High: > 4.5, > 4.9, > 6.3 servings/d

Serum 25-OH, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 18.45, ≤ 14.97, ≤ 19.09 ng/mL, Medium: ≤ 26.11, ≤ 23.81, ≤ 30.86 ng/mL, High: > 26.11, > 23.81, > 30.86 ng/mL

Serum Ca^{2+}, Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 9.2, ≤ 9.0, ≤ 9.3 mg/dL, Medium: ≤ 9.5, ≤ 9.4, ≤ 9.6 mg/dL, High: > 9.5, > 9.4, > 9.6 mg/dL

Total Physical Activity (MET-min/wk)), Low (Male/Female (4-Arm)/Female (2-Arm): ≤ 1440, ≤ 666, ≤ 720, Medium: ≤ 3306, ≤ 1862.5, ≤ 2364, High: > 3306, > 1862.5, > 2364

Supplementary Table 4: Comparison of TLR4 Expression in the Lamina Propria Region by Treatment Type Stratified by Frequency of NSAID Usage[a]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Frequency of NSAID use	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean[d]	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Use of NSAIDs 4 or more times a week														
Whole Region														
No calcium	4	120.46	45.03	322.21	--	166.05	62.08	444.21	--	Ref.	--	--	--	--
Calcium[e]	4	88.56	34.49	227.35	0.599	78.37	30.53	201.22	0.225	0.64	0.15	2.84	0.493	-55.78
No vitamin D	6	100.09	43.17	232.06	--	59.77	25.78	138.57	--	Ref.	--	--	--	--
Vitamin D[f]	6	57.98	23.17	145.07	0.358	67.70	27.06	169.39	0.830	1.96	0.65	5.90	0.206	50.04
Calcium only	5	107.05	62.04	184.71	--	66.86	38.75	115.36	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	3	39.89	18.54	85.85	0.046	27.70	12.87	59.60	0.066	1.11	0.23	5.37	0.875	27.99
Top 20% of Region														
No calcium	4	55.01	18.40	164.51	--	58.17	19.45	173.96	--	Ref.	--	--	--	--
Calcium	4	36.23	12.78	102.69	0.522	27.96	9.86	79.25	0.279	0.73	0.21	2.58	0.564	-11.43
No vitamin D	6	43.94	17.95	107.59	--	22.50	9.19	55.08	--	Ref.	--	--	--	--
Vitamin D	6	22.72	8.50	60.72	0.302	18.44	6.90	49.28	0.750	1.59	0.60	4.22	0.319	17.17
Calcium only	5	46.03	27.29	77.66	--	24.53	14.54	41.39	--	Ref.	--	--	--	--
Vitamin D and Calcium	3	15.58	7.03	34.55	0.037	7.13	3.21	15.80	0.023	0.86	0.25	3.00	0.775	13.05
Upper 40% of Region														
No calcium	4	82.34	28.22	240.21	--	100.33	34.39	292.69	--	Ref.	--	--	--	--
Calcium	4	59.64	21.50	165.41	0.611	47.12	16.99	130.69	0.256	0.65	0.17	2.46	0.457	-30.52
No vitamin D	6	66.43	26.81	164.58	--	37.21	15.02	92.20	--	Ref.	--	--	--	--
Vitamin D	6	36.72	13.61	99.13	0.356	32.51	12.04	87.74	0.830	1.58	0.53	4.70	0.372	24.99
Calcium only	5	72.30	39.30	133.02	--	41.74	22.68	76.79	--	Ref.	--	--	--	--
Vitamin D and Calcium	3	24.28	9.52	61.92	0.062	11.46	4.49	29.23	0.035	0.82	0.20	3.38	0.741	17.75
Lower 60% of Region														
No calcium	4	26.53	9.24	76.20	--	60.01	20.89	172.36	--	Ref.	--	--	--	--
Calcium	4	24.85	8.94	69.12	0.917	25.95	9.33	72.18	0.211	0.46	0.06	3.61	0.393	-32.38
No vitamin D	6	27.79	11.68	66.11	--	18.62	7.83	44.30	--	Ref.	--	--	--	--
Vitamin D	6	15.43	6.07	39.23	0.333	29.24	11.50	74.34	0.453	2.83	0.67	11.87	0.138	22.98
Calcium only	5	30.34	15.49	59.46	--	21.18	10.81	41.50	--	Ref.	--	--	--	--
Vitamin D and Calcium	3	11.24	4.42	28.61	0.084	11.11	4.37	28.28	0.227	1.42	0.20	10.28	0.682	9.04
Use of NSAIDs less than 4 times a week														
Whole Region														
No calcium	25	124.33	81.85	188.86	--	99.22	65.32	150.72	--	Ref.	--	--	--	--
Calcium	30	143.97	96.22	215.42	0.570	123.33	82.42	184.55	0.401	1.07	0.58	1.99	0.819	4.47
No vitamin D	45	124.55	89.36	173.57	--	111.77	80.20	155.77	--	Ref.	--	--	--	--
Vitamin D	48	119.25	87.31	162.86	0.836	77.90	57.03	106.39	0.088	0.73	0.43	1.22	0.225	-28.57
Calcium only	34	157.97	103.85	240.28	--	135.59	89.14	206.25	--	Ref.	--	--	--	--
Vitamin D and Calcium	34	121.90	81.63	182.02	0.292	79.24	53.07	118.33	0.031	0.76	0.40	1.42	0.381	-20.27
Top 20% of Region														
No calcium	25	36.30	23.53	56.00	--	29.88	19.37	46.11	--	Ref.	--	--	--	--
Calcium	30	44.14	29.00	67.17	0.461	34.21	22.48	52.08	0.609	0.94	0.52	1.70	0.839	-3.51
No vitamin D	45	38.64	27.32	54.65	--	34.14	24.14	48.29	--	Ref.	--	--	--	--
Vitamin D	48	34.98	25.15	48.65	0.649	22.19	15.95	30.87	0.051	0.72	0.43	1.21	0.207	-8.29
Calcium only	34	50.63	32.56	78.74	--	39.62	25.48	61.61	--	Ref.	--	--	--	--
Vitamin D and Calcium	34	35.46	23.17	54.27	0.167	23.34	15.25	35.73	0.042	0.84	0.45	1.59	0.590	-1.10
Upper 40% of Region														
No calcium	25	66.78	43.60	102.29	--	55.07	35.95	84.36	--	Ref.	--	--	--	--
Calcium	30	82.76	54.82	124.95	0.415	70.69	46.82	106.72	0.344	1.04	0.57	1.90	0.908	-0.36
No vitamin D	45	68.83	49.04	96.61	--	63.46	45.21	89.08	--	Ref.	--	--	--	--
Vitamin D	48	66.63	48.46	91.60	0.879	43.79	31.85	60.21	0.085	0.71	0.43	1.18	0.188	-17.46
Calcium only	34	91.86	59.96	140.74	--	78.52	51.25	120.31	--	Ref.	--	--	--	--
Vitamin D and Calcium	34	67.70	45.04	101.76	0.220	45.07	29.99	67.74	0.028	0.78	0.42	1.44	0.421	-9.29
Lower 60% of Region														
No calcium	25	47.33	29.86	75.02	--	37.40	23.60	59.28	--	Ref.	--	--	--	--
Calcium	30	50.89	32.70	79.19	0.801	44.32	28.48	68.98	0.554	1.10	0.53	2.30	0.792	3.37
No vitamin D	45	47.84	33.24	68.83	--	38.88	27.02	55.94	--	Ref.	--	--	--	--
Vitamin D	48	43.88	31.17	61.76	0.710	28.17	20.01	39.65	0.167	0.79	0.43	1.44	0.440	-6.75
Calcium only	34	58.08	36.54	92.31	--	45.89	28.87	72.94	--	Ref.	--	--	--	--
Vitamin D and Calcium	34	46.97	30.14	73.20	0.439	28.68	18.41	44.70	0.089	0.77	0.37	1.61	0.487	-6.11

		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Frequency of NSAID use	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Use of NSAIDs 4 or more times a week														
φh[h]														
No calcium	4	68.41	57.59	79.23	--	61.28	50.46	72.10	--	Ref.	--	--	--	--
Calcium	4	67.51	57.14	77.89	0.888	60.61	50.23	70.98	0.916	0.23	-16.45	16.90	0.975	1.00
No vitamin D	6	66.37	59.26	73.48	--	62.69	55.58	69.80	--	Ref.	--	--	--	--
Vitamin D	6	63.97	56.29	71.64	0.624	49.78	42.11	57.46	0.022	-10.51	-21.83	0.81	0.066	0.82
Calcium only	5	67.16	56.73	77.58	--	62.59	52.16	73.01	--	Ref.	--	--	--	--
Vitamin D and Calcium	3	61.35	44.17	78.53	0.530	42.75	25.57	59.93	0.063	-14.03	-31.25	3.20	0.093	0.75
Use of NSAIDs less than 4 times a week														
φh														
No calcium	25	55.52	50.74	60.30	--	56.84	52.06	61.61	--	Ref.	--	--	--	--
Calcium	30	58.55	53.96	63.13	0.311	58.07	53.49	62.66	0.677	-1.79	-9.49	5.92	0.643	0.97
No vitamin D	45	56.32	52.74	59.89	--	57.79	54.22	61.37	--	Ref.	--	--	--	--
Vitamin D	48	56.97	53.61	60.32	0.774	57.37	54.02	60.73	0.852	-1.07	-6.48	4.34	0.695	0.98
Calcium only	34	58.78	54.46	63.11	--	58.87	54.55	63.19	--	Ref.	--	--	--	--
Vitamin D and Calcium	34	56.11	51.99	60.24	0.286	57.54	53.41	61.67	0.594	1.34	-4.67	7.35	0.657	1.02

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[f]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[g]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

Supplementary Table 5: Comparison of TLR4 Expression in the Lamina Propria Region by Treatment Type Stratified by Frequency of Aspirin Usage[a]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Frequency of aspirin use	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Use of aspirin 4 or more times a week														
Whole Region														
No calcium	10	114.78	58.01	227.08	--	106.92	54.04	211.56	--	Ref.	--	--	--	--
Calcium[e]	17	145.91	77.54	274.60	0.536	118.40	62.92	222.81	0.792	0.87	3.09	2.34	0.776	-19.65
No vitamin D	21	106.70	65.53	173.75	--	104.91	64.43	170.84	--	Ref.	--	--	--	--
Vitamin D[f]	17	95.81	56.07	163.73	0.737	90.59	53.01	154.79	0.647	0.96	0.41	2.25	0.926	-3.44
Calcium only	16	140.64	73.05	270.78	--	139.14	72.27	267.90	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	12	90.67	41.50	198.09	0.249	87.50	40.05	191.16	0.224	0.98	0.35	2.71	0.961	-1.67
Top 20% of Region														
No calcium	10	33.81	16.11	70.99	--	28.84	13.74	60.55	--	Ref.	--	--	--	--
Calcium	17	43.42	21.78	86.57	0.552	34.32	17.21	68.42	0.679	0.93	0.33	2.63	0.882	-4.13
No vitamin D	21	31.73	18.99	53.03	--	34.44	20.61	57.56	--	Ref.	--	--	--	--
Vitamin D	17	29.89	17.00	52.55	0.858	23.98	13.64	42.16	0.286	0.74	0.30	1.79	0.494	-8.62
Calcium only	16	43.25	22.37	83.60	--	43.85	22.68	84.76	--	Ref.	--	--	--	--
Vitamin D and Calcium	12	24.92	11.35	54.69	0.155	23.59	10.75	51.78	0.112	0.93	0.33	2.66	0.894	-1.92
Upper 40% of Region														
No calcium	10	63.90	30.66	133.18	--	55.63	26.69	115.94		Ref.	--	--	--	--
Calcium	17	83.77	42.31	165.87	0.516	67.53	34.10	133.70	0.641	0.93	0.33	2.61	0.880	-7.98
No vitamin D	21	59.19	35.19	99.55	--	60.56	36.01	101.85	--	Ref.	--	--	--	--
Vitamin D	17	55.47	31.33	98.22	0.849	47.74	26.96	84.52	0.486	0.84	0.34	2.06	0.697	-9.10
Calcium only	16	80.78	40.83	159.80	--	82.23	41.57	162.67	--	Ref.	--	--	--	--
Vitamin D and Calcium	12	50.16	22.24	113.17	0.231	46.25	20.50	104.36	0.151	0.91	0.31	2.65	0.851	-5.36
Lower 60% of Region														
No calcium	10	39.38	19.73	78.60	--	41.97	21.03	83.76	--	Ref.	--	--	--	--
Calcium	17	53.89	28.55	101.71	0.428	43.10	22.84	81.35	0.946	0.75	0.26	2.15	0.579	-13.37
No vitamin D	21	40.48	24.78	66.14	--	36.35	22.26	59.38	--	Ref.	--	--	--	--
Vitamin D	17	33.32	19.43	57.15	0.548	35.49	20.69	60.86	0.941	1.19	0.49	2.84	0.695	6.30
Calcium only	16	53.58	27.53	104.27	--	48.84	25.09	95.06	--	Ref.	--	--	--	--
Vitamin D and Calcium	12	38.08	17.20	84.33	0.372	37.00	16.71	81.92	0.466	1.07	0.39	2.91	0.897	3.65
Use of aspirin less than 4 times a week														
Whole Region														
No calcium	19	123.25	78.34	193.89	--	101.91	64.77	160.34	--	Ref.	--	--	--	--
Calcium	17	113.74	71.16	181.78	0.798	104.05	65.10	166.30	0.947	1.11	0.55	2.24	0.773	11.65
No vitamin D	30	133.86	88.01	203.61	--	102.79	67.58	156.35	--	Ref.	--	--	--	--
Vitamin D	37	115.45	82.19	162.15	0.576	69.92	49.77	98.20	0.148	0.79	0.44	1.40	0.414	-14.46
Calcium only	23	144.21	83.84	248.07	--	103.86	60.38	178.64	--	Ref.	--	--	--	--
Vitamin D and Calcium	25	120.80	77.10	189.26	0.583	65.48	41.80	102.60	0.157	0.75	0.37	1.53	0.425	-14.95
Top 20% of Region														
No calcium	19	40.20	25.50	63.36	--	34.24	21.72	53.97	--	Ref.	--	--	--	--
Calcium	17	39.39	24.70	62.81	0.948	29.92	18.76	47.71	0.662	0.89	0.50	1.60	0.693	-3.51
No vitamin D	30	46.28	29.99	71.41	--	31.75	20.58	48.98	--	Ref.	--	--	--	--
Vitamin D	37	35.36	24.61	50.81	0.328	20.96	14.59	30.12	0.133	0.86	0.50	1.51	0.601	0.13
Calcium only	23	50.01	28.13	88.91	--	30.07	16.91	53.45	--	Ref.	--	--	--	--
Vitamin D and Calcium	25	37.36	22.94	60.81	0.396	19.77	12.15	32.19	0.224	0.88	0.44	1.77	0.716	2.36
Upper 40% of Region														
No calcium	19	68.35	43.91	106.41	--	59.66	38.32	92.87	--	Ref.	--	--	--	--
Calcium	17	68.53	43.38	108.23	0.994	61.04	38.64	96.41	0.940	1.02	0.55	1.89	0.947	1.21
No vitamin D	30	76.77	50.18	117.44	--	58.84	38.46	90.01	--	Ref.	--	--	--	--
Vitamin D	37	65.01	46.15	91.60	0.534	39.63	28.12	55.83	0.142	0.80	0.47	1.36	0.396	-7.46
Calcium only	23	87.63	50.32	152.58	--	60.34	34.65	105.06	--	Ref.	--	--	--	--
Vitamin D and Calcium	25	68.38	43.36	107.83	0.449	37.36	23.69	58.92	0.147	0.79	0.41	1.53	0.482	-3.73
Lower 60% of Region														
No calcium	19	43.09	24.92	74.52	--	36.43	21.06	63.00	--	Ref.	--	--	--	--
Calcium	17	35.56	20.14	62.78	0.614	35.48	20.10	62.65	0.945	1.18	0.45	3.10	0.728	6.59
No vitamin D	30	48.38	30.11	77.70	--	35.00	21.79	56.22	--	Ref.	--	--	--	--
Vitamin D	37	40.35	27.40	59.42	0.546	24.46	16.61	36.03	0.236	0.84	0.40	1.76	0.636	-2.51
Calcium only	23	48.58	26.66	88.52	--	33.63	18.46	61.28	--	Ref.	--	--	--	--
Vitamin D and Calcium	25	43.36	26.19	71.79	0.754	22.45	13.56	37.17	0.269	0.75	0.30	1.87	0.527	-5.97

		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Frequency of aspirin use	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
Use of aspirin 4 or more times a week														
φh[h]														
No calcium	10	57.63	50.49	64.77	--	53.58	46.44	60.72	--	Ref.	--	--	--	--
Calcium	17	58.11	51.54	64.68	0.906	57.65	51.07	64.22	0.322	3.59	-7.21	14.38	0.500	1.07
No vitamin D	21	56.38	51.69	61.06	--	58.43	53.74	63.11	--	Ref.	--	--	--	--
Vitamin D	17	59.04	53.89	64.18	0.388	53.95	48.81	59.10	0.150	-7.13	-15.12	0.85	0.079	0.88
Calcium only	16	58.05	52.55	63.55	--	59.81	54.31	65.31	--	Ref.	--	--	--	--
Vitamin D and Calcium	12	55.91	49.34	62.47	0.498	53.32	46.76	59.88	0.047	-4.35	-12.79	4.10	0.300	0.93
Use of aspirin less than 4 times a week														
φh														
No calcium	19	56.96	51.25	62.66	--	59.38	53.67	65.08	--	Ref.	--	--	--	--
Calcium	17	61.38	55.46	67.29	0.269	59.28	53.37	65.20	0.982	-4.51	-14.25	5.23	0.353	0.93
No vitamin D	30	58.28	53.61	62.96	--	58.22	53.55	62.90	--	Ref.	--	--	--	--
Vitamin D	37	57.32	53.53	61.12	0.746	57.88	54.09	61.68	0.909	0.62	-6.09	7.33	0.854	1.01
Calcium only	23	61.31	55.73	66.90	--	59.12	53.53	64.71	--	Ref.	--	--	--	--
Vitamin D and Calcium	25	57.27	52.60	61.93	0.232	58.22	53.56	62.88	0.788	3.14	-4.88	11.17	0.434	1.05

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[f]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[g]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

Supplementary Table 6: Comparison of TLR4 Expression in Selected Lamina Propria Region by Treatment Type Stratified by NSAID and/or Aspirin Usage[a]

		Baseline				*1-Yr Follow-Up*				*Relative Tx[b]*				*Absolute Tx[c]*
Frequency of NSAID/aspirin use	*n*	*Geometric Mean[d]*	*95% CI*		*p-value*	*Geometric Mean*	*95% CI*		*p-value*	*Treatment Effect*	*95% CI*		*p-value*	*Treatment Effect*
Regular use of NSAIDs or aspirin[e]														
Whole Region														
No calcium	14	100.59	61.80	163.73	--	105.78	64.99	172.17	--	Ref.	--	--	--	--
Calcium[e]	21	131.04	85.38	201.12	0.395	110.10	71.74	168.98	0.897	0.80	0.36	1.77	0.570	-26.13
No vitamin D	29	109.21	74.70	159.67	--	98.73	67.53	144.34	--	Ref.	--	--	--	--
Vitamin D[f]	23	88.23	57.87	134.50	0.440	84.69	55.55	129.11	0.578	1.06	0.55	2.07	0.857	6.95
Calcium only	22	127.75	79.32	205.78	--	116.49	72.33	187.64	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	16	88.28	50.06	155.66	0.277	75.31	42.71	132.79	0.201	0.94	0.42	2.08	0.867	-1.71
Top 20% of Region														
No calcium	14	34.95	27.16	58.92	--	32.51	19.28	54.81	--	Ref.	--	--	--	--
Calcium	21	44.82	28.26	71.09	0.455	36.43	22.97	57.78	0.731	0.87	0.39	1.97	0.738	-5.95
No vitamin D	29	36.56	24.61	54.33	--	34.42	23.17	51.15	--	Ref.	--	--	--	--
Vitamin D	23	30.26	19.49	46.96	0.511	23.97	15.44	37.20	0.211	0.84	0.43	1.66	0.612	-4.15
Calcium only	22	44.37	27.38	71.90	--	39.71	24.50	64.35	--	Ref.	--	--	--	--
Vitamin D and Calcium	16	28.20	15.88	50.09	0.190	22.44	12.63	39.85	0.101	0.89	0.40	1.99	0.769	-1.10
Non-regular use of NSAIDs or aspirin[e]														
Whole Region														
No calcium	22	147.94	82.23	266.16	--	104.74	58.22	188.44	--	Ref.	--	--	--	--
Calcium[e]	31	119.10	63.77	222.45	0.569	106.69	57.12	199.26	0.961	1.27	0.55	2.92	0.568	30.78
No vitamin D	15	137.57	80.67	234.56	--	108.07	63.38	184.29	--	Ref.	--	--	--	--
Vitamin D[f]	13	135.54	90.88	202.19	0.961	74.47	49.92	111.09	0.225	0.70	0.35	1.40	0.307	-31.58
Calcium only	17	155.43	76.85	314.32	--	111.15	54.96	224.80	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	21	131.87	77.45	224.55	0.649	70.36	41.32	119.81	0.210	0.75	0.31	1.79	0.501	-17.23
Top 20% of Region														
No calcium	22	42.07	23.28	76.02	--	33.04	18.29	59.70	--	Ref.	--	--	--	--
Calcium	31	37.31	20.00	69.60	0.746	26.75	14.34	49.91	0.570	0.91	0.45	1.85	0.793	-1.53
No vitamin D	15	43.37	24.76	75.94	--	30.37	17.34	53.18	--	Ref.	--	--	--	--
Vitamin D	13	37.72	24.41	58.28	0.661	21.29	13.78	32.90	0.267	0.81	0.41	1.59	0.527	-3.43
Calcium only	17	49.91	23.40	106.44	--	29.32	13.75	62.53	--	Ref.	--	--	--	--
Vitamin D and Calcium	21	37.43	20.83	67.25	0.456	20.25	11.27	36.38	0.338	0.92	0.39	2.20	0.849	3.41

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Regular use of NSAIDs/aspirin was dichotomized as usage more or less than 2.07 times a week

[f]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[g]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

Supplementary Table 7: Comparison of TLR4 Expression in the Lamina Propria Region by Treatment Type Stratified by High or Low Calcium Intake[aj]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Calcium Intake	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
High (> 50% median total calcium intake)														
Whole Region														
No calcium	20	89.95	44.62	181.36	--	85.76	42.53	172.90	--	Ref.	--	--	--	--
Calcium[e]	25	129.18	74.98	222.56	0.372	124.90	72.49	215.19	0.354	1.01	0.41	2.54	0.975	-0.08
No vitamin D	10	96.89	59.94	156.62	--	105.67	65.37	170.80	--	Ref.	--	--	--	--
Vitamin D[f]	18	110.91	73.25	167.92	0.657	59.46	39.27	90.03	0.063	0.49	0.23	1.07	0.072	-60.22
Calcium only	17	119.80	76.02	188.80	--	118.05	74.91	186.05	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	18	118.04	75.55	184.42	0.961	56.93	36.44	88.95	0.020	0.49	0.20	1.18	0.108	-59.35
Top 20% of Region														
No calcium	20	28.92	14.02	59.62	--	22.89	11.10	47.20	--	Ref.	--	--	--	--
Calcium	25	43.64	24.89	76.52	0.325	43.93	25.06	77.04	0.124	1.27	0.50	3.26	0.604	6.32
No vitamin D	10	32.46	19.71	53.45	--	36.63	22.24	60.32	--	Ref.	--	--	--	--
Vitamin D	18	34.35	22.35	52.80	0.857	17.28	11.24	26.56	0.020	0.45	0.21	0.96	0.039	-21.25
Calcium only	17	40.90	25.90	64.61	--	39.92	25.28	63.05	--	Ref.	--	--	--	--
Vitamin D and Calcium	18	34.44	21.99	53.94	0.568	17.67	11.28	27.67	0.010	0.53	0.22	1.24	0.138	-15.80
Upper 40% of Region														
No calcium	20	51.84	25.45	105.60	--	46.49	22.82	94.69	--	Ref.	--	--	--	--
Calcium	25	77.72	44.74	135.02	0.325	78.34	45.10	136.09	0.207	1.12	0.45	2.80	0.794	5.98
No vitamin D	10	56.14	34.19	92.19	--	63.99	38.97	105.09	--	Ref.	--	--	--	--
Vitamin D	18	63.79	41.59	97.85	0.683	32.57	21.23	49.96	0.035	0.45	0.21	0.97	0.042	-39.07
Calcium only	17	70.26	44.03	112.13	--	70.41	44.12	112.37	--	Ref.	--	--	--	--
Vitamin D and Calcium	18	65.05	41.12	102.90	0.802	31.36	19.82	49.62	0.012	0.48	0.20	1.16	0.101	-33.83
Lower 60% of Region														
No calcium	20	30.93	14.64	65.34		31.88	15.09	67.35		Ref.	--	--	--	--
Calcium	25	43.98	24.67	78.40	0.418	38.44	21.56	68.53	0.665	0.85	0.28	2.53	0.759	-6.49
No vitamin D	10	34.68	20.40	58.94	--	33.85	19.92	57.53	--	Ref.	--	--	--	--
Vitamin D	18	39.50	24.96	62.53	0.699	21.30	13.45	33.71	0.173	0.55	0.22	1.37	0.193	-17.38
Calcium only	17	42.76	25.44	71.87	--	39.50	23.50	66.40	--	Ref.	--	--	--	--
Vitamin D and Calcium	18	46.45	27.91	77.30	0.809	20.31	12.20	33.80	0.058	0.47	0.17	1.28	0.136	-22.88
Low (≤ 50% median total calcium intake)														
Whole Region														
No calcium	22	121.63	81.38	181.78	--	104.62	69.99	156.35	--	Ref.	--	--	--	--
Calcium	26	108.79	69.33	170.70	0.695	103.93	66.23	163.07	0.982	1.11	0.53	2.34	0.774	12.16
No vitamin D	16	112.38	73.52	171.79	--	104.47	68.34	159.72	--	Ref.	--	--	--	--
Vitamin D	14	107.98	73.97	157.62	0.885	111.09	76.10	162.16	0.825	1.11	0.61	2.01	0.733	11.02
Calcium only	15	130.32	64.00	265.36	--	141.17	69.33	287.46	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	98.62	53.66	181.25	0.496	101.10	55.01	185.81	0.415	0.95	0.44	2.03	0.883	-8.38
Top 20% of Region														
No calcium	22	39.56	26.50	59.06	--	34.81	23.32	51.96	--	Ref.	--	--	--	--
Calcium	26	35.53	22.63	55.77	0.698	24.85	15.83	39.01	0.229	0.80	0.45	1.41	0.420	-5.92
No vitamin D	16	40.46	26.96	60.70	--	33.07	22.04	49.62	--	Ref.	--	--	--	--
Vitamin D	14	33.00	22.61	48.17	0.444	31.67	21.70	46.23	0.871	1.17	0.70	1.96	0.532	6.05
Calcium only	15	44.04	22.09	87.82	--	38.48	19.30	76.73	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	31.71	17.18	58.52	0.403	29.15	15.80	53.80	0.479	1.05	0.52	2.13	0.884	3.01
Upper 40% of Region														
No calcium	22	67.57	45.99	99.26	--	60.29	41.04	88.56	--	Ref.	--	--	--	--
Calcium	26	64.50	41.81	99.51	0.864	57.07	36.99	88.04	0.839	0.99	0.53	1.87	0.978	-0.16
No vitamin D	16	66.26	43.75	100.33	--	57.62	38.05	87.25	--	Ref.	--	--	--	--
Vitamin D	14	60.54	41.81	87.66	0.739	62.70	43.30	90.79	0.755	1.19	0.70	2.03	0.514	10.80
Calcium only	15	81.11	40.33	163.12	--	77.87	38.72	156.60	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	57.43	31.61	104.34	0.389	58.80	32.36	106.83	0.483	1.07	0.53	2.15	0.853	4.60
Lower 60% of Region														
No calcium	22	40.55	23.91	68.78	--	38.24	22.55	64.86	--	Ref.	--	--	--	--
Calcium	26	33.83	18.74	61.08	0.628	39.32	21.78	70.99	0.940	1.23	0.44	3.42	0.677	7.80
No vitamin D	16	37.50	22.91	61.39	--	38.40	23.46	62.86	--	Ref.	--	--	--	--
Vitamin D	14	36.81	23.74	57.08	0.954	40.40	26.05	62.65	0.875	1.07	0.50	2.30	0.855	2.69
Calcium only	15	40.40	18.16	89.90	--	49.54	22.26	110.23	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	33.21	16.71	66.01	0.672	35.06	17.64	69.67	0.457	0.86	0.33	2.27	0.755	-7.29

		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Calcium Intake	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
High (> 50% median total calcium intake)														
φh[i]														
No calcium	20	59.39	53.10	65.68	--	56.67	50.38	62.96	--	Ref.	--	--	--	--
Calcium	25	60.89	56.09	65.69	0.686	62.86	58.06	67.66	0.104	4.69	-7.24	16.62	0.427	1.08
No vitamin D	10	58.85	53.59	64.12	--	61.05	55.79	66.31	--	Ref.	--	--	--	--
Vitamin D	18	58.27	53.72	62.81	0.859	56.73	52.18	61.27	0.197	-3.73	-12.03	4.56	0.369	0.94
Calcium only	17	59.39	54.08	64.70	--	60.29	54.98	65.60	--	Ref.	--	--	--	--
Vitamin D and Calcium	18	55.53	50.30	60.75	0.267	56.49	51.26	61.72	0.274	0.06	-8.37	8.50	0.988	1.00
Low (≤ 50% median total calcium intake)														
φh														
No calcium	22	57.26	50.99	63.52	--	58.07	51.81	64.33	--	Ref.	--	--	--	--
Calcium	26	60.52	53.44	67.59	0.461	55.72	48.65	62.80	0.595	-5.61	-15.42	4.21	0.251	0.91
No vitamin D	16	59.84	55.06	64.63	--	55.89	51.11	60.67	--	Ref.	--	--	--	--
Vitamin D	14	57.43	53.17	61.69	0.443	56.98	52.72	61.24	0.728	3.50	-3.77	10.77	0.337	1.06
Calcium only	15	62.67	56.24	69.11	--	55.97	49.54	62.41	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	59.09	53.54	64.64	0.346	58.55	52.99	64.10	0.497	6.16	-2.98	15.30	0.179	1.11

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Total calcium intake (calcium in the diet and supplemental calcium) was dichotomized into sex-specific high/low categories

[f]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[g]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[i]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

[j]Total calcium was categorized as a sex-specific binomial variable dividing total calcium intake by the median. Median for males was 743.4 mg/d, females (4-arm) was 598.6 mg/d, females (2-arm) was 1099.1 mg/d

Supplementary Table 8: Comparison of TLR4 Expression in the Lamina Propria Region by Treatment Type Stratified by High or Low Vitamin D Intake[aj]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Vitamin D Intake	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
High (> 50% median vitamin D intake)														
Whole Region														
No calcium	21	85.28	37.88	192.00	--	75.21	33.40	169.36	--	Ref.	--	--	--	--
Calcium[e]	21	126.94	75.13	214.46	0.330	147.04	87.03	248.41	0.107	1.31	0.62	2.78	0.461	30.17
No vitamin D	8	95.60	60.26	151.68	--	120.07	75.68	190.51	--	Ref.	--	--	--	--
Vitamin D[f]	19	109.84	68.42	176.32	0.649	87.37	54.42	140.25	0.300	0.63	0.34	1.19	0.152	-46.94
Calcium only	18	106.01	67.40	166.70	--	128.51	81.72	202.09	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	16	97.85	61.46	155.77	0.788	83.78	52.63	133.38	0.158	0.71	0.35	1.44	0.328	-36.57
Top 20% of Region														
No calcium	21	32.13	13.39	77.08	--	23.30	9.71	55.90	--	Ref.	--	--	--	--
Calcium	21	46.39	26.35	81.67	0.406	52.23	29.67	91.95	0.075	1.55	0.63	3.81	0.323	14.66
No vitamin D	8	34.27	20.66	56.84	--	42.18	25.43	69.94	--	Ref.	--	--	--	--
Vitamin D	19	35.89	21.36	60.29	0.890	25.70	15.30	43.18	0.144	0.58	0.29	1.16	0.121	-18.09
Calcium only	18	37.98	23.56	61.24	--	43.59	27.03	70.28	--	Ref.	--	--	--	--
Vitamin D and Calcium	16	30.29	18.55	49.44	0.472	25.81	15.81	42.14	0.102	0.74	0.36	1.53	0.406	-10.08
Upper 40% of Region														
No calcium	21	52.65	22.63	122.46	--	42.94	18.46	99.88	--	Ref.	--	--	--	--
Calcium	21	78.67	45.60	135.75	0.345	91.72	53.15	158.25	0.081	1.43	0.64	3.18	0.367	22.75
No vitamin D	8	57.52	35.23	93.91	--	73.35	44.93	119.76	--	Ref.	--	--	--	--
Vitamin D	19	63.68	38.51	105.29	0.753	48.13	29.11	79.58	0.197	0.59	0.31	1.14	0.114	-31.39
Calcium only	18	64.65	40.34	103.62	--	78.12	48.74	125.20	--	Ref.	--	--	--	--
Vitamin D and Calcium	16	54.85	33.80	89.01	0.596	46.77	28.82	75.91	0.105	0.71	0.34	1.45	0.332	-21.54
Lower 60% of Region														
No calcium	21	26.64	11.40	62.25	--	24.61	10.53	57.51	--	Ref.	--	--	--	--
Calcium	21	41.74	24.12	72.23	0.296	45.95	26.55	79.53	0.150	1.19	0.51	2.78	0.673	6.24
No vitamin D	8	31.42	19.58	50.43	--	37.48	23.35	60.17	--	Ref.	--	--	--	--
Vitamin D	19	38.56	23.75	62.63	0.514	32.26	19.86	52.38	0.632	0.70	0.36	1.38	0.294	-12.37
Calcium only	18	34.14	21.01	55.46	--	40.61	25.00	65.98	--	Ref.	--	--	--	--
Vitamin D and Calcium	16	36.06	21.90	59.39	0.864	30.77	18.69	50.68	0.389	0.72	0.33	1.56	0.391	-11.77
Low (≤ 50% median vitamin D intake)														
Whole Region														
No calcium	19	122.25	81.52	183.35	--	92.67	61.79	138.98	--	Ref.	--	--	--	--
Calcium	25	90.17	54.18	150.08	0.322	77.79	46.74	129.48	0.566	1.14	0.49	2.63	0.752	17.21
No vitamin D	16	124.28	77.66	198.90	--	103.40	64.61	165.47	--	Ref.	--	--	--	--
Vitamin D	12	102.44	70.66	148.50	0.503	80.30	55.40	116.41	0.382	0.94	0.47	1.87	0.862	-1.26
Calcium only	13	172.45	77.05	385.91	--	154.89	69.21	346.61	--	Ref.	--	--	--	--
Vitamin D and Calcium	15	93.01	47.50	182.13	0.122	72.35	36.95	141.67	0.060	0.87	0.34	2.18	0.751	-3.10
Top 20% of Region														
No calcium	21	37.63	25.89	54.70	--	30.32	20.86	44.07	--	Ref.	--	--	--	--
Calcium	21	26.81	16.70	43.01	0.224	17.64	10.99	28.30	0.058	0.82	0.44	1.52	0.509	-1.86
No vitamin D	8	40.07	26.07	61.59	--	33.03	21.49	50.76	--	Ref.	--	--	--	--
Vitamin D	19	30.28	21.24	43.16	0.292	22.75	15.96	32.42	0.163	0.91	0.52	1.59	0.740	-0.49
Calcium only	18	51.87	24.97	107.78	--	44.01	21.18	91.46	--	Ref.	--	--	--	--
Vitamin D and Calcium	16	26.31	14.03	49.32	0.061	18.55	9.90	34.79	0.020	0.83	0.38	1.84	0.636	0.11
Upper 40% of Region														
No calcium	19	66.69	45.59	97.56	--	53.18	36.35	77.79	--	Ref.	--	--	--	--
Calcium	25	51.65	31.89	83.65	0.374	42.09	25.99	68.16	0.415	1.02	0.50	2.10	0.951	3.96
No vitamin D	16	69.68	43.93	110.52	--	56.15	35.40	89.07	--	Ref.	--	--	--	--
Vitamin D	12	56.59	39.35	81.39	0.461	44.70	31.08	64.29	0.419	0.98	0.52	1.84	0.949	1.64
Calcium only	13	103.98	47.26	228.77	--	86.74	39.42	190.83	--	Ref.	--	--	--	--
Vitamin D and Calcium	15	52.46	27.20	101.17	0.080	40.19	20.84	77.51	0.051	0.92	0.39	2.18	0.841	4.97
Lower 60% of Region														
No calcium	19	43.16	25.34	73.52	--	34.46	20.23	58.70	--	Ref.	--	--	--	--
Calcium	25	28.77	14.79	55.99	0.315	29.38	15.10	57.16	0.690	1.28	0.40	4.04	0.663	9.30
No vitamin D	16	45.80	26.54	79.03	--	37.83	21.93	65.27	--	Ref.	--	--	--	--
Vitamin D	12	36.07	23.40	55.62	0.478	29.90	19.39	46.09	0.484	1.00	0.41	2.43	0.994	1.79
Calcium only	13	60.26	24.05	150.99	--	53.30	21.27	133.54	--	Ref.	--	--	--	--
Vitamin D and Calcium	15	33.98	15.74	73.37	0.216	27.64	12.80	59.68	0.158	0.92	0.28	3.01	0.886	0.62

		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Vitamin D intake	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
High (> 50% median vitamin D intake)														
φh[i]														
No calcium	21	62.96	54.30	71.63	--	60.26	51.59	68.92	--	Ref.	--	--	--	--
Calcium	21	62.45	56.85	68.06	0.910	62.65	57.05	68.26	0.596	2.90	-9.26	15.07	0.627	1.05
No vitamin D	8	60.93	55.81	66.05	--	61.71	56.59	66.83	--	Ref.	--	--	--	--
Vitamin D	19	58.59	53.35	63.82	0.493	57.15	51.91	62.38	0.185	-2.23	-10.40	5.94	0.585	0.96
Calcium only	18	61.64	56.68	66.61	--	61.55	56.58	66.52	--	Ref.	--	--	--	--
Vitamin D and Calcium	16	56.67	51.56	61.79	0.138	57.26	52.15	62.38	0.199	0.68	-7.86	9.22	0.872	1.01
Low (≤ 50% median vitamin D intake)														
φh														
No calcium	19	56.08	50.18	61.98	--	57.82	51.92	63.72	--	Ref.	--	--	--	--
Calcium	25	58.95	51.42	66.49	0.517	54.87	47.33	62.40	0.505	-5.83	-16.04	4.37	0.250	0.90
No vitamin D	16	57.06	51.85	62.28	--	55.03	49.82	60.25	--	Ref.	--	--	--	--
Vitamin D	12	56.60	52.48	60.73	0.885	56.24	52.12	60.37	0.705	1.67	-6.31	9.65	0.675	1.03
Calcium only	13	60.82	52.56	69.08	--	56.79	48.53	65.05	--	Ref.	--	--	--	--
Vitamin D and Calcium	15	57.39	50.47	64.31	0.405	55.98	49.06	62.90	0.843	2.62	-8.01	13.26	0.616	1.04

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Total vitamin D intake (vitamin D in the diet and supplemental vitamin D) was dichotomized into sex-specific high/low categories

[f]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[g]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[i]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

[j]Total vitamin D intake was categorized as a sex-specific binomial variable dividing total vitamin D intake by the median. Median for males was 508.8 IU/d, females (4-arm) was 123.1 IU/d, females (2-arm) was 504.6 IU/d

Supplementary Table 9: Comparison of TLR4 Expression in the Lamina Propria Region by Treatment Type Stratified by High or Low Serum Levels of Vitamin D[aj]

		Baseline				1-Yr Follow-Up				Relative Tx[b]				Absolute Tx[c]
Vitamin D Intake	n	Geometric Mean[d]	95% CI		p-value	Geometric Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
High (> 50% median serum vitamin D)														
Whole Region														
No calcium	23	129.52	71.99	232.99	--	83.33	46.32	149.92	--	Ref.	--	--	--	--
Calcium[e]	29	159.49	86.50	294.09	0.604	107.49	58.30	198.20	0.526	1.05	0.41	2.70	0.921	-5.82
No vitamin D	14	124.49	79.14	195.82	--	97.19	61.79	152.89	--	Ref.	--	--	--	--
Vitamin D[f]	17	107.96	73.55	158.48	0.620	63.35	43.16	92.99	0.140	0.75	0.35	1.61	0.454	-17.33
Calcium only	18	142.47	80.34	252.60	--	108.13	60.98	191.73	--	Ref.	--	--	--	--
Vitamin D and Calcium[g]	20	105.64	64.90	171.93	0.354	63.93	39.28	104.05	0.108	0.80	0.31	2.03	0.626	-7.37
Top 20% of Region														
No calcium	23	38.49	21.23	69.76	--	24.36	13.44	44.16	--	Ref.	--	--	--	--
Calcium	29	55.41	29.97	102.42	0.368	29.64	16.03	54.78	0.626	0.85	0.36	1.96	0.686	-11.65
No vitamin D	14	42.99	27.08	68.24	--	31.81	20.04	50.51	--	Ref.	--	--	--	--
Vitamin D	17	33.01	22.20	49.08	0.367	17.21	11.58	25.59	0.039	0.70	0.35	1.43	0.327	-4.62
Calcium only	18	51.14	28.54	91.63	--	34.39	19.19	61.62	--	Ref.	--	--	--	--
Vitamin D and Calcium	20	32.64	19.78	53.84	0.166	17.68	10.72	29.16	0.043	0.81	0.34	1.91	0.614	1.79
Upper 40% of Region														
No calcium	23	72.00	39.81	130.20	--	47.01	25.99	85.00	--	Ref.	--	--	--	--
Calcium	29	93.55	50.24	174.18	0.519	58.31	31.32	108.56	0.595	0.95	0.39	2.31	0.915	-10.26
No vitamin D	14	73.83	46.10	118.26	--	56.63	35.36	90.70	--	Ref.	--	--	--	--
Vitamin D	17	60.54	40.65	90.16	0.505	33.78	22.68	50.30	0.086	0.73	0.35	1.53	0.393	-9.55
Calcium only	18	88.08	48.02	161.56	--	63.92	34.85	117.25	--	Ref.	--	--	--	--
Vitamin D and Calcium	20	59.42	35.60	99.18	0.242	33.71	20.19	56.26	0.061	0.78	0.31	1.96	0.590	-1.55
Lower 60% of Region														
No calcium	23	46.79	24.27	90.21	--	30.80	15.98	59.38	--	Ref.	--	--	--	--
Calcium	29	52.37	26.69	102.75	0.801	41.85	21.33	82.12	0.494	1.21	0.37	4.01	0.742	5.47
No vitamin D	14	41.93	25.71	68.37	--	32.55	19.96	53.08	--	Ref.	--	--	--	--
Vitamin D	17	38.52	25.42	58.37	0.785	24.90	16.43	37.74	0.391	0.83	0.34	2.02	0.680	-4.24
Calcium only	18	46.56	25.11	86.33	--	35.60	19.20	66.01	--	Ref.	--	--	--	--
Vitamin D and Calcium	20	38.34	22.61	65.01	0.580	25.95	15.30	44.01	0.370	0.89	0.30	2.57	0.818	-1.43
Low (≤ 50% median serum vitamin D)														
Whole Region														
No calcium	28	98.85	62.97	155.17	--	114.39	72.87	179.54	--	Ref.	--	--	--	--
Calcium	25	109.02	71.84	165.42	0.723	117.94	77.73	178.95	0.912	0.93	0.50	1.73	0.825	-6.61
No vitamin D	15	116.04	76.52	175.99	--	106.55	70.25	161.58	--	Ref.	--	--	--	--
Vitamin D	17	113.42	73.49	175.05	0.935	96.46	62.50	148.87	0.724	0.93	0.51	1.67	0.796	-7.48
Calcium only	21	150.39	86.37	261.88	--	132.21	75.92	230.21	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	125.49	69.39	226.97	0.620	89.79	49.65	162.39	0.292	0.81	0.39	1.70	0.574	-17.52
Top 20% of Region														
No calcium	28	34.01	21.24	54.44	--	38.25	23.90	61.23	--	Ref.	--	--	--	--
Calcium	25	33.20	21.31	51.74	0.934	37.26	23.91	58.07	0.928	1.00	0.55	1.83	0.994	-0.19
No vitamin D	15	36.97	23.81	57.40	--	33.62	21.66	52.20	--	Ref.	--	--	--	--
Vitamin D	17	35.82	22.52	56.98	0.915	30.27	19.03	48.14	0.724	0.93	0.49	1.75	0.818	-2.20
Calcium only	21	49.35	27.23	89.45	--	40.14	22.14	72.75	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	37.75	19.99	71.30	0.496	29.32	15.53	55.38	0.425	0.96	0.42	2.16	0.910	0.79
Upper 40% of Region														
No calcium	28	55.51	34.90	88.28	--	64.64	40.64	102.81	--	Ref.	--	--	--	--
Calcium	25	63.32	41.22	97.26	0.643	71.43	46.50	109.72	0.725	0.97	0.52	1.81	0.918	-1.02
No vitamin D	15	63.69	41.96	96.69	--	61.07	40.23	92.71	--	Ref.	--	--	--	--
Vitamin D	17	65.29	42.25	100.88	0.930	55.73	36.07	86.12	0.745	0.89	0.50	1.58	0.687	-6.94
Calcium only	21	86.92	50.24	150.37	--	77.26	44.66	133.66	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	71.16	39.63	127.78	0.579	53.83	29.98	96.65	0.318	0.85	0.42	1.73	0.648	-7.68
Lower 60% of Region														
No calcium	28	32.40	19.26	54.54	--	41.52	24.67	69.87	--	Ref.	--	--	--	--
Calcium	25	38.97	24.09	63.06	0.565	38.42	23.74	62.17	0.808	0.77	0.37	1.59	0.467	-9.67
No vitamin D	15	45.02	28.16	71.97	--	36.38	22.76	58.16	--	Ref.	--	--	--	--
Vitamin D	17	38.85	23.83	63.35	0.643	32.08	19.67	52.30	0.692	1.02	0.49	2.12	0.953	1.87
Calcium only	21	55.61	29.99	103.11	--	43.81	23.63	81.23	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	47.69	24.62	92.36	0.707	28.08	14.50	54.39	0.280	0.75	0.30	1.84	0.517	-7.81

		Baseline				1-Yr Follow-Up				Absolute Tx				Relative Tx
Vitamin D Intake	n	Mean	95% CI		p-value	Mean	95% CI		p-value	Treatment Effect	95% CI		p-value	Treatment Effect
High (> 50% median serum vitamin D)														
φh[i]														
No calcium	23	57.48	51.39	63.57	--	58.20	52.11	64.29	--	Ref.	--	--	--	--
Calcium	29	59.61	53.37	65.86	0.608	55.19	48.94	61.44	0.470	-5.14	-16.27	5.99	0.352	0.91
No vitamin D	14	60.23	55.08	65.39	--	59.60	54.44	64.75	--	Ref.	--	--	--	--
Vitamin D	17	57.22	52.87	61.57	0.354	54.87	50.52	59.22	0.149	-1.71	-9.36	5.94	0.655	0.97
Calcium only	18	62.02	55.46	68.59	--	60.08	53.51	66.64	--	Ref.	--	--	--	--
Vitamin D and Calcium	20	56.64	51.16	62.12	0.129	53.68	48.20	59.16	0.074	-1.01	-9.51	7.50	0.812	0.98
Low (≤ 50% median serum vitamin D)														
φh														
No calcium	28	57.63	51.50	63.76	--	57.13	51.00	63.26	--	Ref.	--	--	--	--
Calcium	25	59.16	53.50	64.83	0.686	60.89	55.23	66.56	0.323	2.23	-6.78	11.25	0.616	1.04
No vitamin D	15	55.88	51.65	60.10	--	57.93	53.71	62.15	--	Ref.	--	--	--	--
Vitamin D	17	58.47	54.07	62.87	0.369	58.57	54.17	62.96	0.825	-1.96	-8.98	5.07	0.579	0.97
Calcium only	21	58.60	53.52	63.69	--	59.23	54.14	64.31	--	Ref.	--	--	--	--
Vitamin D and Calcium	17	57.53	52.06	62.99	0.751	60.57	55.11	66.04	0.692	2.43	-5.81	10.66	0.554	1.04

[a]TLR4 by treatment type was modeled using a mixed linear model in SAS 9.4 (Cary, NC), controlling for age, gender (by study arm), and study center

[b]Relative Tx = Relative Treatment Effect = [(Tx Yr.1)/(Tx Baseline)]/[(Placebo Yr.1)/(Placebo Baseline)]

[c]Absolute Tx = Absolute Treatment Effect = [(Tx Yr.1) - (Tx Baseline)] - [(Placebo Yr.1) - (Placebo Baseline)]

[d]The TLR4 measurement variable was log-transformed. Reported values are geometric means of optical density

[e]Serum vitamin D levels was dichotomized into sex-specific high/low categories

[f]Includes patients that were assigned to either calcium (4-Arm) or calcium + vitamin D (4-Arm). No patients from the 2-Arm treatment were included

[g]Includes patients that were assigned to either vitamin D (4-Arm) or calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[h]Includes patients that were assigned to either calcium + vitamin D (4-Arm) or vitamin D (2-Arm)

[i]Defined as the expression in the upper 40% of the representative crypt area of the lamina propria region sampled over the expression of the entire representative crypt area of the lamina propria region

[j]Serum levels of vitamin D was categorized as a sex-specific binomial variable dividing serum levels of vitamin D by the median. Median for males was 22.69 ng/mL , females (4-arm) was 16.83 ng/mL, females (2-arm) was 24.23 ng/mL