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Associations of Diet and Lifestyle with Cell Cycle Biomarkers in the Normal-appearing Colorectal Mucosa of Colorectal Adenoma Patients

By

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Master of Public Health

Epidemiology

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By

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M.Sc.
University College London
2016

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An abstract of
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Abstract

Associations of Diet and Lifestyle with
Cell Cycle Biomarkers in the
Normal-appearing Colorectal Mucosa
of Colorectal Adenoma Patients
By Xuan Guang

Background

Expression of cell cycle biomarkers of proliferation (Mib-1), differentiation (p21), apoptosis-promotion (BAX) and apoptosis-inhibition (Bcl-2) in the normal-appearing colorectal mucosa were associated with colorectal adenoma. However, there are few data on associations of diet, lifestyle or other factors with expression of these biomarkers in the normal colorectal mucosa of humans.

Methods

We measured Mib-1, p21, BAX, Bcl-2 expression in the normal-appearing rectal mucosa, using automated IHC and image analysis, in a subset of 104 participants in a large chemoprevention trial. Diet was assessed using a Block Brief 2000 Food Frequency Questionnaire. Physical activity was assessed by asking participants how much time they spent in moderate or vigorous activities during the past week. Cross-sectional associations of participants' characteristics with the biomarkers at baseline were assessed using multivariable general linear models to compare adjusted mean biomarker expression across categories of the participants' characteristics.

Results

Whole crypt adjusted mean BAX expression was 49.4% higher (P=0.11) among patients with higher relative to lower physical activity. Adjusted Bcl-2 expression was 45.3% lower (P=0.05) among patients with higher relative to lower total calcium intakes. Adjusted Mib-1 expression was 19.4% lower (P=0.10) among patients with higher relative to lower fruit intakes. Adjusted P21 expression was 37.5% higher (P=0.18) among patients with higher relative to lower serum 25-OH vitamin D concentrations.

Conclusions

Our findings support 1) a possible direct association of physical activity with BAX expression; 2) an inverse association of total calcium intake with Bcl-2 expression; 3) inverse associations of fruit intake with Mib-1 expression; and 4) a direct association of serum 25-OH vitamin D concentrations with p21 expression in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients.

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Introduction

Colorectal cancer is the second leading cause of cancer-related deaths among men and women combined in the United States. Though the colorectal cancer mortality rate has been dropping in both men and women for several decades due to screening and improving treatment, colorectal cancer is still the third most common cancer diagnosed in the United States. The risk of developing colorectal cancer in women is slightly lower than in men (4.15% vs. 4.49%) (American Cancer Society, 2018).

The evolution of normal colonic mucosa to life-threatening invasive carcinoma involves unbalanced proliferation and apoptosis as well as a number of molecular changes (Mayer, 2012). Pathology examination of the biopsies of gastrointestinal mucosa from colorectal cancer patients revealed aberrant colorectal epithelial cell proliferation, differentiation, and/or decreased apoptosis (Fleming, Ravula, Tatishchev, & Wang, 2012). Altered proliferative patterns are associated with changes in a set of biomarkers: Mib-1, $p21^{waf1/cip1}$, BAX, and BCL-2.

This set of cell-cycle biomarkers is well supported by basic science literature. BAX promotes apoptosis by controlling mitochondrial outer membrane integrity, while BCL-2 , known as B cell lymphoma 2 protein family, inhibits apoptosis (Tait & Green, 2010). BAX gene expression elicits tumor-specific apoptosis of human colon, lung, and cervical cancer cells *in vitro*, and suppressed tumor cell growth *in vivo* (Gu et al., 2000). The Bcl-2 gene family inhibits apoptosis and promotes tumorigenesis. Bcl-2 over-expression was found in a large number of epithelial tumors, such as breast cancer, neuroblastoma, non-small cell lung cancer, and colon cancer (Coultas & Strasser, 2003; Poincloux et al., 2009). Elevated Ki-67 clone Mib-1 was associated with a higher mitotic index and increased proliferation (Martin et al., 2004; Spyratos et al., 2002). $p21^{waf1/cip1}$ is a cyclin-dependent kinase inhibitor that expresses in fully differentiated cells of colorectal crypts (Eldeiry et al., 1995; Fedirko et al., 2009).

Epidemiological evidence suggests that cell cycle biomarkers are associated with colon cancer. A cohort study that followed 168 patients for 15 years reported an inverse association of BAX expression with a '*survivin score*', which reflects the degree of tumor dedifferentiation (Hernandez et al., 2011). Also, higher BCL-2 and Mib-1 expression were associated with advanced tumor grade, clinical stage, and reduced overall survival in colorectal cancer patients (J. S. Wang et al., 2018). The validity of Bcl-2, BAX, Mib-1, and p21 as biomarkers of risk for colorectal neoplasms was supported by the results from a previous pilot case-control study (Bostick, 2015; Fedirko et al., 2009).

Diet is an important risk factor for colorectal adenoma and cancer. Mortality from colorectal cancer is positively associated with the consumption of total energy, red meat, processed meats, and fat (Mayer, 2012). High intakes of fruits, vegetables, fiber, and calcium are associated with lower risk of colorectal cancer (Baron et al., 1999). Low physical activity level, smoking, and alcohol intake are associated with increasing cancer-specific mortality after colorectal cancer diagnosis (Banck-Petersen et al., 2018; Mayer, 2012). All of these findings taken together suggest that higher intakes of fruit, vegetables, and calcium and lower intake of fat may be associated with higher expression of p21 and BAX and lower expression of Mib-1 and Bcl-2 in the normal colorectal mucosa.

Although current literature supports associations of pre-neoplastic biomarkers of risk for colorectal neoplasms with colorectal cancer, few reported studies focused on associations of diet and lifestyle with those biomarkers. In our study, we investigated associations of diet and lifestyle with cell cycle biomarkers in the colorectal epithelium of colorectal adenoma patients.

Methods

The 104 participants in this cross-sectional study were recruited from two of 11 academic medical centers conducting a randomized, double-blind, placebo-controlled chemoprevention clinical trial (n=2,259) designed to test the effects of calcium and vitamin D_3 on colorectal

adenoma recurrence. Enrollment of patients was from July 2004 through July 2008. Eligibility criteria for the clinical trial included being in general good health, aged 45-75, and having at least one histologically-verified neoplastic polyp ≥ 2 mm in diameter removed within 120 days of study entry. We excluded participants with a history of invasive carcinoma, familial colonic polyposis syndromes, and other serious intestinal diseases, serum calcium concentrations outside the normal range, and serum 25(OH)D concentrations <12 ng/ml or >90 ng/ml. All participants provided written informed consent, and the research was approved by the institutional review board at each center.

Participants provided self-reported information regarding demographics, diet (using Block Brief 2000 food frequency questionnaires), lifestyle, and medical history. Physical activity was measured by asking participants how much time they spend on moderate and vigorous activities during the past week, and the results were converted to metabolic equivalents of task minutes (MET). Serum 25(OH)D concentrations were measured using a radioimmunoassay kit from Immunodiagnostic Systems.

Participants underwent "non-prep" (i.e., no prior colon-cleansing preparations) biopsies of normal-appearing rectal mucosa at enrollment. A rigid sigmoidoscope and jumbo cup flexible biopsy forceps mounted on a semi-rigid rod were used to take six approximately 1 mm-thick biopsy specimens from the rectal mucosa 10 cm proximal to the external anal aperture. To avoid possible field effects, all biopsies were taken at least 4 cm from any polypoid lesions. Biopsies were teased onto a strip of bibulous paper and immediately placed in normal saline, oriented, and then transferred to 10% normal buffered formalin for 24 hours; they were then transferred to 70% ethanol, and, within a week, processed and embedded in paraffin blocks (two blocks of three biopsies per participant per biopsy visit). Sufficient biopsy tissue for biomarker measurements was obtained at baseline on 104 patients. (Gao et al., 2018).

Immunohistochemistry Protocol

The four biomarkers—Mib-1, p21, BAX, and Bcl-2—were measured using automated immunohistochemistry and image analysis. First, to uncover epitopes, the slides were placed in a preheated Pretreatment Module (Lab Vision Corp., Fremount, CA) with 100× Citrate Buffer pH 6.0 (DAKO S1699, DAKO Corp., Carpinteria, CA [hereafter referred to as DAKO]) and steamed for 40 minutes (Gao et al., 2018). The slides were then placed in a DakoCytomation Autostainer Plus System (DAKO) automated immunostainer and immunohistochemically processed using a labeled streptavidin-biotin method (LSAB2 Detection System [DAKO K0675]) for Mib-1, p21, and Bcl-2, and a polymer system (Envision Plus Rabbit System [DAKO K4003]) for BAX. Monoclonal antibodies were used for each biomarker as follows at the following concentrations: Mib-1 (DAKO M7240) – 1:350; p21 (DAKO M7202) – 1:40; BAX (DAKO A3533) – 1:200; and Bcl-2 (Santa Cruz sc-509) – 1:100) (Gao et al., 2018).

Quantifying Staining Density of Immunohistochemically Detected Biomarkers in Colon Crypts Protocol ("Scoring")

A quantitative image analysis procedure ("scoring") was used to describe and measure the detected biomarkers in colon crypts (Figure 1) (Gao et al., 2018). The unit of analysis was a "hemicrypt," defined as one-half of a longitudinally bisected crypt. A crypt defined as "scorable" was one that extended from the muscularis mucosae to the colon lumen(Gao et al., 2018).

The major equipment and software used for the image analysis procedures were: Scanscope CS Digital scanner (Aperio Technologies, Inc., CA), computer, digital drawing board, MatLab Software (MathWorks, Inc., MA), CellularEyes Image Analysis Suite (DivEyes LLC, GA), and MySQL (Sun Microsystems Inc., Santa Clara, CA). Throughout the scoring procedures, standardized settings were used for all equipment, and negative and positive control slides were checked for staining adequacy before analysis (Gao et al., 2018).

First, digital images of the slides were acquired using the Aperio Scanscope CS digital scanner, and then the CellularEyes program was used to review the electronic images and identify colon crypts acceptable for analysis (Gao et al., 2018). Following a strict protocol, trained technicians "scored" hemicrypts for analysis. One technician scored all participants for a given biomarker. The borders of each selected hemicrypt were traced using the digital drawing board, and the program divided the outline into 50 equal-width segments of approximately the average width of normal colonocytes. (Gao et al., 2018). The program then measured the background-corrected optical density of the biomarker labeling across the entire hemicrypt and within each segment. The resulting data were transferred automatically into the MySQL database (Gao et al., 2018). The previously described steps were then repeated for each subsequently identified hemicrypt, with a goal of scoring 32 hemicrypts (Gao et al., 2018). Subsamples of blinded slides were re-scored at intervals and after completing scoring to assess scoring reliability (Gao et al., 2018).

Statistical Analysis

Participants' characteristics were summarized using descriptive statistics, such as means (SD) and proportions as percentages. Continuous variables that were not normally distributed were log transformed to meet normality assumptions. We assessed mean biomarker expression as labeling optical densities within whole crypts, the upper 40% of crypts (differentiation zone), the lower 60% of the crypts (proliferation zone), as well as the ratios of expression in the upper 40% of the crypts to the whole crypts (Φ_h). We also calculated a BAX/BCL-2 ratio to represent the balance of pro- to anti-apoptosis factors, a BAX/Mib-1 ratio to represent the balance of apoptosis to proliferation, and a p21/Mib-1 ratio to represent the balance of differentiation to proliferation.

Continuous exposure variables, such as dietary variables and physical activity, were categorized into tertiles for further data analysis. For total energy, red meat, processed meat, fruit, and dietary fiber intake, tertile categorization was sex specific. Macronutrients were analyzed as percentage of total energy contributed by the macronutrient (e.g., g of fat * 9 kcal /

total energy [kcal]* 100%). Micronutrients were analyzed as nutrient densities (e.g., mg of calcium / 1,000 kcal). Non-aspirin NSAID use was categorized as no current use, < once a week, and ≥ once a week. BMI was categorized as < 25, 25 – 29.9, and ≥ 30 kg/m^2.

Multivariable general linear models were used to compare adjusted mean biomarker expression among categories of exposure variables. Potential confounders included sex, age, race, education, smoking, alcohol, physical activity, and multivitamin supplement and non-aspirin NSAID use, BMI, total energy, total fat, saturated fat, red meat, processed meats, fruits, vegetables, dietary fiber, and calcium intakes, 25-(OH)-D concentrations, and selected adenoma characteristics. These potential confounders were assessed through observing whether inclusion/exclusion of these covariates, individually or in combination, affected the magnitude of mean biomarker difference between the upper and lower categories of the exposure variable by ≥10%. Two-sided P values < 0.05 were considered statistically significant. Statistical analyses were conducted using SAS software, version 9.4 (SAS Institute).

Results

Selected characteristics of the 104 participants are summarized in Table 1. Of the participants, 46% were men, 79% were white, 7.7% were current smokers, 66.4% regularly took a non-aspirin NSAID once a week or more, and 32.7% had multiple adenomas, and 20.4% had an advanced adenoma removed at their most recent colonoscopy. The participants' ages ranged from 47 – 75 years (mean 59 years), their BMIs ranged from 21.0 – 54.1 kg/m^2 (mean 29.6 kg/m^2), and their serum 25-OH-vitamin D concentrations ranged from 12.9 – 68.8 ng/dL (mean 24.1 ng/dL).

Mean expression of BAX, Bcl-2, Mib-1, and p21 in different areas of crypts, by categories of participant characteristics, are presented in Tables 2 - 6. In Table 2 we summarize the strongest findings for all of the biomarkers. The criteria for inclusion in this table were: estimated proportional mean differences in biomarker expression between the highest and lowest categories

of the exposure variable of ≥20% and/or a p-value of <0.2, plus at least an approximate dose-response pattern. More comprehensive findings and exact values are provided in Tables 3 – 6. Findings for the BAX/BCL-2 ratio (ratio of pro to anti-apoptosis), BAX/Mib-1 ratio (ratio of apoptosis to proliferation), the p21/Mib-1 ratio (ratio of differentiation to proliferation), and the ϕh of crypts for all biomarkers are presented in Supplement Table 2.

Apoptosis-promoting (BAX)

Among those in the highest relative to the lowest tertile of physical activity, mean BAX expression was estimated to be 49.4% (p = 0.11), 95.6% (p = 0.02), 22.6% (p = 0.55), 44.8% (p = 0.02) higher in the whole, upper 40%, lower 60%, and ϕh of crypts, respectively (Table 3 and Supplement Table 2). Among those in the highest relative to the lowest tertile of processed meat intake, mean Bax expression was estimated to be 36.2% (p = 0.25) lower in the upper 40% of crypts. Among those in the upper relative to the lower tertile of red meat intake, bax expression was estimated to be 23.8% (p = 0.37) lower in the lower 60% of crypts. Among regular non-aspirin NSAID users, mean bax expression was estimated to be 45.9% (p = 0.27) higher in the upper 40% of crypts and 33.8% (p = 0.11) higher in the ϕh of crypts. Also, among those in the highest relative to the lowest tertile of circulating 25-OH vitamin D concentrations, bax expression was estimated to be 24.4% (p = 0.41), 20.7% (p = 0.66), and 21.5% (p = 0.52) higher in the whole, upper 40% and lower 60% of crypts, respectively. However, among those in the upper relative to the lowest tertile of dietary fiber intake, bax expression was estimated to be lower in all crypt parameters, and among those in the upper tertile of total calcium intake, it was also estimated to be lower in the whole and upper 40% and lower 60% of crypts; none of these estimates was statistically significant.

Apoptosis-inhibiting (Bcl- 2)

Among those in the highest relative to the lowest tertile of total calcium intake, mean Bcl-2 expression was estimated to be 45.3% (p = 0.05), 78.3% (p = 0.20), 41.8% (p = 0.06), 43.9% (p=0.36) lower in the whole, upper 40%, lower 60% and ϕh of crypts, respectively (Table 4 and Supplement Table 2). Among those use Non-aspirin NSAID more than once a week relative to those do not use Non-aspirin NSAID, mean Bcl-2 expression was estimated to be 45.7% (p = 0.26) and 32.9% (p = 0.37) lower in the upper 40% and ϕh of crypts. Among those in the highest relative to the lowest tertile of current serum 25-OH vitamin D, mean Bcl-2 expression was estimated to be 40.0% (p = 0.64) lower in the upper 40% of crypts. However, among those with relative to without multivitamin supplement use, mean Bcl-2 expression was estimated to be 49.8% (p = 0.04), 86.6% (p = 0.26), 45.7%(p=0.03) higher in the whole, upper 40% and lower 60% of crypts. Among those in the highest relative to the lowest tertile of saturated fat intake, mean Bcl-2 expression was estimated to be 26.3% (p = 0.17) and 25.0% (p = 0.17) lower in the whole and lower 60% of crypts. Among those in the highest relative to the lowest tertile of processed meat, mean Bcl-2 expression was estimated to be 85.2% (p = 0.11) and 30.4% (p = 0.60) lower in the upper 40% and ϕh of crypts. Among those in the highest relative to the lowest tertile of physical activity and fruit intake, mean Bcl-2 expression was estimated to be 52.6% (p = 0.65) and 39.4% (p = 0.83) higher in the upper 40% of crypts. Among those in the highest relative to the lowest tertile of current alcohol intake, mean Bcl-2 expression was estimated to be 44.1% (p = 0.17) lower in the upper 40% of crypts.

Proliferation (Mib-1)

Among those in the highest relative to the lowest tertile of fruit intake, mean Mib-1 expression was estimated to be 19.4% (p = 0.10), 46.1% (p = 0.03), 17.6% (p = 0.15), and 33.0% (p = 0.11) lower in the whole, upper 40%, lower 60%, and ϕh of crypts, respectively (Table 5 and Supplement Table 2). Among those in the highest relative to the lowest tertile of dietary fiber

intake, mean Mib-1 expression was estimated to be 27.0% (p = 0.02), 24.2% (p = 0.44), and 27.2% (p = 0.02) lower in the whole, upper 40%, and lower 60% of crypts. Among those with relative to without multivitamin supplement use, mean Mib-1 expression was estimated to be 14.2% (p = 0.09), 26.3% (p = 0.12) and 13.4% (p = 0.10) lower in the whole, upper 40%, and lower 60% of crypts. Among those in the highest relative to the lowest tertile of alcohol intake, mean Mib-1 expression was estimated to be 27.6% (p = 0.07), 68.9% (p = 0.10), and 25.7% (p = 0.10) higher in the whole, upper 40%, and lower 60% of crypts. Among those with highest relative to the lowest tertile of total calcium intake, mean Mib-1 expression in the whole and upper 40% of crypts was estimated to be 20.6% (p = 0.20) and 44.3% (p = 0.14) lower. Among those with highest relative to the lowest tertile of total fat and processed meat intakes, mean Mib-1 expression in the upper 40% of crypts was estimated to be 20.6% (p = 0.80) and 49.9% (p = 0.37) higher. Among those with highest relative to the lowest tertile of non-aspirin NSAID use, mean Mib-1 expression in the upper 40% of crypts was estimated to be 22.9% (p = 0.71) lower.

Differentiation (p21)

Among those in the highest relative to the lowest tertile of serum 25(OH) vitamin D, mean p21 expression was estimated to be 37.5% (p = 0.18), 34.2% (p = 0.16), and 123.2% (p = 0.22) higher in the whole, upper 40%, and lower 60% of crypts, respectively (Table 6). Mean p21 expression in the lower 60% of crypts was estimated to be 136.9% (p = 0.26) and 36.2% (p = 0.54) higher among those in the highest relative to the lowest tertile of dietary fiber intake and multivitamin supplement use. Mean p21 expression in the lower 60% of crypts was estimated to be 27.2 (p = 0.78), 41.9% (p = 0.29) lower among those in the highest relative to the lowest tertile of current alcohol and saturated fat intakes. However, among those in the highest relative to the lowest tertile of non-aspirin NSAID use, mean p21 expression was estimated to be 42.9% (p = 0.50) and 39.6% (p = 0.58) lower in the whole and lower 60% of crypts. Among those in the highest relative to the lowest tertile of red meat intake, mean p21 expression was estimated to be

39.7% (p = 0.75) higher in the lower 60% of crypts; none of these estimates was statistically significant.

Discussion

Our results suggest that NSAID use, physical activity, vitamin D exposure, and intakes of calcium, processed meats, fruit, fiber, and alcohol, may be associated with biomarkers of the cell cycle in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients. These associations, although cross-sectional, suggest that the exposures may affect the cell cycle in the colorectal epithelium, and thus risk for colorectal neoplasms, and so support further investigation in larger studies.

More specifically, our results suggest that, based on their estimated associations with bax and bcl-2 expression, the following may be associated with higher apoptosis: NSAID use, higher physical activity, higher vitamin D exposure, and lower alcohol intake. However, our results for apoptosis in relation to saturated fat and red and processed meat intakes were mixed, with red and processed meat intakes being associated with lower bax expression, but with saturated fat and processed meats being associated with lower bcl-2 expression. Similarly, total calcium intakes were associated with lower bcl-2 expression, but also with lower bax expression. Contrary to our hypotheses, higher dietary fiber intake was associated with lower bax expression, and multivitamin/mineral use and higher fruit intakes were associated with higher bcl-2 expression.

Also, our results suggest that, based on their estimated associations with mib-1 expression, the following may be associated with higher colorectal epithelial cell proliferation: alcohol, total fat intake, and processed meat intakes. The following may be associated with lower colorectal epithelial cell proliferation: multivitamin/mineral supplement use, NSAID use, and higher fruit, fiber, and calcium intakes.

Our results also suggest that, based on their estimated associations with p21 expression, the following may be associated with higher colorectal epithelial cell differentiation: higher vitamin

D exposures and calcium and fiber intakes, and lower alcohol, saturated fat and processed meat intakes. However, contrary to our hypotheses, NSAID use was associated with lower p21 expression.

Finally, in this small, preliminary study, we found no strong suggestions that smoking, BMI, or vegetable intakes were associated with the expression of cell cycle biomarkers in the normal-appearing colorectal mucosa of adenoma patients.

Apoptosis

As noted above, our results suggest that the following dietary and lifestyle exposures may be associated with bax and bcl-2 expression in ways that suggest that they may increase apoptosis in colorectal crypts: NSAID use, higher physical activity, higher vitamin D exposure, and higher calcium intakes, and to a lesser extent, lower red and processed meat intakes. Impaired apoptosis is one of the hallmarks of colorectal carcinogenesis. NSAID use may induce gastrointestinal cell apoptosis via cyclooxygenase (COX) inhibition, down-regulating the mitochondrial membrane potential, and up-regulating pro-apoptotic BAX, caspase- 8, caspase- 9, and caspase- 3 activity. (Cheng, Lin, Jhang, & Yen, 2019; Musumba, Pritchard, & Pirmohamed, 2009). The underlying mechanisms for possible protective effects of physical activity have not been clearly defined, but may involve decreased expression of COX-2 and INOS in the colon mucosa (Buehlmeyer, Doering, Daniel, Schulz, & Michna, 2007). Vitamin D and calcium may promote apoptosis by modulating the vitamin D receptor and the calcium sensing receptor in mucosa, and activating inflammation signaling (Ahearn et al., 2011; Li, Chen, & Du, 2015). Red and processed meats may reduce apoptosis by heme-induced signaling from surface to crypt cells. Heme in red and processed meat may upregulate the expression of apoptosis inhibitors, including survivin (Birc5), Xiap, and Bcl2 (N et al., 2012).

There are few data on associations or intervention effects of dietary and lifestyle factors with/on apoptosis in the normal-appearing colorectal mucosa of humans. In chemoprevention trials in colorectal adenoma patients, calcium combined with vitamin D increased apoptosis in the

normal human colorectal epithelium. In a randomized controlled trial (n = 202 healthy sedentary participants), BAX expression increased (+0.87 log BAX density; p = 0.05) in bottom of crypts of men after 12 months of exercise, a finding that is consistent with our results (Campbell et al., 2007). A second, small randomized trial (N = 20) reported 11.2% (p>0.05) increased BAX expression after 12 weeks physical activity (Dimauro et al., 2016).

Proliferation

As noted above, our results suggest that multiple factors may affect colorectal epithelial cell proliferation. Increased proliferation is one of the hallmarks of colorectal carcinogenesis. Multivitamin/mineral supplement use may decrease proliferation via multiple mechanisms related to the properties of the multiple micronutrients, such as the antioxidant effects of vitamins C and E, beta-carotene, and lutein. Vitamin E may inhibit mucosal cell proliferation by blocking adaptive changes in cell NADH/NAD^+ redox states and mitochondrial function (Olguin-Martinez, Hernandez-Espinosa, & Hernandez-Munoz, 2013). Fruits may decrease proliferation via the effects of their fiber, antioxidant, and disaccharidase constituents on the human intestinal mucosa (Turco et al., 2016). Alcohol intake may increase proliferation by generating reactive oxygen species (ROS) and inhibiting the expression of antioxidant and cytoprotective enzymes and upregulating the expression of the metabolic activator CYP2E1 (Na & Lee, 2017).

There are few data on associations or intervention effects of dietary and lifestyle factors with/on proliferation in the normal-appearing colorectal mucosa of humans. In chemoprevention trials in colorectal adenoma patients, Mib-1 expression decreased by 24% (p=0.06) in the upper 40% of crypts with supplemental vitamin D3 (1,000 IU/day [25 μg/day]) and calcium (1,200 mg/day) treatment (Gao et al., 2018). Observational studies also support the association of dietary and lifestyle factors with proliferation. In a cross-sectional study of 44 heavy drinkers and 26 controls found that alcohol abusers had 1.2 – 1.5 times higher proliferative cell nuclear antigen (PCNA) and Mib-1 expression in the lower and middle sections of crypts (Simanowski et al., 2001). In a randomized trial, there were no significant effects of high fruit and vegetable diets on

Mib-1 expression in Barrett's esophagus patients over 36 months (Kristal et al., 2005). In an animal study, reported significant decrease of Mib-1 and Bcl-2 expression significantly decreased with a high dose of fruit peel polyphenols (Kubatka et al., 2016)

Differentiation

As noted above, our results suggest that higher fiber intake, multivitamin supplement use, and vitamin D exposures, lower saturated fat and alcohol intakes may increase differentiation of colorectal crypt epithelial cells. Decreased differentiation is one of the hallmarks of colorectal carcinogenesis. A dietary-induced adenoma model on mouse suggested that western-style diet decreased differentiation and apoptosis in histologically normal villus cells. The altered cell-cycle of villus cells may be caused by inheritance of an *Apc* mutation or inactivation of $p21^{WAF1/cip1}$ (D. Wang et al., 2011; Yang et al., 2001). After 36 weeks of feeding with western diet, the differentiated goblet cells decreased by 25% ($p<0.05$) in $p21^{+/-}$ type and 39% ($p<0.001$) in the $^{p21-/-}$ type (Yang et al., 2001).

There are few data on associations or intervention effects of dietary and lifestyle factors with/on epithelial cell differentiation in the normal-appearing colorectal mucosa of humans. In chemoprevention trials in colorectal adenoma patients, calcium and vitamin D alone or combined promoted colorectal crypt epithelial cell differentiation (Gao et al., 2018).

Strengths and Limitations

Strengths of the study include that it is first study of associations of multiple dietary and lifestyle factors with a set of biomarkers profiling cell proliferation, differentiation, and apoptosis within a single study, and the automated immunohistochemistry and novel image analysis methods, which allowed measuring various aspects of the cell cycle in colorectal crypts, the high measurement reliability, and the high protocol adherence by study participants. Limitations include the small sample size; however, as noted, our findings in this pilot study support more

definitive study of dietary and lifestyle factors that may affect the cell cycle in the normal-appearing colorectal epithelium, and thus could help favorably modulate risk for colorectal neoplasms. Other limitations include the known limitations of FFQs (recall error, limited number of food items, etc.); however, the Block Brief 2000 is a well-developed, validated FFQ (Delgado et al., 2014). Finally, all study participants were colorectal adenoma patients in a randomized controlled trial, thus potentially limiting the generalizability of our findings to the general population. Another limitation was that we investigated associations of single dietary and lifestyle factors with the biomarkers. It is possible that larger dietary and lifestyle patterns may be more strongly associated with the cell cycle in the colorectal mucosa

Conclusions

In conclusion, our findings, taken in context with those from previous studies, 1) suggest that multiple dietary and lifestyle factors, including NSAID use, physical activity, vitamin D exposure, and intakes of calcium, processed meats, fruit, fiber, and alcohol, may affect the cell cycle in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients, and 2) support their further investigation in larger observational studies and/or intervention trials with cell cycle biomarker and colorectal neoplasm endpoints.

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Figure

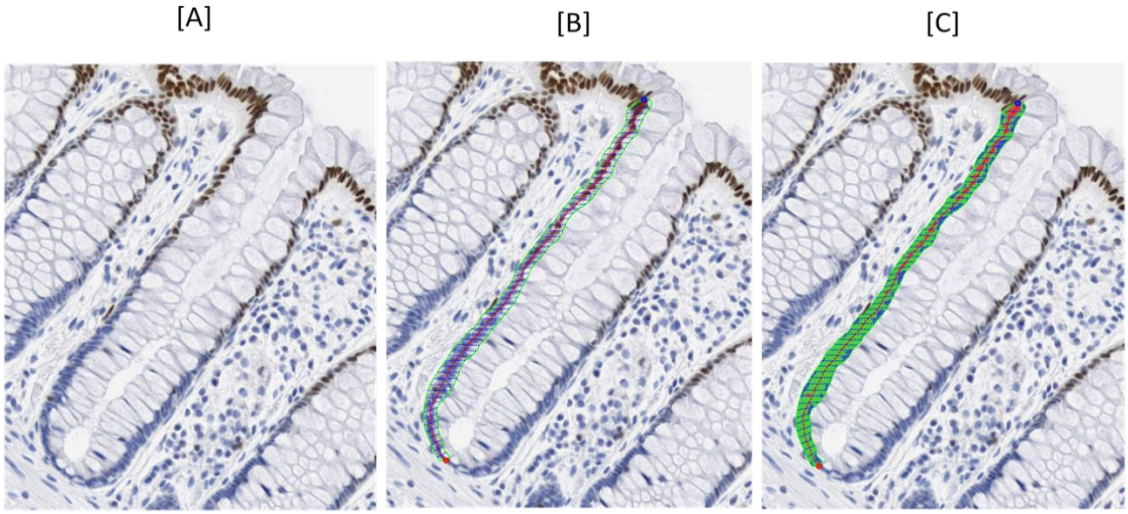

Figure 1. Depicts image analysis biomarker (p21) expression in crypts of normal-appearing rectal mucosa using custom-designed quantitative image analysis software. [A] A full length hemicrypt is identified. [B] The hemicrypt is manually outlined. [C] p21 labeling optical density is quantified, overall and within each of the 50 segments of the hemicrypt.

Tables

Table 1. Selected characteristics of the study participants (n = 104) [a]

Characteristic	Mean or proportion	SD	Range
Demographics			
Age (yr.)	58.9	6.7	47 - 75
Male (%)	46.2		
White (%)	78.9		
≥ High school education (%)	81.7		
Lifestyle and medical history			
Currently smoke (%)	7.7		
Alcohol (drinks/day)	0.7	0.9	0.0 - 3.6
Multivitamin supplement (n/week)	4.7	3.3	0 - 7
Take non-aspirin NSAID (%) [b]	66.4		
BMI (kg/m^2)	29.6	5.6	21.0 - 54.1
Physical activity (MET-min/week)	2,472	2,484	0 - 15,198
Dietary intakes			
Total energy (kcal/day)	1,456	554	630 - 2,936
Total fat (g/day)	59.6	28.1	15.8 - 145.7
Saturated fat (g/day)	19.2	9.5	5.0 - 46.0
Red meat (servings/day)	0.5	0.5	0 - 2.3
Processed meat (servings/day)	0.3	0.4	0 - 2.3
Fruits (servings/day)	1.3	0.7	0.1 - 3.6
Vegetables (serving/day)	3.4	1.9	0.6 - 11.7
Dietary fiber (gm/day)	14.6	5.7	3.8 - 32.2
Total calcium (mg/d) [c]	879	450	213 - 2,512
Serum concentration			
25-OH vitamin D (ng/ml)	24.1	9.3	12.9-68.8
Cell cycle biomarkers expression (OD) [d]			
BAX (apoptosis-promoting)	446	118	31 - 2,756
Bcl-2 (apoptosis-inhibiting)	796	474	157 - 2,583
Mib-1 (proliferation)	1,267	518	416 - 3,302
p21 (differentiation)	708	435	166 - 2,680
Adenoma			
>1 adenoma (%)	32.7		
Advanced adenoma (%)	20.4		

Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; NSAID, nonsteroidal anti-inflammatory drug; OD, optical density.

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

[b] Regularly take non-aspirin nonsteroidal anti-inflammatory drug at least once a week

[c] Dietary plus supplemental calcium intake

[d] Optical density of biomarker expression in whole crypts of normal-appearing rectal mucosa

Table 2. Summary of differences [a] in mean cell cycle biomarker expression in the normal-appearing colorectal mucosa, across categories of selected participant characteristics.

Participants characteristics	BAX				Bcl-2				Mib-1				P21 [b]		
	Whole crypts	Upper 40% of crypts	Lower 60% of crypts	ϕh[c]	Whole crypts	Upper 40% of crypts	Lower 60% of crypts	ϕh[c]	Whole crypts	Upper 40% of crypts	Lower 60% of crypt	ϕh[c]	Whole crypt	Upper 40% of crypts	Lower 60% of crypt
Lifestyle and medical history															
Current alcohol intake						(↓)			↑	↑	↑				↓
Multivitamin supplement use					(↑)*	(↑)	(↑)*		↓	↓	↓				↑
Regularly take non-aspirin NSAID		↑		↑		↓		↓		↓			(↓)		(↓)
Physical activity	↑	↑*	↑	↑		(↑)									
Dietary intakes															
Total fat										↑					
Saturated fat					(↓)		(↓)								↓
Red meat			↓												(↑)
Processed meat		↓				(↓)		(↓)		↑					
Fruit						(↑)			↓	↓*	↓	↓			
Dietary fiber	(↓)	(↓)	(↓)	(↓)					↓*	↓	↓*				↑
Total calcium	(↓)	(↓)	(↓)		↓*	↓	↓	↓	↓	↓					
Serum concentrations															
25-OH Vitamin D	↑	↑	↑			↓							↑	↑	↑

Abbreviations: MET, metabolic equivalent of task; NSAID, nonsteroidal anti-inflammatory drug.

[a] Criteria for inclusion in this table are: estimated proportional mean difference ≥ 20% and/or a p-value < 0.2 for the estimated difference. Up/down arrows indicate the direction (higher or lower, respectively) of the mean biomarker difference between a higher relative to the reference exposure category. Arrows in brackets [e.g., (↑)] indicate that the direction of the difference was opposite that hypothesized. * Indicates a statistically significant ($p < 0.05$) finding.

[b] p21 ϕh is not included in this table because no findings met the criteria for inclusion

[c] ϕh is the ratio of mean biomarker expression in upper 40% of crypts relative to that in whole crypts

Table 3. BAX expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to categories of participant characteristics (n = 104) [a].

Participant characteristics	Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Current alcohol intake [f]												
None	477.9	60.4			148.7	25.9			335.6	39.8		
Low	358.6	54.6	-25.0%	0.25	133.7	23.4	-10.0%	0.87	226.7	35.9	-32.4%	0.08
High	454.7	60.9	-4.9%	0.95	167.9	26.1	12.9%	0.83	278.5	40.1	-17.0%	0.51
Multivitamin use												
No	416.5	398.6			139.9	25.3			258.7	39.8		
Yes	442.6	438.6	6.3%	0.73	153.6	17.5	9.8%	0.66	285.0	27.5	10.2%	0.60
Non-aspirin NSAID use												
None	454.4	58.6			145.5	24.5			292.1	39.3		
Once a week	405.3	47.7	-10.8%	0.77	135.0	19.9	-7.2%	0.95	265.5	32.0	-9.1%	0.83
> once a week	487.3	92.9	7.3%	0.94	212.3	38.8	45.9%	0.27	276.2	62.3	-5.5%	0.97
Physical activity [d]												
Low	354.0	65.9			106.4	27.5			250.4	43.9		
Medium	407.9	59.5	15.2%	0.77	126.1	24.8	18.5%	0.81	271.2	39.6	8.3%	0.91
High	528.9	60.7	49.4%	0.11	208.0	25.3	95.6%	0.02	307.0	40.4	22.6%	0.55
Total fat intake												
Low	445.9	59.4			151.5	25.2			270.2	39.7		
Medium	415.7	58.0	-6.8%	0.91	142.6	24.6	-5.9%	0.98	283.7	38.7	5.0%	0.96
High	440.1	60.9	-1.3%	1.00	153.5	25.8	1.4%	0.61	274.8	40.7	1.7%	1.00
Saturated fat intake												
Low	501.0	56.1			176.7	24.1			321.1	37.7		
Medium	316.1	57.3	-36.9%	0.05	102.8	24.6	-41.8%	0.07	198.5	38.5	-38.2%	0.05
High	473.0	56.1	-5.6%	0.92	164.5	24.1	-6.9%	0.91	303.9	37.7	-5.3%	0.93

Red meat intake												
Low	451.4	58.1			146.5	25.1			309.6	39.9		
Medium	479.3	60.9	6.2%	0.93	171.9	25.9	17.3%	0.71	281.3	41.1	-9.1%	0.84
High	366.0	62.8	-18.9%	0.54	130.0	26.1	-11.2%	0.87	235.8	41.5	-23.8%	0.37
Processed meat intake												
Low	445.7	65.5			185.9	27.5			275.4	43.9		
Medium	417.9	59.0	-6.3%	0.93	141.5	24.7	-23.9%	0.35	272.0	39.6	-1.3%	1.00
High	437.5	71.8	-1.8%	1.00	118.5	30.1	-36.2%	0.25	281.7	48.2	2.3%	0.99
Fruit intake												
Low	463.4	65.4			150.3	27.8			288.9	43.7		
Medium	423.9	57.2	-8.5%	0.87	148.4	24.3	-1.3%	1.00	275.2	38.2	-4.8%	0.96
High	422.5	55.0	-8.8%	0.85	148.9	23.4	-0.9%	1.00	268.5	36.8	-7.1%	0.91
Dietary fiber intake												
Low	509.0	63.3			158.2	26.9			329.7	42.1		
Medium	415.9	58.1	-18.3%	0.45	164.8	24.7	4.2%	0.98	248.6	38.6	-24.6%	0.27
High	374.2	63.9	-26.5%	0.28	124.0	27.2	-21.7%	0.61	248.8	42.5	-24.5%	0.35
Total calcium intake [e]												
Low	520.8	65.9			174.7	28.2			319.3	43.7		
Medium	405.9	63.1	-22.1%	0.37	154.1	27.0	-11.8%	0.82	261.6	41.8	-18.0%	0.53
High	395.9	67.3	-24.0%	0.37	134.2	28.8	-23.2%	0.54	249.4	44.6	-21.9%	0.47
Serum 25-OH vitamin D [f]												
Low	421.3	51.0			138.0	21.7			265.9	34.1		
Medium	389.7	60.8	-7.5%	0.90	151.6	25.9	9.9%	0.90	257.2	40.6	-3.3%	0.98
High	524.3	69.6	24.4%	0.41	166.6	29.7	20.7%	0.66	323.2	46.5	21.5%	0.52

Abbreviations: OD, optical density; SE, standard error; NSAID, nonsteroidal anti-inflammatory drug.

[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.

[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.

[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.

[d] Physical activity was measured by asking participants how much time they spend on moderate and vigorous activities during the past week, the results were converted to metabolic equivalents of task minutes (MET).
[e] Total calcium defined as dietary calcium plus supplement calcium intake.
[f] Low, medium, and high categories were defined as: current alcohol intake (drinks/day), none, ≤0.8, >0.8; Serum 25-OH vitamin D (ng/ml), <20, 20 – 30, ≥30;

Table 4. Bcl-2 expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to categories of participant characteristics (n = 104) [a].

Participant characteristics	Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Current alcohol intake [f]												
None	745.5	112.1			78.8	24.8			688.5	96.6		
Low	955.6	96.8	28.2%	0.32	77.6	21.4	-1.6%	1.00	858.5	84.2	24.7%	0.35
High	791.7	105.1	6.2%	0.94	44.1	23.3	-44.1%	0.52	737.1	91.7	7.1%	0.92
Multivitamin use												
No	632.7	109.9			42.9	24.0			589.2	92.9		
Yes	947.9	73.2	49.8%	0.04	80.1	16.0	86.6%	0.26	858.6	62.5	45.7%	0.03
Non-aspirin NSAID use												
None	1,029.9	211.5			119.1	46.8			908.2	84.8		
Once a week	707.1	75.5	-31.3%	0.03	36.9	16.7	-69.0%	0.01	672.2	63.2	-26.0%	0.07
> once a week	877.4	349.3	-14.8%	0.57	64.6	77.3	-45.7%	0.26	791.6	119.9	-12.8%	0.66
Physical activity [d]												
Low	711.3	107.4			47.8	23.8			664.9	93.4		
Medium	936.9	93.8	31.7%	0.21	79.7	20.8	66.8%	0.51	854.0	81.3	28.4%	0.24
High	847.3	92.3	19.1%	0.55	72.9	20.5	52.6%	0.65	776.3	80.3	16.8%	0.59
Total fat intake												
Low	819.7	95.8			57.1	21.8			762.6	82.9		
Medium	1,023.7	91.4	24.9%	0.20	86.4	20.8	51.5%	0.50	937.2	79.1	22.9%	0.21
High	660.5	101.4	-19.4%	0.48	58.2	23.0	2.0%	1.00	602.3	87.7	-21.0%	0.38
Saturated fat intake												
Low	957.0	98.6			72.0	21.5			885.0	85.7		

Medium	846.3	95.6	-11.6%	0.63	92.0	20.8	27.8%	0.72	754.3	83.1	-14.8%	0.44
High	705.5	97.6	-26.3%	0.17	41.8	21.3	-42.0%	0.56	663.7	84.8	-25.0%	0.17
Red meat intake												
Low	897.6	103.4			70.5	22.5			827.1	89.8		
Medium	788.1	99.4	-12.2%	0.67	53.8	21.7	-23.7%	0.82	734.3	86.4	-11.2%	0.69
High	810.9	101.2	-9.7%	0.80	76.5	22.0	8.5%	0.98	734.4	87.9	-11.2%	0.71
Processed meat intake												
Low	873.4	107.4			91.9	23.5			773.1	96.1		
Medium	927.3	102.7	6.2%	0.91	100.7	22.5	9.6%	0.95	834.9	91.0	8.0%	0.85
High	711.9	118.3	-18.5%	0.59	13.6	25.9	-85.2%	0.11	700.5	104.6	-9.4%	0.87
Fruit intake												
Low	707.7	112.2			40.8	24.2			666.9	98.1		
Medium	941.3	92.2	33.0%	0.22	98.2	19.9	140.9%	0.15	843.0	80.6	26.4%	0.31
High	820.8	92.1	16.0%	0.66	56.9	19.9	39.4%	0.83	763.9	80.5	14.5%	0.67
Dietary fiber intake												
Low	809.5	109.6			51.5	22.6			758.0	96.1		
Medium	923.8	93.7	14.1%	0.65	113.0	19.3	119.4%	0.09	810.8	82.2	7.0%	0.88
High	750.1	130.2	-7.3%	0.93	31.6	26.9	-38.7%	0.83	718.5	114.1	-5.2%	0.96
Total calcium intake [e]												
Low	981.6	119.1			87.4	26.2			894.5	103.7		
Medium	978.8	94.9	-0.3%	1.00	95.3	20.9	9.0%	0.96	880.7	82.6	-1.5%	0.99
High	537.1	119.0	-45.3%	0.05	18.9	26.2	-78.3%	0.20	520.6	103.7	-41.8%	0.06
Serum 25-OH vitamin D [f]												
Low	880.0	88.3			78.3	19.3			799.5	76.8		
Medium	835.6	95.7	-5.1%	0.92	65.9	20.9	-15.8%	0.88	766.8	83.2	-4.1%	0.94
High	733.2	134.1	-16.7%	0.62	47.0	29.1	-40.0%	0.64	694.7	116.7	-13.1%	0.73

Abbreviations: OD, optical density; SE, standard error; NSAID, nonsteroidal anti-inflammatory drug.
[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.
[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.
[d] Physical activity was measured by asking participants how much time they spend on moderate and vigorous activities during the past week, the results were converted to metabolic equivalents of task minutes (MET).
[e] Total calcium defined as dietary calcium plus supplement calcium intake.
[f] Low, medium, and high categories were defined as: current alcohol intake (drinks/day), none, ≤0.8, >0.8; Serum 25-OH vitamin D (ng/ml), <20, 20 – 30, ≥30;

Table 5. Mib-1 expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to categories of participant characteristics (n = 104) [a].

Participant characteristics	Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Current alcohol intake [f]												
None	1,119.9	101.4			50.6	12.2			1,069.4	96.0		
Low	1,179.6	85.4	5.3%	0.86	60.5	10.3	19.7%	0.76	1,119.1	80.9	4.6%	0.89
High	1,429.1	97.5	27.6%	0.07	85.4	11.8	68.9%	0.10	1,343.7	92.3	25.7%	0.10
Multivitamin use												
No	1,370.9	91.5			79.4	11.0			1,291.5	86.3		
Yes	1,176.5	64.0	-14.2%	0.09	58.5	7.7	-26.3%	0.12	1,118.0	60.4	-13.4%	0.10
Non-aspirin NSAID use												
None	1,323.9	90.5			64.4	10.9			1,259.5	85.3		
Once a week	1,205.8	74.5	-8.9%	0.52	70.1	8.9	8.9%	0.89	1,135.6	70.2	-9.8%	0.45
> once a week	1,153.1	146.7	-12.9%	0.53	49.7	17.6	-22.9%	0.71	1,103.4	138.3	-12.4%	0.55
Physical activity [d]												
Low	1,157.6	104.3			63.8	12.6			1,093.8	98.3		
Medium	1,317.9	92.5	13.8%	0.42	71.9	11.2	12.6%	0.85	1,246.1	87.2	13.9%	0.41
High	1,242.6	92.0	7.3%	0.77	60.7	11.1	-4.8%	0.98	1,181.9	86.7	8.1%	0.73
Total fat intake												
Low	1,351.2	116.0			60.5	14.0			1,290.7	109.3		
Medium	1,198.6	90.5	-11.3%	0.45	62.9	10.9	4.0%	0.98	1,135.7	85.2	-12.0%	0.40
High	1,173.3	118.5	-13.2%	0.54	72.9	14.3	20.6%	0.80	1,100.4	111.6	-14.7%	0.46

Saturated fat intake												
Low	1,346.7	94.0			63.2	41.0			1,283.5	88.5		
Medium	1,218.8	94.8	-9.5%	0.53	73.5	51.0	16.3%	0.74	1,145.4	89.3	-10.8%	0.44
High	1,154.5	95.2	-14.3%	0.29	60.1	37.5	-5.0%	0.97	1,094.4	89.7	-14.7%	0.26
Red meat intake												
Low	1,215.1	96.3			68.3	11.6			1,146.8	90.9		
Medium	1,147.2	95.6	-5.6%	0.84	56.1	11.6	-17.9%	0.69	1,091.1	90.2	-4.9%	0.88
High	1,357.6	97.8	11.7%	0.51	71.1	11.8	4.1%	0.98	1,286.5	92.2	12.2%	0.49
Processed meat intake												
Low	1,205.6	107.2			52.8	12.8			1,152.8	101.2		
Medium	1,192.1	94.5	-1.1%	0.99	64.6	11.3	22.2%	0.70	1,127.6	89.2	-2.2%	0.97
High	1,325.1	116.8	9.9%	0.73	79.2	13.9	49.9%	0.37	1,245.9	110.3	8.1%	0.80
Fruit intake												
Low	1,378.0	104.6			86.1	12.4			1,291.9	99.1		
Medium	1,274.2	87.7	-7.5%	0.65	69.9	10.4	-18.8%	0.49	1,204.3	83.1	-6.8%	0.71
High	1,111.3	86.5	-19.4%	0.10	46.4	10.2	-46.1%	0.03	1,064.9	82.0	-17.6%	0.15
Dietary fiber intake												
Low	1,429.4	96.4			82.1	11.8			1,347.3	90.8		
Medium	1,242.0	90.5	-13.1%	0.27	51.2	11.1	-37.6%	0.11	1,190.8	85.3	-11.6%	0.35
High	1,043.1	98.3	-27.0%	0.02	62.2	12.0	-24.2%	0.44	980.9	92.6	-27.2%	0.02
Total calcium intake [e]												
Low	1,440.8	118.5			89.5	14.0			1,351.3	112.2		
Medium	1,195.5	96.8	-17.0%	0.20	55.1	11.4	-38.5%	0.11	1,140.4	91.7	-15.6%	0.26
High	1,143.6	114.8	-20.6%	0.20	49.9	13.6	-44.3%	0.14	1,093.7	108.8	-19.1%	0.25
Serum 25-OH vitamin D [f]												
Low	1,197.1	85.9			62.0	10.4			1,135.0	81.1		

Medium	1,216.1	93.3	1.6%	0.98	64.8	11.2	4.5%	0.98	1,151.3	88.0	1.4%	0.99
High	1,357.4	120.0	13.4%	0.50	72.5	14.5	16.9%	0.81	1,284.9	113.2	13.2%	0.50

Abbreviations: OD, optical density; SE, standard error; NSAID, nonsteroidal anti-inflammatory drug.
[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.
[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.
[d] Physical activity was measured by asking participants how much time they spend on moderate and vigorous activities during the past week, the results were converted to metabolic equivalents of task minutes (MET).
[e] Total calcium defined as dietary calcium plus supplement calcium intake.
[f] Low, medium, and high categories were defined as: current alcohol intake (drinks/day), none, ≤0.8, >0.8; Serum 25-OH vitamin D (ng/ml), <20, 20 – 30, ≥30;

Table 6. P21 expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to categories of participant characteristics (n = 104) [a].

Participant characteristics	Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Current alcohol intake [f]												
None	680.8	81.6			581.7	60.9			102.8	31.8		
Low	733.5	75.2	7.7%	0.85	623.3	56.1	7.1%	0.83	105.7	29.3	2.9%	1.00
High	675.1	83.2	-0.8%	1.00	577.9	62.0	-0.7%	1.00	74.8	32.4	-27.2%	0.78
Multivitamin use												
No	753.0	753.0			657.2	65.3			76.7	34.2		
Yes	668.4	668.4	-11.2%	0.47	562.7	44.1	-14.4%	0.28	104.5	23.1	36.2%	0.54
Non-aspirin NSAID use												
None	890.4	178.7			664.1	62.8			132.4	32.8		
Once a week	639.0	64.3	-28.2%	0.29	567.8	45.6	-14.5%	0.39	76.5	23.8	-42.2%	0.32
> once a week	508.6	294.6	-42.9%	0.50	546.0	88.4	-17.8%	0.47	79.9	46.1	-39.6%	0.58
Physical activity [d]												
Low	720.1	86.3			628.0	63.8			73.1	33.2		
Medium	692.5	78.0	-3.8%	0.96	547.3	57.6	-12.9%	0.53	125.2	30.0	71.2%	0.39
High	686.6	79.5	-4.6%	0.95	610.4	58.8	-2.8%	0.97	87.9	30.6	20.1%	0.93
Total fat intake												
Low	739.6	78.6			620.2	58.8			69.4	29.8		
Medium	731.0	78.0	-1.2%	1.00	590.3	58.3	-4.8%	0.91	148.0	29.5	113.1%	0.12
High	632.2	77.6	-14.5%	0.53	577.5	58.1	-6.9%	0.83	67.3	29.4	-3.0%	1.00
Saturated fat intake												

Low	780.7	78.0			646.4	58.6			142.3	30.5		
Medium	589.0	78.0	-24.5%	0.16	508.4	58.6	-21.3%	0.18	57.6	30.5	-59.6%	0.10
High	712.2	73.8	-8.8%	0.75	622.4	55.4	-3.7%	0.94	82.8	28.8	-41.9%	0.29
Red meat intake												
Low	588.8	86.7			547.4	64.6			77.6	33.9		
Medium	798.3	85.5	35.6%	0.20	629.5	63.8	15.0%	0.60	98.3	33.5	26.7%	0.88
High	713.0	81.4	21.1%	0.49	610.1	60.7	11.5%	0.72	108.4	31.9	39.7%	0.75
Processed meat intake												
Low	617.7	85.7			573.2	63.1			108.6	33.6		
Medium	789.6	88.1	27.8%	0.27	685.8	64.9	19.6%	0.34	82.7	34.6	-23.9%	0.81
High	713.1	97.6	15.4%	0.76	546.4	71.9	-4.7%	0.96	90.0	38.3	-17.1%	0.93
Fruit intake												
Low	708.4	84.4			554.9	63.0			96.4	33.2		
Medium	744.5	73.2	5.1%	0.93	660.3	54.6	19.0%	0.34	118.4	28.8	22.8%	0.83
High	643.2	74.5	-9.2%	0.80	562.1	55.6	1.3%	0.99	70.2	29.3	-27.1%	0.78
Dietary fiber intake												
Low	678.1	84.1			602.2	62.4			57.8	32.4		
Medium	730.1	76.5	7.7%	0.86	632.9	56.8	5.1%	0.91	100.9	29.5	74.7%	0.52
High	685.1	96.9	1.0%	1.00	540.1	72.0	-10.3%	0.78	136.9	37.4	136.9%	0.26
Total calcium intake [e]												
Low	722.2	95.9			592.7	51.3			113.8	37.5		
Medium	623.8	78.6	-13.6%	0.63	578.9	59.4	-2.3%	0.98	74.8	30.7	-34.3%	0.63
High	752.6	93.3	4.2%	0.97	614.7	78.9	3.7%	0.97	96.0	36.4	-15.6%	0.93
Serum 25-OH vitamin D [f]												
Low	598.2	68.7			514.2	71.3			67.6	27.2		
Medium	753.7	79.6	26.0%	0.26	643.5	58.5	25.1%	0.20	93.9	31.5	38.9%	0.77
High	822.6	105.7	37.5%	0.18	690.1	69.4	34.2%	0.16	150.9	41.8	123.2%	0.22

Abbreviations: OD, optical density; SE, standard error; NSAID, nonsteroidal anti-inflammatory drug.
[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.
[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.
[d] Physical activity was measured by asking participants how much time they spend on moderate and vigorous activities during the past week, the results were converted to metabolic equivalents of task minutes (MET).
[e] Total calcium defined as dietary calcium plus supplement calcium intake.
[f] Low, medium, and high categories were defined as: current alcohol intake (drinks/day), none, ≤0.8, >0.8; Serum 25-OH vitamin D (ng/ml), <20, 20 – 30, ≥30;

Supplements

Supplement Table 1. Ratios of mean biomarker expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to categories of participant characteristics (n = 104) [a].

Participant characteristics	BAX/BCL-2			BAX/mib-1			p21/mib-1		
	Ratio of mean biomarker expression	Proportional difference [b]	*P* value [c]	Ratio of mean biomarker expression	Proportional difference [b]	*P* value [c]	Ratio of mean biomarker expression	Proportional difference [b]	*P* value [c]
Current alcohol intake [f]									
None	0.74			0.49			0.62		
Low	0.52	-30.1%	0.40	0.33	-33.3%	0.19	0.63	0.8%	1.00
High	0.65	-12.3%	0.87	0.36	-27.8%	0.38	0.42	-32.2%	0.04
Multivitamin use									
No	0.70			0.34			0.59		
Yes	0.60	-13.9%	0.63	0.41	22.9%	0.38	0.54	-8.5%	0.50
Non-aspirin NSAID use									
None	0.56			0.38			0.60		
Once a week	0.66	18.8%	0.78	0.37	-1.8%	1.00	0.53	-11.6%	0.58
> once a week	0.69	24.4%	0.81	0.48	27.2%	0.65	0.61	1.5%	0.99
Physical activity [d]									
Low	0.63			0.31			0.56		
Medium	0.79	25.4%	0.60	0.36	16.7%	0.85	0.55	-2.8%	0.97
High	0.45	-29.4%	0.52	0.48	55.1%	0.22	0.57	1.9%	0.99
Total fat intake									
Low	0.65			0.32			0.52		
Medium	0.44	-31.6%	0.43	0.40	23.4%	0.69	0.64	22.0%	0.28
High	0.80	22.8%	0.68	0.44	36.5%	0.61	0.53	1.0%	1.00

Saturated fat intake									
Low	0.71			0.41			0.44		
Medium	0.33	-52.7%	0.08	0.28	-31.9%	0.33	0.54	21.2%	0.40
High	0.82	15.2%	0.79	0.48	17.7%	0.71	0.69	56.5%	0.05
Red meat intake									
Low	0.57			0.37			0.51		
Medium	0.87	52.8%	0.22	0.46	23.5%	0.60	0.63	22.7%	0.22
High	0.47	-16.7%	0.84	0.33	-10.7%	0.90	0.54	5.5%	0.90
Processed meat intake									
Low	0.65			0.41			0.55		
Medium	0.70	8.9%	0.94	0.42	1.9%	1.00	0.67	21.1%	0.22
High	0.56	-13.8%	0.91	0.34	-16.6%	0.84	0.48	-12.7%	0.68
Fruit intake									
Low	0.76			0.37			0.53		
Medium	0.53	-29.7%	0.41	0.37	0.4%	1.00	0.61	14.9%	0.51
High	0.64	-15.9%	0.77	0.43	17.1%	0.78	0.54	2.3%	0.98
Dietary fiber intake									
Low	0.70			0.39			0.51		
Medium	0.66	-6.1%	0.96	0.40	0.7%	1.00	0.62	23.1%	0.22
High	0.50	-28.6%	0.96	0.38	-3.1%	0.99	0.55	9.3%	0.82
Total calcium intake [e]									
Low	0.79			0.40			0.58		
Medium	0.48	-38.8%	0.18	0.41	2.0%	1.00	0.56	-2.7%	0.97
High	0.63	-19.9%	0.65	0.35	-11.6%	0.89	0.55	-4.5%	0.95
Serum 25-OH vitamin D [f]									
Low	0.61			0.38			0.48		
Medium	0.55	-9.9%	0.93	0.35	-7.1%	0.95	0.63	30.0%	0.08
High	0.80	30.6%	0.64	0.47	24.2%	0.61	0.63	30.9%	0.15

Abbreviations: OD, optical density; SE, standard error; NSAID, nonsteroidal anti-inflammatory drug.

[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.
[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.
[d] Physical activity was measured by asking participants how much time they spend on moderate and vigorous activities during the past week, the results were converted to metabolic equivalents of task minutes (MET).
[e] Total calcium defined as dietary calcium plus supplement calcium intake.
[f] Low, medium, and high categories were defined as: current alcohol intake (drinks/day), none, ≤0.8, >0.8; Serum 25-OH vitamin D (ng/ml), <20, 20 – 30, ≥30;

Supplement Table 2. Mean biomarker expression in ϕh[a] of crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to categories of participant characteristics (n = 104).

	BAX			Bcl-2			Mib-1			p21		
Participant characteristics	**ϕh**	**Proportional difference [b]**	***P* value [c]**	**ϕh**	**Proportional difference [b]**	***P* value [c]**	**ϕh**	**Proportional difference [b]**	***P* value [c]**	**ϕh**	**Proportional difference [b]**	***P* value [c]**
Current alcohol intake [f]												
None	0.30			0.09			0.05			0.89		
Low	0.32	3.7%	0.95	0.07	-23.0%	0.61	0.05	-1.0%	1.00	0.90	1.2%	0.91
High	0.37	20.0%	0.32	0.06	-34.7%	0.39	0.06	17.4%	0.68	0.91	2.7%	0.65
Multivitamin use												
No	0.33			0.08			0.06			0.92		
Yes	0.33	-0.7%	0.95	0.07	-6.9%	0.82	0.05	-13.7%	0.38	0.89	-2.6%	0.44
Non-aspirin NSAID use												
None	0.32			0.11			0.05			0.87		
Once a week	0.31	-5.1%	0.89	0.06	-44.8%	0.05	0.05	10.9%	0.79	0.92	6.0%	0.11
> once a week	0.43	33.8%	0.11	0.07	-32.9%	0.37	0.04	-20.3%	0.68	0.89	2.8%	0.75
Physical activity [d]												
Low	0.27			0.07			0.05			0.92		
Medium	0.31	15.5%	0.53	0.09	27.1%	0.63	0.05	-1.1%	1.00	0.88	-3.9%	0.36
High	0.39	44.8%	0.02	0.06	-11.0%	0.92	0.05	-12.8%	0.75	0.90	-2.4%	0.69
Total fat intake												
Low	0.35			0.07			0.05			0.92		
Medium	0.31	-13.0%	0.47	0.08	18.4%	0.80	0.05	0.0%	1.00	0.86	-7.0%	0.04
High	0.32	-9.0%	0.71	0.08	17.3%	0.87	0.06	20.9%	0.71	0.92	-0.1%	1.00
Saturated fat intake												
Low	0.32			0.07			0.04			0.87		

Medium	0.31	-3.3%	0.96	0.08	14.0%	0.87	0.06	31.7%	0.27	0.92	4.7%	0.27
High	0.35	10.7%	0.67	0.08	9.1%	0.95	0.05	10.4%	0.87	0.91	4.2%	0.34
Red meat intake												
Low	0.32			0.06			0.05			0.92		
Medium	0.32	-0.1%	1.00	0.07	19.6%	0.83	0.05	-5.3%	0.95	0.90	-1.9%	0.82
High	0.34	6.3%	0.88	0.09	45.1%	0.44	0.05	-3.2%	0.98	0.89	-3.2%	0.56
Processed meat intake												
Low	0.36			0.09			0.04			0.89		
Medium	0.29	-18.6%	0.24	0.07	-20.5%	0.68	0.05	18.9%	0.67	0.91	1.6%	0.87
High	0.33	-6.8%	0.88	0.06	-30.4%	0.60	0.06	47.0%	0.27	0.90	0.8%	0.97
Fruit intake												
Low	0.33			0.08			0.06			0.90		
Medium	0.34	2.9%	0.97	0.09	18.7%	0.79	0.05	-14.8%	0.58	0.88	-2.2%	0.71
High	0.31	-7.1%	0.81	0.06	-16.2%	0.83	0.04	-33.0%	0.11	0.91	1.0%	0.94
Dietary fiber intake												
Low	0.36			0.07			0.05			0.93		
Medium	0.37	2.9%	0.96	0.11	66.8%	0.09	0.04	-16.0%	0.64	0.89	-3.9%	0.37
High	0.26	-25.8%	0.13	0.04	-40.0%	0.57	0.06	8.2%	0.91	0.87	-5.8%	0.23
Total calcium intake [e]												
Low	0.34			0.09			0.06			0.89		
Medium	0.34	1.7%	0.98	0.08	-10.3%	0.90	0.04	-27.6%	0.27	0.91	2.3%	0.73
High	0.35	5.1%	0.88	0.05	-43.9%	0.36	0.05	-18.5%	0.64	0.91	2.1%	0.84
Serum 25-OH vitamin D [f]												
Low	0.33			0.07			0.05			0.91		
Medium	0.30	-10.6%	0.65	0.07	0.5%	1.00	0.05	10.5%	0.83	0.90	-1.0%	0.93
High	0.37	11.7%	0.62	0.08	14.1%	0.92	0.05	4.8%	0.97	0.88	-3.6%	0.58

Abbreviations: SE, standard error; NSAID, nonsteroidal anti-inflammatory drug.

[a] ϕh is the ratio of mean biomarker expression in upper 40% of crypts relative to that in whole crypts, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.
[b] Proportional difference defined as [(comparison group value - reference group value)/ reference group value] *100%.
[c] P value for the difference between the comparison group and the reference group, from general linear model.
[d] Physical activity was measured by asking participants how much time they spend on moderate and vigorous activities during the past week, the results were converted to the metabolic equivalent of task minutes (MET).
[e] Total calcium defined as dietary calcium plus supplement calcium intake
[f] Low, medium, and high categories were defined as: current alcohol intake (drinks/day), none, ≤0.8, >0.8; Serum 25-OH vitamin D (ng/ml), <20, 20 – 30, ≥30;

Supplement Table 3. BAX expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to additional categories of participant characteristics (n = 104) [a].

Participant characteristics		Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
		Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Age [e]													
	Low	366.8	61.3			139.9	26.6			243.1	40.7		
	Medium	365.9	55.1	-0.3%	1.00	127.1	23.9	-9.2%	0.91	227.5	36.6	-6.4%	0.94
	High	559.0	57.8	52.4%	0.05	180.1	25.0	28.7%	0.46	356.7	38.4	46.7%	0.10
Sex													
	Male	408.6	51.4			154.2	21.8			250.6	34.2		
	Female	456.5	48.6	11.7%	0.53	144.5	20.7	-6.3%	0.76	299.7	32.4	19.6%	0.33
Race													
	White	451.0	38.7			155.5	16.4			280.8	25.9		
	Other race	378.4	74.1	-16.1%	0.40	128.0	31.4	-17.7%	0.45	261.4	49.6	-6.9%	0.74
Education [e]													
	Low	536.6	57.8			151.6	24.8			359.4	37.7		
	Medium	364.0	56.9	-32.2%	0.08	125.5	24.4	-17.2%	0.69	233.6	37.2	-35.0%	0.04
	High	390.2	65.3	-27.3%	0.21	175.5	28.0	15.7%	0.76	221.9	42.7	-38.2%	0.04
BMI [e]													
	Low	469.6	72.3			161.7	30.7			292.5	48.4		
	Medium	448.5	53.1	-4.5%	0.96	157.2	22.5	-2.8%	0.99	288.2	35.5	-1.5%	1.00
	High	397.2	55.2	-15.4%	0.64	133.2	23.4	-17.6%	0.67	254.3	36.9	-13.1%	0.74
Vegetable intake													
	Low	422.1	55.6			132.5	23.0			289.6	37.4		

	Medium	473.6	59.6	12.2%	0.76	196.1	24.7	48.0%	0.12	277.6	40.1	7.4%	0.96
	High	379.5	60.9	-10.1%	0.83	120.9	25.2	-8.7%	0.92	258.6	41.0	9.6%	0.91
Current smoker													
	Low	408.7	44.2			142.2	18.8			265.6	29.6		
	Medium	467.6	51.4	14.4%	0.40	158.4	21.8	11.4%	0.58	290.8	34.4	9.5%	0.58
Adenoma [d]													
	Low risk	391.3	43.7			142.8	18.6			249.3	29.2		
	High	478.8	55.6	22.4%	0.24	158.9	23.6	11.2%	0.61	318.5	37.2	27.8%	0.16

Abbreviations: OD, optical density; SE, standard error; BMI, body mass index;
[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.

[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.

[d] Low-risk adenoma defined as with less or equal than one and no advanced adenoma, high-risk adenoma defined as with multiple adenomas or with at least one advanced adenoma .
[e] Low, medium, and high categories defined as: age (yr.), 47 - 54, 55 – 63, >63; education (yr.), 0 - 10,11 – 13, >13; BMI (kg/m2), <25, 25 – 30, ≥30;

Supplement Table 4. Bcl-2 expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to additional categories of participant characteristics (n = 104) [a].

Participant characteristics		Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
		Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Age [e]													
	Low	850.0	104.5			62.3	22.9			787.7	90.6		
	Medium	763.8	99.3	-10.1%	0.78	70.1	21.7	12.5%	0.96	693.7	86.1	-11.9%	0.68
	High	884.3	98.9	4.0%	0.96	68.5	21.6	9.9%	0.97	815.8	85.7	3.6%	0.97
Sex													
	Male	783.6	84.1			42.2	18.3			741.5	73.1		
	Female	882.7	85.5	12.6%	0.46	93.0	18.6	120.5%	0.09	789.7	74.3	6.5%	0.68
Race													
	White	825.0	62.5			73.6	13.5			751.3	54.3		
	Other race	860.2	135.7	4.3%	0.83	43.7	29.4	-40.7%	0.39	816.6	117.8	8.7%	0.64
Education [e]													
	Low	912.6	94.2			67.8	20.5			844.8	81.6		
	Medium	795.0	96.2	-12.9%	0.62	51.8	20.9	-23.5%	0.82	743.2	83.3	-12.0%	0.62
	High	772.0	109.1	-15.4%	0.55	85.6	23.7	26.2%	0.81	686.5	94.4	-18.7%	0.38
BMI [e]													
	Low	820.0	120.8			50.5	26.6			769.5	105.2		
	Medium	873.1	92.2	6.5%	0.91	63.0	20.3	24.8%	0.90	810.1	80.3	5.3%	0.93
	High	802.3	84.0	-2.2%	0.99	79.7	18.5	58.0%	0.54	722.5	73.2	-6.1%	0.90
Vegetable intake													
	Low	732.1	92.2			40.1	19.7			692.0	80.4		
	Medium	836.5	100.5	14.3%	0.69	82.0	21.5	104.6%	0.30	754.5	87.6	9.0%	0.84

	High	955.7	103.1	30.5%	0.20	85.1	22.0	112.3%	0.24	870.7	89.8	25.8%	0.26
Current smoker													
	Low	856.7	74.0			59.8	16.2			796.9	64.2		
	Medium	801.7	84.8	-6.4%	0.65	76.7	18.5	28.3%	0.52	725.0	73.6	-9.0%	0.49
Adenoma [d]													
	Low risk	822.4	73.3			58.9	15.9			763.5	63.7		
	High	846.7	89.5	3.0%	0.84	79.1	19.4	34.2%	0.45	767.7	77.8	0.5%	0.97

Abbreviations: OD, optical density; SE, standard error; BMI, body mass index;
[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.

[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.

[d] Low-risk adenoma defined as with less or equal than one and no advanced adenoma, high-risk adenoma defined as with multiple adenomas or with at least one advanced adenoma .
[e] Low, medium, and high categories defined as: age (yr.), 47 - 54, 55 – 63, >63; education (yr.), 0 - 10,11 – 13, >13; BMI (kg/m2), <25, 25 – 30, ≥30;

Supplement Table 5. Mib-1 expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to additional categories of participant characteristics (n = 104) [a].

Participant characteristics	Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Age [e]												
Low	1,148.8	99.1			64.5	11.9			1084.3	93.5		
Medium	1,306.0	89.6	13.7%	0.40	74.4	10.7	15.4%	0.76	1231.6	84.5	13.6%	0.40
High	1,254.6	91.4	9.2%	0.65	56.8	10.9	-11.9%	0.85	1197.8	86.2	10.5%	0.58
Sex												
Male	1,269.8	79.9			62.6	9.6			1207.2	75.3		
Female	1,213.9	75.8	-4.4%	0.63	67.9	9.1	8.5%	0.70	1146.0	71.4	-5.1%	0.57
Race												
White	1,248.3	61.5			63.6	7.4			1184.6	58.0		
Other race	1,214.7	117.9	-2.7%	0.81	71.1	14.1	11.7%	0.65	1143.7	111.2	-3.5%	0.75
Education [e]												
Low	1,298.7	87.8			60.6	10.5			1238.1	82.9		
Medium	1,305.4	89.1	0.5%	1.00	81.9	10.6	35.1%	0.27	1223.4	84.2	-1.2%	0.99
High	1,082.8	102.6	-16.6%	0.21	50.7	12.2	-16.4%	0.77	1032.1	97.0	-16.6%	0.21
BMI [e]												
Low	1,153.8	116.1			62.0	13.8			1091.8	109.5		
Medium	1,170.1	85.2	1.4%	0.99	63.3	10.1	2.1%	1.00	1106.8	80.3	1.4%	0.99
High	1,365.8	88.4	18.4%	0.25	69.6	10.5	12.3%	0.86	1296.2	83.3	18.7%	0.23
Vegetable intake												
Low	1,279.6	88.6			66.0	10.5			1213.6	83.7		
Medium	1,181.3	97.8	-7.7%	0.70	53.5	11.6	-18.9%	0.66	1127.8	92.3	-7.1%	0.73
High	1,253.6	97.5	-2.0%	0.97	77.0	11.6	16.6%	0.71	1176.6	92.0	-3.0%	0.94
Current smoker												

Low	1,196.3	69.6			64.5	8.4			1131.8	65.6		
Medium	1,299.9	80.8	8.7%	0.34	66.5	9.7	3.1%	0.88	1233.3	76.2	9.0%	0.32
Adenoma [d]												
Low risk	1,192.5	69.7			65.4	8.4			1127.1	65.7		
High	1,315.5	89.0	10.3%	0.30	65.4	10.7	0.0%	1.00	1250.1	83.9	10.9%	0.27

Abbreviations: OD, optical density; SE, standard error; BMI, body mass index;
[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.

[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.

[d] Low-risk adenoma defined as with less or equal than one and no advanced adenoma, high-risk adenoma defined as with multiple adenomas or with at least one advanced adenoma .
[e] Low, medium, and high categories defined as: age (yr.), 47 - 54, 55 – 63, >63; education (yr.), 0 - 10,11 – 13, >13; BMI (kg/m2), <25, 25 – 30, ≥30;

Supplement Table 6. P21 expression in crypts of normal-appearing rectal mucosa of sporadic colorectal adenoma patients according to additional categories of participant characteristics (n = 104) [a].

Participant characteristics	Whole crypts				Upper 40% of crypts				Lower 60% of crypts			
	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]	Mean biomarker expression (OD)	SE	Proportional difference [b]	*P* value [c]
Age [e]												
Low	692.3	85.3			625.4	63.6			66.9	33.0		
Medium	698.2	76.6	0.9%	1.00	578.7	57.1	-7.5%	0.81	119.5	29.6	78.6%	0.40
High	681.1	75.9	-1.6%	0.99	587.1	56.6	-6.1%	0.87	94.0	29.4	40.6%	0.77
Sex												
Male	644.4	66.8			577.4	50.0			66.9	25.8		
Female	737.5	67.8	14.5%	0.37	613.9	50.8	6.3%	0.64	123.6	26.2	84.6%	0.16
Race												
White	707.4	49.8			604.4	37.1			103.0	19.5		
Other race	627.3	101.2	-11.3%	0.49	562.3	75.4	-7.0%	0.63	65.0	39.6	-36.9%	0.41
Education [e]												
Low	773.1	75.5			630.6	56.4			142.5	29.1		
Medium	678.1	77.9	-12.3%	0.61	620.7	58.2	-1.6%	0.99	57.5	30.0	-59.7%	0.10
High	595.1	86.7	-23.0%	0.24	517.4	64.8	-17.9%	0.34	77.7	33.4	-45.5%	0.27
BMI [e]												
Low	651.0	99.1			574.2	73.6			76.8	38.6		
Medium	681.7	77.7	4.7%	0.96	566.2	57.7	-1.4%	0.99	115.4	30.2	50.3%	0.65
High	718.4	71.4	10.3%	0.79	632.8	53.1	10.2%	0.73	85.7	27.8	11.5%	0.97
Vegetable intake												
Low	623.3	74.8			573.0	56.0			50.3	28.8		
Medium	765.7	79.1	22.8%	0.35	646.6	59.2	12.8%	0.60	119.2	30.4	137.0%	0.21
High	689.9	82.6	10.7%	0.78	565.8	61.9	-1.3%	0.99	124.1	31.8	146.8%	0.17
Current smoker												

	Low	674.5	59.0			587.9	44.0			86.6	23.0		
	Medium	710.5	66.8	5.3%	0.69	605.0	49.8	2.9%	0.80	105.5	26.1	21.8%	0.59
Adenoma [d]													
	Low risk	711.4	59.5			606.5	44.4			104.8	23.2		
	High	660.5	72.4	-7.2%	0.61	579.7	54.0	-4.4%	0.72	80.8	28.3	-22.9%	0.54

Abbreviations: OD, optical density; SE, standard error; BMI, body mass index;
[a] Results presented as geometric means, adjusted for batch, age, race, total calcium, and total energy intake, multivitamin supplement use, non-aspirin NSAID use, and adenoma characteristics.

[b] Proportional difference defined as [(comparison group mean - reference group mean) / reference group mean] *100%.
[c] P value for the difference between the comparison group and the reference group, from multivariable general linear model.

[d] Low-risk adenoma defined as with less or equal than one and no advanced adenoma, high-risk adenoma defined as with multiple adenomas or with at least one advanced adenoma .
[e] Low, medium, and high categories defined as: age (yr.), 47 - 54, 55 – 63, >63; education (yr.), 0 - 10,11 – 13, >13; BMI (kg/m2), <25, 25 – 30, ≥30;