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Association of Dietary Polyamines with Incident, Sporadic Colorectal Adenomas

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Master of Public Health

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2009

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Abstract

Association of Dietary Polyamines with Incident, Sporadic Colorectal Adenomas

By, Kehinde Oladunni Raji

PURPOSE: To investigate the association of dietary polyamines with risk of incident, sporadic colorectal adenoma.

METHODS: To investigate the association of dietary polyamines and risk for incident, sporadic colorectal adenoma, data from the Minnesota Cancer Prevention Research Unit case-control study were analyzed. The colonoscopy-/community-based case-control study conducted 1991-1994, enrolled 30-74 year old 564 incident, sporadic, colorectal adenomas participants, 684 polyp-free controls, and 535 community controls. Diet was assessed using a 153-food item semi-quantitative Willet food frequency questionnaire (FFQ). Polyamine exposure was quantified based on responses to the food frequency questionnaire and a previously published report on the polyamine content of select food items. Polyamine intakes were categorized according to the quartiles based on the distribution among the community controls and analyzed using unconditional multivariate logistic regression.

RESULTS: Polyamine intake was inversely associated with risk for colorectal adenomas. The odds ratios (OR) for the highest relative to the lowest category of polyamine intake were 0.57 (95% confidence interval (CI), 0.33 – 1.00; p trend 0.0001) and 0.76 (CI 0.43 – 1.33; p trend 0.04) in the comparisons with the colonoscopy and community-based controls, respectively. The inverse associations tended to be stronger among those with a more positive oxidative balance, no family history of colorectal neoplasms, and women who did not take hormone replacement therapy, and for smaller and distal adenomas.

CONCLUSIONS: These findings suggest that higher polyamine intakes may be associated with lower risk for incident, sporadic colorectal adenomas, perhaps especially for smaller and distal adenomas.

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CHAPTER I: BACKGROUND AND LITERATURE REVIEW

Colorectal adenomas are benign polyps that form along the walls of the colon and can, over time, lead to colorectal cancer (1-6). There are several lines of evidence supporting the adenoma-carcinoma sequence. Most adenomas appear 7-8 years earlier than carcinomas (3, 5). Countries with higher incidences of colorectal cancer also have a higher prevalence of adenomas than do countries with lower CRC incidence rates (2, 5, 7). Analyses of colon tumors containing carcinoma were also found to contain adenomatous tissue (4, 5, 8). These studies provide evidence for the lineage hypothesis of colorectal adenomas as precursors to CRC, and thereby highlight the importance of investigating avenues to potentially prevent or reduce the risk of colorectal adenomas, in order to decrease the incidence of colorectal cancer.

Descriptive epidemiology

Colorectal cancer is the third most common cancer in the United Sates among both men and women (9, 10), with a reported 75,590 men and 71,380 women diagnosed in 2009 (11). Colorectal cancer declined 2.8% in men and 2.2% in women in the U.S. from 1998 to 2005 (10-12), but this decline was specific to adults aged 50 years and older. However, a recently published article on incidence trends among younger adults (ages 20-49 years) found, using data from 13 SEER cancer registries, that overall incidence rates of colorectal cancer per 100,000 young adults increased 1.5% per year in men and 1.6% per year in women (12). This changing trend is interesting given that age

older than 50 is one of the known risk factors for CRC (11), and 92% of diagnoses are in people age 50 and above (9, 11, 12).

The distribution of CRC incidence varies across different sub-populations. For instance, a study that utilized data from the National Program of Cancer Registries (NPCR) and SEER from 1994 to 2004 found that, overall, men had a higher age-adjusted incidence rate (61.1 per 100,000) than did women (44.2 per 100,000), and the greatest difference in rates were seen among those who were ≥50 years of age (13). Incidence rates were higher in blacks (57.2 per 100,000) than in Asian/Pacific Islanders, American Indian/Alaska Natives, and whites (50.8 per 100,000) (13). These results suggest that race and sex need to be considered as potential effect modifiers in our analyses.

Studies on U.S. migrants also suggest that environmental exposures play a major role in colorectal cancer etiology. For instance, in Israel, male Jews born in Europe or in the U.S. have a higher risk of developing colorectal cancer than those born in Africa or Asia (14). In addition, a study of Japanese immigrants to the U.S. found that the incidence of CRC, previously a low-incidence disease in Japan, eventually increased to a rate similar to that in native-born Americans. Also, the incidence of CRC increased dramatically from 1950 to 1970 in Japan, and was thought to be related to the increased westernization of their diet (9, 15). These findings highlight the major role that environmental exposures, especially diet, play in increasing the risk of developing CRC and the importance of investigating various preventative measures for reducing the burden of this avoidable disease.

International trends

The worldwide disease burden of colorectal cancer is also an important factor that cannot be ignored given the high morbidity and mortality of this disease. With about 850,000 people being diagnosed with and 500,000 dying annually of the disease, its impact both on a population's health and its economy is vast. It is the fourth most common cancer among men and the third most common among women worldwide, and represents about 20% of the cancer burden (9, 11, 16, 17). Although the advent and implementation of screening and advances in treatment options has led to a modest decline in the United States, this same decline has not been noted in other populations, especially in lesser developed populations. A study of international trends in incidence rates of CRC found that there were statistically significant increases in incidence rates for both males and females in 27 of 51 cancer registries across the world that were considered in the analysis (10, 11, 16, 18). Most of the registries that found this trend were mostly countries in Eastern Europe, most of Asia, and a few countries in South America, all of which are less economically developed than the U.S. (16, 18). Thus, investigating ways to lessen the incidence of colorectal cancers, not only locally, but globally is crucial to the health status of the world population.

Financial cost burden

Another important burden that high incidence diseases, such as CRC, place on a population is that of the huge financial cost of managing the disease from screening, to diagnosis, to treatment. This reason highlights the importance of investigating possible targets for prevention. Just in the U.S. alone, colorectal cancer is estimated to account for

about 12% of cancer costs and 1% of the total health care costs (11, 19). This is a huge financial burden that can be avoided since most cases of colorectal cancer are thought to be preventable given the modifiable environmental nature of most of the risk factors. The national cost of managing CRC is estimated to be from $4.5 billion to $9.6 billion, a staggering sum of money considering the already unsustainable nature of our health care system (11, 20). Models have projected that if these current trends in disease burden continue, the national cost of CRC management could rise to 14.1 billion dollars by 2020 (11, 21). Although screening measures such as colonoscopies and sigmoidoscopies have lessened the incidence of this disease, they have also contributed to the financial costs of management (22-24). It is therefore important to consider other measures of prevention that would simultaneously reduce disease burden and not add to the financial cost of management leading to decreases in incidence rates and costs.

Analytic epidemiology

Identification of risk factors and exposures associated with an increased (or decreased) risk of colorectal cancer is crucial to determining realistic preventive strategies. Similar to most other cancers, sporadic cases of colorectal cancer are multifactorial. Epidemiologic studies have provided consistent evidence that diets high in fat and red meat consumption are associated with higher risk of CRC (14, 25). The U.S. Nurses' Health Study found that after adjustment for total energy intake, animal fat consumption was associated with increased risk of CRC (relative risk[RR] 1.89, 95% CI: 1.13 to 3.15) comparing the highest to the lowest quintile. No association was found with vegetable fat (14, 26).

Dietary fiber is another factor proposed to account for differences in colorectal cancer rates between Africa and westernized countries. Although there have been inconsistent findings on the association between dietary fiber overall and CRC risk, when this association was analyzed according to fiber sources, fiber derived from fruits and vegetables were inversely associated with risk of CRC, while a null association was found between cereal fiber and CRC risk (14). This indicates there may be other component of fruits and vegetables responsible for this association. Although there are numerous repeated studies of the association between diet and CRC (27, 28), the association between diet and adenoma has received far less attention. Dietary factors may influence different stages of adenoma formation from incidence, to recurrence, to progression of adenomas to malignancy (28). Fruits and vegetables have been hypothesized to be associated with lower risk for colorectal adenomas. Of nine (6, 29-36) case-control studies that were reviewed, six (6, 29, 31, 32, 34, 36) found significant inverse association between fruits and vegetable consumption and colorectal adenomas.

An inverse association of physical activity with colorectal cancer risk has been consistently found. Numerous occupational cohort studies and case-control studies found evidence that men who engage in greater occupational or recreational physical activity tend to have lower risk of developing CRC, and this association remains even after confounding factors are controlled for (14). Other factors such as the waist-to-hip ratio and BMI have been found to be directly associated with CRC risk (14, 37). Furthermore, obesity has also been found to be directly associated with colorectal adenomas (14, 25).

There has also been increasing evidence for an inverse association of hormone replacement therapy (HRT) with risk of CCR. Ten of nineteen published studies on HRT

and risk of colorectal cancer support an inverse association (statistically significant in five), and long-term users (5-10 years) were at the lowest risk, approximately 50% lower.(14) An inverse association of non-steroidal inflammatory drugs (NSAIDs) use with colorectal cancer risk is also well established. Multiple randomized clinical trials (RCT) found that NSAIDs reduced adenoma recurrence by about 40-50%, while numerous observational studies, including cohort and case-control studies, consistently found NSAID use to be inversely associated with invasive CRC (38).

Several *in vivo* and *in vitro* studies have suggested that vitamin D and calcium have anti-colon carcinogenetic effects (39-43). Analytic epidemiological studies have extensively examined the association of these nutrients with colorectal cancer risk. Studies that combined multiple sources of vitamin D or measured serum 25-OH D_3 levels consistently found that intake or serum levels that are above average to be associated with lower risk for colorectal cancer (43, 44). However, studies that measured only dietary vitamin D intake tend to yield mixed results, probably because diet provides only 5 – 10% of total vitamin D (44). A pooled analysis of 10 cohort studies found calcium and milk consumption to be inversely associated with colorectal cancer risk (43).

Reactive oxygen species (ROS) have also been implicated as initiators and promoters of carcinogenesis. They directly alter nucleic acids, damage cell membrane lipids and proteins, and modify expression of genes responsible for cell differentiation and growth (45-47). The balance between pro-oxidant and anti-oxidant factors (which determine ROS production and neutralization) determine the degree and extent of ROS-induced damage. Several environmental exposures, such as diet (high fat and red meat intake) and inhaled tobacco smoke increase the oxidative burden (46). Whereas

associations of individual antioxidant micronutrients with colorectal neoplasms have been inconsistent, Goodman et al found an oxidative balance score to be strongly inversely associated with risk for colorectal adenomas.

Molecular basis of colorectal cancer

Colorectal tumors develop as a result of accumulated alterations in oncogenes and tumor suppressor genes (48-51). Vogelstein and colleagues found four genetic alterations (APC, *K-ras*, and p53 gene mutations, and an allelic deletion in chromosome 18) that were involved in the various stages of colorectal tumor development. These alterations occurred in a parallel fashion to the clinical progression of tumors supporting the model of colorectal tumorigenesis occurring as a result of activation of an oncogene coupled with subsequent inactivation of tumor-suppressor genes (48, 52). Additional support for this model was generated from studies conducted on the well-defined hereditary syndromes FAP and HNPCC which significantly increase the risk of developing colorectal cancer.

Individuals with familial adenomatous polyposis (FAP) have an almost 100% risk of developing CRC by age 40 years while those with hereditary non-polyposis colon cancer syndrome (HNPCC) carry a 40% lifetime risk of developing CRC (52, 53). FAP is an autosomal dominant genetic disorder that affects about 1 in every 7,000 people (52, 53). Individuals with FAP develop anywhere from hundreds to thousands of adenomatous polyps in their colon as early as 20 to 30 years of age, and if left untreated, ultimately develop malignancy (52). Truncating mutations of the *APC* gene located on chromosome 5 causes FAP (52, 54-56). HNPCC is another genetic disorder caused by mutations in one

of three DNA mismatch repair (MMR) genes (*MSH2, MLH1*, and *PMS2*), which ultimately leads to microsatellite instability in other genes such as *BAX* and *TGFBRII* (52, 57, 58). Although, individuals with HNPCC develop polyps at a normal rate, their progression through the stages of carcinogenesis occurs rapidly (1, 52). FAP and HNPCC together account for about 6% of CRC cases. About 10%-20% of cases have a family history of the disease, whereas ~75% of cases have no family history of the disease (52) (Fig 1). The study population on which our investigation will be focused excludes individuals with FAP or HNPCC, since our outcome of interest is sporadic colorectal adenomas.

Biological plausibility

Dietary polyamines are involved in different stages of colorectal cancer development. Polyamines are naturally occurring aliphatic polycations found ubiquitously in all mammalian cells. Their essential role in the regulation of cellular proliferation and differentiation is well established (59, 60). The main polyamines, spermidine and spermine, are derived from the conversion of ornithine to putrescine by the rate-limiting enzyme ornithine decarboxylase (ODC), and, subsequently, the conversion of putrescine first to spermidine, and then to spermine by polyamine synthetases (60, 61). To control intracellular levels of polyamines, their biosynthesis is highly regulated through various pathways involving ODC, which catalyzes the first step in polyamine metabolism (60, 62). Although naturally produced, polyamines can also be introduced exogenously via the gastrointestinal tract (63). Therefore, diet can influence the levels of polyamines available to cells at any one time. For instance, increased

consumption of dietary arginine leads to increased polyamine levels due to the conversion of arginine to ornithine in the urea cycle (64) (Fig.2).

ODC activity is thought to be involved in carcinogenesis, in part since higher activity and polyamine levels were found in tumors relative to normal tissue, including colorectal cancer compared to normal-appearing colorectal mucosa (62). However, studies of colorectal adenomas found either higher, the same, or lower ODC activity in adenoma tissue than in normal tissue, but these conflicting results may have been due to the various methodologies in the different studies for measuring ODC activity. Hixson and colleagues, using a more sensitive means of quantifying polyamine content and ODC activity in neoplastic polyps, found significant differences between these polyps and normal colorectal tissue, which led them to conclude that the hyperproliferation of these colorectal adenomas is due to the higher ODC activity and polyamine content located in the cells, which in turn promotes tumor progression. Other studies reported that most dietary polyamines are degraded in the gut and only 20% are released into the systemic circulation in healthy adults. Interestingly, the presence of tumors in the intestinal mucosal was significantly associated with enhanced uptake of dietary polyamines (61, 65). Also, several investigators found that adenomatous and cancerous tissues had significantly elevated levels of ODC activity and polyamine concentrations (60). These findings suggest that polyamines may play a role in tumor proliferation and progression, but there are little to no data on whether or not they may play a role in tumor incidence.

The main focus of this study is to investigate whether dietary polyamines are associated with incident, sporadic colorectal adenomas, while a secondary association of interest is between polyamine intake and various adenoma characteristics such as polyp

size, location, histologic type (villous, tubular, tubulovillous), and degree of dysplasia (mild, moderate, or severe). A polyamine index score will be constructed using the information reported by subjects on a food questionnaire in conjunction with the polyamine content contained in certain food items reported by Zoumas-Morse et al (66).

CHAPTER II: MANUSCRIPT

Title: Association of Dietary Polyamines with Incident, Sporadic Colorectal Adenomas.

Author: Kehinde Oladunni Raji, Roberd M. Bostick

Abstract

PURPOSE: To investigate the association of dietary polyamines with risk of incident, sporadic colorectal adenoma.

METHODS: To investigate the association of dietary polyamines and risk for incident, sporadic colorectal adenoma, data from the Minnesota Cancer Prevention Research Unit case-control study were analyzed. The colonoscopy-/community-based case-control study conducted 1991-1994, enrolled 30-74 year old 564 incident, sporadic, colorectal adenomas participants, 684 polyp-free controls, and 535 community controls. Diet was assessed using a 153-food item semi-quantitative Willet food frequency questionnaire (FFQ). Polyamine exposure was quantified based on responses to the food frequency questionnaire and a previously published report on the polyamine content of select food items. Polyamine intakes were categorized according to the quartiles based on the distribution among the community controls and analyzed using unconditional multivariate logistic regression.

RESULTS: Polyamine intake was inversely associated with risk for colorectal adenomas. The odds ratios (OR) for the highest relative to the lowest category of polyamine intake were 0.57 (95% confidence interval (CI), 0.33 – 1.00; p trend 0.0001) and 0.76 (CI 0.43 – 1.33; p trend 0.04) in the comparisons with the colonoscopy and community-based controls, respectively. The inverse associations tended to be stronger among those with a more positive oxidative balance, no family history of colorectal neoplasms, and women who did not take hormone replacement therapy, and for smaller and distal adenomas.

CONCLUSIONS: These findings suggest that higher polyamine intakes may be associated with lower risk for incident, sporadic colorectal adenomas, perhaps especially for smaller and distal adenomas.

Introduction

Colorectal cancer (CRC) is the third most common cancer in the United Sates among both men and women (9, 10). Recent advances in screening methodology have increased early detection and modest declines in incidence rates (18). Ecologic and migration studies point to the importance of modifiable environmental factors, especially diet (9, 67, 68) and physical activity (9, 69), in the etiology, and thus the prevention of the disease. A dietary factor that has received little attention is polyamines. Polyamines are naturally occurring polycations found in all mammalian cells, although they can be introduced endogenously into the human gut through diet (61, 65). The main polyamines, spermidine and spermine, are derived from the conversion of ornithine to putrescine by the rate-limiting enzyme ornithine decarboxylase (ODC) (60, 61). They function in the proliferation and differentiation of both prokaryotic and eukaryotic cells and are thought to play a role in tumor progression and carcinogenesis (62). Several experimental studies found that colorectal adenomatous and cancerous tissues had substantially higher levels of ODC activity and polyamine concentrations compared to normal mucosa (60).

To our knowledge, there are no published human epidemiologic studies of the association between dietary polyamines and colorectal neoplasms. However, observational studies of the association between endogenous polyamines and colon cancer exist. A case-control study analyzing 4-6 multiple rectal biopsies from normal controls and case patients with colon cancer found that the odds ratios for spermine and spermidine levels, in comparison to levels in controls, were 4.8, [95% CI, 1.6 – 33.7] and 2.3, (95% CI, 1.2 – 6.3) respectively (70) . Studies targeting endogenous polyamine synthesis by oral administration of difluoromethylornithine (DMFO) and sulindac for

chemoprevention of colon cancer, and assessing the effect of polyamine-restricted diets in prevention of colorectal neoplasm (59, 71-74) found that decreasing levels of endogenous polyamines resulted in a 70% reduction in recurrence of all adenomas, and was associated with lower risk for colorectal neoplasms. In a recent abstract presented at the 2010 American Society of Clinical Oncology (ASCO) annual meeting, it was reported that there was a statistically significant direct association of dietary polyamine intake with tissue polyamine concentrations, and that high dietary polyamine was associated with adenomas >1cm, high-grade adenomas, and advanced adenomas. (75). This was the only human epidemiologic study to investigate the association of dietary polyamines with colorectal adenomas, but since it was reported in abstract form, our findings will be the first full report on the association of dietary polyamines with incident, sporadic colorectal adenomas.

The main objective was to investigate the association of dietary polyamines with incident, sporadic colorectal adenomas, while a secondary objective was to investigate the association with adenomas with various characteristics such as polyp size, location, histologic type (villous, tubular, tubulovillous), and degree of dysplasia (mild, moderate, or severe). We analyzed data from the colonoscopy-/community-based Minnesota Cancer Prevention Research Unit case-control study. A polyamine index score was constructed using the information reported by subjects on a food questionnaire in conjunction with the polyamine content contained in certain food items reported by Zoumas-Morse et al (66).

Methods

This study was a secondary analysis of data collected in the Minnesota Cancer Prevention Research Unit (CPRU) case-control study from 1991-1994. The case-control study was a collaborative effort of units within the University of Minnesota and a large multi-clinic private gastroenterology practice (Digestive Healthcare (DH)). At the time of the study, the practice was responsible for about 60% of all colonoscopies conducted in metropolitan Minneapolis. The original study was approved by the Emory University Institutional Review Board (IRB), but the analysis conducted here was not subject to IRB review because the dataset was de-identified.

Study subjects

The participants included in this study were patients between 30-74 years of age who were scheduled for colonoscopies between April 1991 and April 1994. Patients who fulfilled specific eligibility criteria (see below) were recruited into the study and completed questionnaires prior to their scheduled colonoscopy, and thus diagnosis, to reduce bias.

Eligibility criteria for cases and controls included, residency in Twin Cities metropolitan area within specific zip codes, age 30-74 years, able to speak English, no history of a predisposition to colonic neoplasia, no history of inflammatory bowel disease (IBD), and no individual history of cancer (with the exception of non-melanoma skin cancer). Cases were patients with first diagnosis of incident colon or rectal adenomatous polyps on colonoscopy, and those who were polyp-free on colonoscopy were deemed colonoscopy-negative controls.

The recruitment protocol was undertaken utilizing staff and resources of both DH and University of Minnesota Divisions of Epidemiology and Biostatistics. The DH patient coordinators scheduled colonoscopies, screened patients for initial eligibility, and mailed out an introductory letter explaining the study along with questionnaires and consent forms. A few days later, a DH nurse called the study subject to confirm that they received the study material, and asked for verbal permission for them to be contacted by University of Minnesota study staff, who would then explain the study in more detail and answer any questions patients had. At the time of the scheduled colonoscopy the forms were collected and the patients had their blood drawn. All polyps detected during the colonoscopy were removed and placed in separate containers. The size of the polyp as well as the site where it was detected was recorded. Determination of polyp size was carried out by comparing the *in vivo* polyps with fully opened standard-sized flexible colonoscopy forceps. Histologic examination of all polyps removed during colonoscopy was carried out by a study index pathologist, and utilizing the diagnostic criteria from the National Polyp Study (76). Histologic type (adenoma, hyperplastic, or other), and if an adenoma, histologic subtype (tubular, villous, or tubulovillous), and degree of dysplasia was also recorded. Study participants were assigned into one of three groups according to the colonoscopy results. The adenomatous polyp group consisted of 574 participants (cases), the hyperplastic-polyp only group was not included in the control for this analysis, and the colonoscopy-negative group (n=707) served as one of our control groups. The participation rate for all patients who underwent colonoscopy was 68%.

A second control group, a population-based control group, was selected utilizing the 1991 Minnesota State Drivers License Registry. Each control was frequency matched

to the age (5-year interval), sex, and zip code of the case population. After an initial phone call with the prospective participants, those who fulfilled the eligibility criteria were mailed a package identical to the one mailed to the colonoscopy population. Incomplete questionnaires resulted in call-backs to all participants for specific questions. The community control participants were asked to self-report on their colonic neoplasia status. Blood samples were not collected from this control group. The participation rate for the community controls was 65%.

Data collection

A modified (expanded) 153-food item version of the Willett food frequency questionnaire was utilized to collect information on usual dietary intake over the previous 12 months. Participants were also asked to provide data on demographic characteristics, personal medical history, physical activity levels, family history of colorectal polyps, and for women only, reproductive history, use of oral contraceptives (OC), and hormone replacement therapy (HRT).

Data Analysis

Polyamine index score

Dietary polyamine intake, our study main exposure of interest, was not originally an output of the Willett Food Frequency Questionnaire. Therefore, we created the variable using dietary information collected from study participants in conjunction with information on the polyamine content of food items published by Zoumas-Morse et al (66), who reported the polyamine content in each food item two different ways. Polyamine content in some food items was reported according to serving size (nmol/serving size), while information on some food items only included the average

polyamine contribution to the daily diet per day (nmol/day). For food items that Zoumas-Morse et al reported the polyamine content by serving size (nmol/serving size), the value of a participant's frequency of daily intake was simply multiplied by the reported polyamine content. For instance, individuals who reported eating corn 2-4 times a day were determined to have a daily intake of 3 servings/day, and this value was then multiplied by the polyamine content in corn (902,880 nmol/serving size) yielding a daily polyamine intake of 2,708,620 nmol from corn. This same methodology was applied to all food items for which polyamine content was reported in a similar manner.

For food items for which only information on the average polyamine contribution to the daily diet was available, we used information on the average frequencies of intakes of these select food items reported by community controls in our study to calculate the average daily intake of the food item. For instance, baked potato was reportedly eaten on average once per week by community controls, yielding an average daily intake value of 1 serving/7 days or 0.0667servings/day. We then divided each individual study participant's daily intake by the study population's average daily intake and multiplied it by the food item's average daily polyamine contribution (nmol/day) to the diet as reported by Zoumas-Morse et al, to yield the daily amount of dietary polyamines that the food item contributed to a participant's total daily polyamine intake. For example, for an individual who reported eating baked potato 2 to 3 times per week (2.5 servings/7 days) = 0.357servings/day, the polyamine intake from potatoes was calculated as divided by the study population's average daily consumption of baked potato (0.0667 servings/day) multiplied by the average daily polyamine contribution of baked potato (2,388 nmol/day) as reported by Zoumas-Morse et al. Thus, the individual's baked potato intake will have

contributed, on average, 12, 278 nmol of polyamines to this individual's diet per day. The same procedure was repeated for all food items in which polyamine content was reported in a similar manner

Next, the amounts of polyamines contributed by each food item were summed up for each study participant. The resulting continuous variable was then categorized into quartiles based on the distribution among the community controls ranging from lowest intake to highest intake.

Participants missing more than 10% of the FFQ information were excluded from the analysis. Those who had missing values for some of the selected food items were determined to have a polyamine value of 0 for that particular food item; that is, the food item was deemed not to have contributed to their total daily polyamine intake. A total of 564 cases, 684 colonoscopy-negative controls, and 535 community-based controls were included in the analysis.

Other key analytic variables

Several factors *a priori* known to be pro-oxidant exposures (saturated fat intake, total iron intake, and smoking history) and anti-oxidant exposures (tocopherol (vitamin E), carotene, vitamin C, lycopene, lutein, and regular aspirin and NSAID use) were selected and used to create a single oxidative balance score (OBS) as reported by Goodman et al (46). Continuous variables reflecting pro-oxidant exposures were categorized into high (0 points) and low (1 point) categories according to the median value among the community controls. By contrast, anti-oxidant exposure variables were given 1 point for each high level exposure and 0 points for each low level exposure (high

(1 point); low (0 points)). For dichotomous variables, study participants were assigned a point for every antioxidant exposure and 1 point for the absence of each pro-oxidant exposure. The points assigned to each individual OBS component were summed to yield an overall score, with higher values indicating mostly antioxidant exposures and lower values indicating predominantly pro-oxidant exposures.

Factors that were considered as covariate include age, sex, BMI, waist/hip ratio, physical activity METs-hours/week, daily alcohol consumption (grams/day), pack years of smoking (defined as pack of cigarettes smoked per day multiplied by years of smoking), history of colon cancer in a first-degree relative (father, mother, sibling, or child), serum 25-OH vitamin D (only measured in cases and clinic controls), dietary vitamin D (IU/day), calcium intake (IU/day), ever use of hormone replacement therapy (HRT), and regular NSAID use. Dietary covariates included total daily energy intake, intakes of fat, total fruits/vegetables (derived by adding total fruits and vegetable intake values measured in dataset), dietary fiber, and red meat.

Statistical analysis

Unadjusted mean baseline characteristics for cases and controls were compared using analysis of covariance (ANOVA) for continuous variables and the chi-square test for categorical variables. All continuous variables were assessed for normality, and if found to violate normality assumption were either transformed to meet the normality assumptions, or in instances where transformation did not normalize the variable, categorized. Categorization of continuous variables, when necessary, was based on the median value of the variable among the community controls. Variables that were

transformed (log-transformation) included intakes of alcohol, total fat, fruits and vegetables, red meat, calcium, vitamin D, total polyamine intake, and years of HRT use. Continuous variables that were categorized included pack-years of smoking, physical activity METs hours/week, and waist-to-hip ratio. These variables were categorized according to the median distribution among the community controls.

Multivariate unconditional logistic regression was used to assess the association between polyamine intake and incident, sporadic adenomas while appropriately controlling for confounding. Variables considered as potential covariates were age, sex, physical activity, pack-years of smoking, oxidative balance score, waist-to-hip ratio, BMI, total daily intake of alcohol, fat, red meat, dietary fiber, fruits and vegetables, calcium, dietary vitamin D (cases vs. community controls), 25-OH vitamin D (cases vs. colonoscopy-negativecontrols), and family history of CRC in a first degree relative. Interaction assessment was conducted by first screening variables for effect modification utilizing multiple, individual logistic regression models that contained the main exposure (E), each potential effect modifier (C), and the multiplicative interaction term (E x C). A chunk test for significance was carried out, and if found to be statistically significant (p-value ≤ 0.1), was included in the next step of the interaction assessment process. After screening, variables found to be statistically significant in the analysis of cases versus colonoscopy-negative controls, included age, sex, pack-years of smoking, alcohol intake, family history of CRC, regular NSAID use, hormone replacement therapy, waist-to-hip ratio, oxidative balance score, and red meat intake.

All remaining eligible variables were assessed for confounding by running multiple bivariate (exposure and potential confounder) and minimally-adjusted (age, sex,

and total energy intake) models. Total daily intake of fat and dietary fiber, pack-years of smoking, and 25-OH vitamin D were found to confound our measure of association (odds ratio) by ≥10%. Correlation analyses, using a *proc corr* procedure to generate correlation coefficients, were conducted on all continuous variables to check for any association among the variables, which would indicate collinearity problems ($r \geq 0.6$), but none were detected. All potential effect modifiers and confounders were then assessed in one comprehensive logistic model, and the backwards elimination procedure was used to generate the final model.

After screening for effect modification in the comparison of the cases to the community controls, the product terms of age, sex, alcohol intake, family history of CRC and polyamines were found to be statistically significant (p-value ≤0.1). All remaining eligible variables were assessed for confounding and intake of fruits/vegetables, dietary fiber, calcium, fat, red meat, dietary vitamin D, HRT use, physical activity, pack-years of smoking, BMI, regular NSAID use, waist-to-hip ratio, and the oxidative balance score were found to confound our association of interest by ≥10%. Correlation analyses were also conducted on all continuous variables in order to assess any collinearity problems ($r \geq 0.6$). Calcium and dietary vitamin D intake, intake of fat and red meat, and intake of dietary fiber and total fruits/vegetables were found to be highly correlated; therefore dietary vitamin D, red meat intake, and fruits/vegetables intake were dropped from further analyses. Backwards elimination procedures conducted on a comprehensive model was used to generate the final model. Effect modifiers were appropriately addressed through stratification and confounders were adjusted for in the model. Several variables that were found to be effect modifiers in the assessment comparing cases to

clinic controls were also found to confound the association when comparing cases to community controls. These variables were appropriately addressed either through stratification or adjustment.

The inclusion criteria for variables in the final models were biological plausibility, whether they fit the model at statistical significance at p-value <0.05, and/or whether inclusion of the variable changed the association by ≥10%. Hormone replacement therapy could not be assessed in the same model containing sex due to complete collinearity. The final models were adjusted for age, sex, total energy intake, physical activity, pack-years of smoking, calcium and dietary fiber intake, and regular NSAID use. The final model for the assessment between cases and colonoscopy-negative controls was further adjusted for serum 25-OH vitamin D.

Separate analyses were conducted for the cases and the two control groups and results are presented as cases versus colonoscopy-negative controls and cases versus community-based controls.

Results

Selected descriptive characteristics of the cases (n = 564), colonoscopy-negative controls (n = 684), and community-based control (n = 535) groups are presented in Table 1. The cases, on average, were older, more likely to be male, smokers, consume more alcohol, and be less physically active than controls. On average, cases also tended to have lower polyamine intake, higher total energy, fat, and red meat intakes, and lower intakes of dietary fiber (versus community controls), fruits and vegetables, calcium, dietary vitamin D (versus community controls), and lower levels of serum 25-OH vitamin D

(versus colonoscopy-negative controls). On average, they also tended to have a higher BMI and waist-to-hip-ratio, and were less likely to regularly take an NSAID. Female cases were less likely to have ever used hormone replacement therapy (HRT) than controls.

The crude and multivariate-adjusted associations of polyamine intake with incident, sporadic adenomas are presented in Table 2. In the crude analysis, relative to the lowest quartile of polyamine intake, the highest quartile of polyamine intake was inversely associated with colorectal adenomas (OR, 0.72, 95% CI 0.53 – 0.99) in the comparison of the cases to the colonoscopy-negative controls. After multivariable adjustments, we observed a stronger inverse association of the highest quartile of intake with colorectal adenoma risk (OR, 0.57, 95% CI, 0.33 – 1.00). There was no change in the OR for the comparison of the cases to the community controls after multivariable adjustments although, the multivariate-adjusted association of the third quartile of polyamine intake with adenomas was stronger (OR 0.68, 95% CI, 0.40 – 1.15) than the crude estimates (OR, 0.88, 95% CI, 0.63 – 1.22), however this pattern was not reflected among the other quartiles of polyamine intake. The multivariate-adjusted inverse associations between quartiles of polyamine intake and colorectal adenomas were much weaker in the comparison involving the community controls than in the comparison involving the colonoscopy-negative controls. A statistically significant trend with increasing polyamine intake was observed across both control group comparisons (P_{trend}, 0.0001; colonoscopy-negative controls and 0.04; community controls)

The multivariate-adjusted association between polyamine intake and risk for adenomas was assessed according to factors established to be associated with colorectal

cancer risk and the results are presented in Table 3. Among individuals with lower oxidative balance scores (OBS < 4), there was a consistent pattern of lower adenoma risk for those in the highest quartile of polyamine intake across comparisons with both control groups, although the inverse association was much stronger in the comparison of the cases to the colonoscopy-negative controls (OR 0.60, 955 CI, 0.26 – 1.38), than in the comparison of the cases to the community controls (OR, 0.88, 95% CI, 0.40 – 1.93). The reverse was observed among individuals with a more positive OBS (≥ 4). The highest quartile of polyamine intake was associated with a stronger reduction in risk of adenomas in the comparison of the cases to the community controls (OR, 0.39, 95% CI, 0.18 – 0.84) than in the comparison of cases to colonoscopy-negative controls (OR, 0.51, 0.22 – 1.20). Overall, there were much stronger inverse associations of polyamine intake with adenoma risk among individuals with a more positive OBS than individuals with a lower OBS.

There were no notable differences in the association of quartiles of polyamine intake with adenoma risk, between levels of waist-to-hip ratios and alcohol intake for both control group comparisons. However, statistically significant lower risks for adenoma, across increasing quartiles of polyamine intake, were observed among individuals with no family history of colorectal cancer. A statistically significant 60 percent lower risk for adenoma among those in the highest quartile of polyamine intake (OR, 0.40, 95% CI, 0.19 – 0.86) was noted in the comparison of the cases to the colonoscopy-negative controls, while a halving of risk in this same level of intake was observed in the comparison of the cases to the community controls (OR, 0.52, 95% CI, 0.29 – 1.00).

Among females who did not take hormone replacement therapy (HRT) and consumed high amount of polyamines (quartile 4), there was 60 percent lower risk for adenoma in the comparison of the cases to the colonoscopy-negative controls (OR, 0.11 – 1.32) and a 70 percent lower risk for adenomas in the comparison of the cases to the community controls (OR 0.30, 95% CI, 0.09 – 1.03). Females who took HRT and had high polyamine consumption were at higher risk for colorectal adenomas in the comparison of the cases to the colonoscopy-negative controls (OR, 1.22, 95% CI 0.32 – 4.56), and the association was inverse, although weak, in the comparison of the cases to the community controls.

There was evidence that the association of polyamines with colorectal adenomas differed according to adenoma characteristics. The highest quartile of polyamine intake was associated with the lowest risk for distal and rectal polyps in the comparison of the cases to colonoscopy-negative controls (OR, 0.52, 95% CI, 0.28 – 0.98), as well as in the comparisons of the cases to community controls (OR, 0.50, 95% CI, 0.28 – 0.91); both of these associations were statistically significant. This same pattern was noted for polyps <10 mm in diameter and with tubular histology across comparisons with both control groups. The highest quartile of polyamine intake was also associated with a low risk for mild-to moderate dysplasia (OR, 0.48, 95% CI, 0.24 – 0.97) and pedunculated polyp shape (OR, 0.60, 95% CI, 0.31 – 1.16), although this was only evident in the comparison of the cases to community controls. There was no discernable pattern or notable difference in the association of quartiles of polyamine intake with adenomas with advanced characteristics (polyps located in proximal colon, polyp size ≥10 mm in

diameter, tubulovillous/villous histology, or moderate/severe atypia), in the comparisons with both control groups.

Discussion

Our findings suggest that a higher polyamine intake may be associated with lower risk for incident, sporadic colorectal adenomas. Our findings also suggest that this association may be stronger among those with a more positive oxidative balance, no family history of CRC, and, among women, no use of hormone replacement therapy. The pattern in the data observed was in contrast to the hypothesis of a positive association between dietary polyamine intake and colorectal adenomas. Instead, an inverse, dose-response association was observed. Notably, there was a more enhanced reduction in adenoma risk in the comparison of the cases to the colonoscopy-negative controls, than in the comparison of the cases to the community controls. There was also evidence that the highest polyamine intake was associated with the lowest risk for distal and nonadvanced adenomas and adenomas that were small (<10 mm), or had mild/moderate dysplasia, or tubular histology, while there was little evidence for an association with proximal or advanced adenoma characteristics. These results are incongruent with the role that polyamines have been shown be play in tumor growth and progression (60, 62, 71).

High polyamine intake was associated with lower risk for colorectal adenomas among individuals with more positive oxidative balance (higher exposure to anti-oxidants than pro-oxidants) than among individuals with lower oxidative balance (higher exposure to pro-oxidants). This suggests anti-oxidants may work in concert with polyamines to

further decrease adenoma risk. This is plausible given that one study suggests that polyamines may have anti-oxidant properties (77).

Although the association of polyamine intake with colorectal adenomas could differ across high (≥0.925) and low (< 0.925) levels of waist-to-hip ratios (WHR), given that at least two studies found evidence of greater waist-to-hip ratios to be associated with risk of adenomas (37, 78), we found no evidence to suggest that WHR modified the polyamine-adenoma association. However, there was evidence to suggest that family history of CRC modified this association. Among individuals with a family history of CRC in a first degree relative, a strong risk factor for CRC (6, 79), a low polyamine intake was associated with an increase in colorectal adenoma risk, although this finding should be interpreted cautiously given the small numbers in the strata, and subsequently, the large confidence intervals. On the other hand, consistent strong inverse associations were noted among individuals with no family history of colorectal cancer, across quartiles of polyamine intake. Women who took hormone replacement therapy and had high polyamine consumption also had lower risk for colorectal adenomas.

The association of dietary polyamines with colorectal cancer is relatively under-investigated, and even more so are their association with colorectal adenomas. Most of the studies measured *in vitro* levels of polyamines and ODC activity in colorectal polyps and cancer tissue specimens and compared the levels to those in normal-appearing mucosa tissue (60, 62). Almost consistently, polyamine and ODC activity levels were found to be higher in the tumor tissue samples than in the normal tissue. There is evidence suggesting that the presence of tumors enhance the uptake of polyamines (61, 65). Therefore it is possible that polyamines do not play a role in the etiology of

adenomas, as originally hypothesized, but, instead, only play a role in carcinogenesis after the occurrence of advanced adenomas, by promoting tumor growth (60, 62). Our findings are therefore, biologically plausible.

Also supporting the biological plausibility of our findings is that the main sources of exogenous polyamines are fruits, vegetables, and cheeses (28, 66, 80). Given that fruits and vegetables are generally inversely associated with colorectal adenomas (28, 33, 35, 36), and that they are the major dietary contributors of polyamines, it is possible that other macronutrients in these food items, such as folate, carotenoids, vitamin C, flavonoids, organosulfides, isothiocyanates, and protease inhibitors that may prevent DNA damage and mutations (27, 28), may be responsible for the inverse association observed with colorectal adenomas. Also, the fermentable fiber in fruits and vegetables, which increases bulk, reduces colon transit time, produces anti-carcinogenic short-chain fatty acids, and lowers pH (27, 28), may also have contributed to the inverse association observed between polyamine intake and adenoma.

Although our hypothesis was not supported by our study results, there are several possible reasons for this. Polyamine exposure was quantified primarily from food items thought to be healthy, such as fruits and vegetables, are fairly consistently inversely associated with adenoma risk (28, 33, 35, 36). This may be the primary reason for the inverse association observed between polyamine content and adenoma risk. Also, studies of exogenous polyamines found a paradox of high supply/low utilization of luminal polyamines; that is, only about 20% of the relatively small amount of polyamines introduced into the intestinal lumen is utilized for growth support throughout the body (65, 81, 82). It is plausible that the amounts of exogenous polyamines, gained from diet,

do not substantially contribute to the role that endogenous polyamines play in tumor progression (60, 62, 63); therefore, the *a priori* established hypothesis of the association of endogenous polyamines with higher colorectal adenoma risk (60, 62, 63) would not be observed in analyses utilizing dietary polyamines. Perhaps much higher polyamine intake than that measured in our study population would be positively associated with adenomas, but further studies would be required to elucidate this.

Strengths and limitations

This study had several strengths. It is one of the first to assess the association of dietary polyamines with adenoma risk. Although the risk estimates were in contrast to the direction hypothesized, they are biologically plausible, and most of the associations were statistically significant. Also, a relatively large sample size (N = 1783) was employed in the analyses assessing the association of dietary polyamines with incident, sporadic colorectal adenomas.

Standard limitations associated with case-control studies are not excluded from this study. The design of the community- and colonoscopy-based case-control study here led to selecting colonoscopy-negative controls from individuals who may have been referred for colonoscopy, due to the presence of signs and symptoms that suggest they were at high risk for colorectal adenomas. Controls may have had similar dietary exposures as cases, which could have led to underestimation of adenoma risk. Also, there is some suggestion of misclassification bias since most of the associations were slightly weaker in the analyses using community controls, some of whom may have had undiagnosed adenomas. As in most dietary epidemiologic studies, utilization of food

frequency questionnaire (FFQ) for measurement of dietary exposures has inherent limitations such as recall error (83). The validity of using quartile values among community controls as a cutoff for our continuous variables is also questionable, especially for exposures that produce a threshold effect or when the controls may not be representative of the general population (46, 84, 85).

Furthermore, as much as utilizing actual quantified amounts of polyamine intake for the main exposure measurement is one of the strengths of our investigation, the method of quantification is subject to some limitations. Since we utilized a previously published dietary polyamine database to measure intake among our study population, our measurements were limited to the food items for which information on polyamine content was presented. That is, we could only estimate polyamine levels of food items investigated in both the Zoumas-Morse et al study and our study. This could potentially have led to an underestimation of polyamine intake in our study population. Also, utilization of information from another study subjects our analyses to the limitations and measurement errors present in the other study.

Conclusions

Overall, taking into account the strengths and limitations of this study, our findings suggest dietary polyamines intake (derived primarily from fruit and vegetable sources) may be inversely associated with adenomas, perhaps especially for small and distal adenomas.

References

1. Ahlquist DA. Aggressive polyps in hereditary nonpolyposis colorectal cancer: Targets for screening. Gastroenterology 1995;108(5):1590-1592.
2. Bremner CG, Ackerman LV. Polyps and carcinoma of the large bowel in the South African Bantu. Cancer 1970;26(5):991-999.
3. Enterline HT. Pathology of the gastrointestinal tract: polyps and cancer of the large bowel. Current Topics in Pathology 1976;63:97.
4. Fenoglio CM, Lane N. The Anatomic Precursor of Colorectal Carcinoma. JAMA: The Journal of the American Medical Association 1975;231(6):640-642.
5. Gloor FJ. The adenoma-carcinoma sequence of the colon and rectum. Sozial-und Praventivmed 1986;31(2):74-75.
6. Kune GA KS, Read A, MacGowan K, Penfold C, Watson LF. Colorectal polyps, diet, alcohol, and family history of colorectal cancer: a case-control study. Nutrition and Cancer 1991;16(1):26-30.
7. Correa P SJ, Reif A, Johnson WD. The epidemiology of colorectal polyps. Prevalence in New Orleans and International Comparisons. Cancer (Phila) 1977;39(5):2258-2264.
8. Muto T, Bussey HJR, Morson BC. The evolution of cancer of the colon and rectum. Cancer 1975;36(6):2251-2270.
9. Benson A. Epidemiology, disease progression, and economic burden of colorectal cancer. Journal of Managed Care Pharmacy 2007;7(6):S5-S18.
10. American C, Society. Global Cancer Facts & Figures. Atlanta, GA: American Cancer Society; 2011.

11. Gellad ZF, Provenzale D. Colorectal Cancer: National and International Perspective on the Burden of Disease and Public Health Impact. Gastroenterology 2010;138(6):2177-2190.

12. Siegel RL, Jemal A, Ward EM. Increase in Incidence of Colorectal Cancer Among Young Men and Women in the United States. Cancer Epidemiology Biomarkers & Prevention 2009;18(6):1695-1698.

13. Rim SH, Seeff L, Ahmed F, King JB, Coughlin SS. Colorectal cancer incidence in the United States, 1999-2004. Cancer 2009;115(9):1967-1976.

14. Boyle P, Langman JS. Epidemiology. BMJ 2000;321(7264):805-808.

15. Kuriki K TK. The increasing incidence of colorectal cancer and the preventive strategy in Japan. Asian Pacific Journal of Cancer Prevention 2006;7(3):495-501.

16. Center MM, Jemal A, Ward E. International Trends in Colorectal Cancer Incidence Rates. Cancer Epidemiology Biomarkers & Prevention 2009;18(6):1688-1694.

17. Parkin D, Bray, F., Ferlay, J., Pisani, P. Global cancer statistics, 2002. CA a cancer journal for clinicians 2005;55(2):74-108.

18. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: A Cancer Journal for Clinicians 2011;61(2):69-90.

19. National C, Institute. Cancer Trends Progress Report-2007 update. Bethseda, MD: National Cancer Institute, NIH, DHHS.

20. Yabroff KR, Warren JL, Banthin J, Schrag D, Mariotto A, Lawrence W, et al. Comparison of Approaches for Estimating Prevalence Costs of Care for Cancer

Patients: What Is the Impact of Data Source? Medical Care 2009;47(7_Supplement_1):S64-S69 10.1097/MLR.0b013e3181a23e25.

21. Yabroff KR MA, Feuer E, Brown ML. Projections of the costs associated with colorectal cancer care in the United States, 2000-2020. Health Economics 2008;17(8):947-959.
22. Marshall JR, Fay D, Lance P. Potential costs of flexible sigmoidoscopy-based colorectal cancer screening. Gastroenterology 1996;111(6):1411-1417.
23. Sonnenberg A, Delco F. Cost-effectiveness of a Single Colonoscopy in Screening for Colorectal Cancer. Arch Intern Med 2002;162(2):163-168.
24. Sonnenberg A, Delcò F, Inadomi JM. Cost-Effectiveness of Colonoscopy in Screening for Colorectal Cancer. Annals of Internal Medicine 2000;133(8):573-584.
25. Bruce WR, Wolever TMS, Giacca A. Mechanisms Linking Diet and Colorectal Cancer: The Possible Role of Insulin Resistance. Nutrition & Cancer 2000;37(1):19-26.
26. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of Meat, Fat, and Fiber Intake to the Risk of Colon Cancer in a Prospective Study among Women. New England Journal of Medicine 1990;323(24):1664-1672.
27. Glade M. Conference Paper. Nutrition 1999;15(6):523-526.
28. Michels KB, Giovannucci E, Chan AT, Singhania R, Fuchs CS, Willett WC. Fruit and Vegetable Consumption and Colorectal Adenomas in the Nurses' Health Study. Cancer Research 2006;66(7):3942-3953.

29. Benito E, Cabeza E, Moreno V, Obrador A, Bosch FX. Diet and colorectal adenomas: A case-control study in majorca. International Journal of Cancer 1993;55(2):213-219.

30. Hoff G MI, Trygg K, Frølich W, Sauar J, Vatn M, Gjone E, Larsen S. Epidemiology of polyps in the rectum and sigmoid colon: evaluation of nutritional factors. Scandanavian Journal of Gastroenterology 1986;21(2):199-204.

31. Kato I, Tominaga S, Matsuura A, Yoshii Y, Shirai M, Kobayashi S. A Comparative Case-Control Study of Colorectal Cancer and Adenoma. Cancer Science 1990;81(11):1101-1108.

32. Macquart-Moulin G RE, Cornée J, Kaaks R, Berthezène P. Colorectal polyps and diet: a case-control study in Marseilles. International Journal of Cancer 1987;40(2):179-188.

33. Mathew A, Peters U, Chatterjee N, Kulldorff M, Sinha R. Fat, fiber, fruits, vegetables, and risk of colorectal adenomas. International Journal of Cancer 2004;108(2):287-292.

34. Sandler RS, Lyles CM, Peipins LA, McAuliffe CA, Woosley JT, Kupper LL. Diet and Risk of Colorectal Adenomas: Macronutrients, Cholesterol, and Fiber. Journal of the National Cancer Institute 1993;85(11):884-891.

35. Smith-Warner SA, Elmer PJ, Fosdick L, Randall B, Bostick RM, Grandits G, et al. Fruits, Vegetables, and Adenomatous Polyps. American Journal of Epidemiology 2002;155(12):1104-1113.

36. Witte JS, Longnecker MP, Bird CL, Lee ER, Frankl HD, Haile RW. Relation of Vegetable, Fruit, and Grain Consumption to Colorectal Adenomatous Polyps. American Journal of Epidemiology 1996;144(11):1015-1025.

37. Giovannucci E, Ascherio A, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Physical Activity, Obesity, and Risk for Colon Cancer and Adenoma in Men. Annals of Internal Medicine 1995;122(5):327-334.

38. Lamont E, Dias L, Lauderdale D. NSAIDs and Colorectal Cancer Risk: Do Administrative Data Support a Chemopreventive Effect? Journal of General Internal Medicine 2007;22(8):1166-1171.

39. Holt PR. Studies of Calcium in Food Supplements in Humans. Annals of the New York Academy of Sciences 1999;889(1):128-137.

40. Holt PR, Wolper C, Moss SF, Yang K, Lipkin M. Comparison of Calcium Supplementation or Low-Fat Dairy Foods on Epithelial Cell Proliferation and Differentiation. Nutrition and Cancer 2001;41(1):150 - 155.

41. Lamprecht SA, Lipkin M. Cellular Mechanisms of Calcium and Vitamin D in the Inhibition of Colorectal Carcinogenesis. Annals of the New York Academy of Sciences 2001;952(1):73-87.

42. Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer 2003;3(8):601-614.

43. Park S-Y, Murphy SP, Wilkens LR, Nomura AMY, Henderson BE, Kolonel LN. Calcium and Vitamin D Intake and Risk of Colorectal Cancer: The Multiethnic Cohort Study. American Journal of Epidemiology 2007;165(7):784-793.

44. Grant WB, C.F. Garland. A critical review of studies on vitamin D in relation to colorectal cancer. Nutrition and Cancer 2004;48(2):115-123.

45. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, et al. Oxygen Radicals and Human Disease. Annals of Internal Medicine 1987;107(4):526.

46. Goodman M, Bostick RM, Dash C, Flanders WD, Mandel JS. Hypothesis: Oxidative Stress Score as a Combined Measure of Pro-oxidant and Antioxidant Exposures. Annals of Epidemiology 2007;17(5):394-399.

47. Kovaci P, Jacintho JD. Mechanisms of Carcinogenesis Focus on Oxidative Stress and Electron Transfer. Current Medicinal Chemistry 2001;8(7):773.

48. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic Alterations during Colorectal-Tumor Development. New England Journal of Medicine 1988;319(9):525-532.

49. Bos JL, Fearon ER, Hamilton SR, Vries MV-d, van Boom JH, van der Eb AJ, et al. Prevalence of ras gene mutations in human colorectal cancers. Nature 1987;327(6120):293-297.

50. Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M. Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. Nature 1987;327(6120):298-303.

51. Nowell PC. Mechanisms of tumor progression. Cancer Research 1986;46(5):2203-2207.

52. Yang V. The molecular genetics of colorectal cancer. Current Gastroenterology Reports 1999;1(5):449-454.

53. McKusick VA. Adenomatous Polyposis of the Colon; APC. In online Mendelian Inheritance in Man. Edited by VA McKusick. 1999;Baltimore: The Johns Hopkins University; (http://www.ncbi.nlm.nih.gov/omim/).

54. Joslyn G, Carlson M, Thliveris A, Albertsen H, Gelbert L, Samowitz W, et al. Identification of deletion mutations and three new genes at the familial polyposis locus. Cell 1991;66(3):601-613.

55. Kinzler K, Nilbert M, Su L, Vogelstein B, Bryan T, Levy D, et al. Identification of FAP locus genes from chromosome 5q21. Science 1991;253(5020):661-665.

56. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 1991;253(5020):665-669.

57. Aaltonen L, Peltomaki P, Leach F, Sistonen P, Pylkkanen L, Mecklin J, et al. Clues to the pathogenesis of familial colorectal cancer. Science 1993;260(5109):812-816.

58. Papadopoulos N, Lindblom A. Molecular basis of HNPCC: Mutations of MMR genes. Human Mutation 1997;10(2):89-99.

59. Pegg AE. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. Cancer Research 1988;48(4):759-774.

60. Weiss T, Bernhardt G, Buschauer A, Thasler W, Dolgner D, Zirngibl H, et al. Polyamine levels of human colorectal adenocarcinomas are correlated with tumor stage and grade. International Journal of Colorectal Disease 2002;17(6):381-387.

61. Murphy GM. Polyamines in the human gut. European Journal of Gastroenterology & Hepatology 2001;13(9):1011-1014.

62. Hixson LJ, Garewal HS, McGee DL, Sloan D, Fennerty MB, Sampliner RE, et al. Ornithine decarboxylase and polyamines in colorectal neoplasia and mucosa. Cancer Epidemiology Biomarkers & Prevention 1993;2(4):369-374.

63. Seiler N SS, Grauffel C, Jones R, Knödgen B, Moulinoux, JP. Endogenous and Exogenous polyamines in support of tumor growth. Cancer Research 1990;50(16):5077-5083.

64. Rial NS, Meykens, F.L, Gerner, E.W. Polyamines as mediators of APC-dependent intestinal carcinogenesis and cancer chemoprevention. Essays in Biochemistry 2009;46:111-124.

65. Milovic V. Polyamines in the gut lumen: bioavailability and biodistribution. European Journal of Gastroenterology & Hepatology 2001;13(9):1021-1025.

66. Zoumas-Morse C, Rock CL, Quintana EL, Neuhouser ML, Gerner EW, Meyskens FL. Development of a Polyamine Database for Assessing Dietary Intake. Journal of the American Dietetic Association 2007;107(6):1024-1027.

67. Morotomi M GJ, LoGerfo P, Weinstein IB. Production of diacylglycerol, an activator of protein kinase C, by human intestinal microflora. Cancer Research 1990;50(12):3595-3599.

68. Bandaru S. Reddy BS, Neha Patel, et al. Effect of amount and types of dietary fat on intestinal bacterial 7 alpha-dehydroxylase and phosphatidylinositol-specific phospholipase C and colonic mucosal diacylglycerol kinase and PKC activities during stages of colon tumor promotion. Cancer Research;56(10):2314-2320.

69. Weber RV, Stein DE, Scholes J, Kral JG. Obesity Potentiates AOM-Induced Colon Cancer. Digestive Diseases and Sciences 2000;45(5):890-895.

70. Wang W, Liu LQ, Higuchi, CM. Mucosal polyamine measurements and colorectal cancer risk. Journal of Cell Biochemistry 1996;63(2):252-257.

71. Wallace HM, Caslake R. Polyamines and colon cancer. European Journal of Gastroenterology & Hepatology 2001;13(9):1033-1039.

72. Thompson PA, Wertheim BC, Zell JA, Chen W-P, McLaren CE, LaFleur BJ, et al. Levels of Rectal Mucosal Polyamines and Prostaglandin E2 Predict Ability of DFMO and Sulindac to Prevent Colorectal Adenoma. Gastroenterology;139(3):797-805.e1.

73. Gerner EW, Meyskens FL. Combination Chemoprevention for Colon Cancer Targeting Polyamine Synthesis and Inflammation. Clinical Cancer Research 2009;15(3):758-761.

74. Meyskens FL, Gerner EW, Emerson S, Pelot D, Durbin T, Doyle K, et al. Effect of Î±-Difluoromethylornithine on Rectal Mucosal Levels of Polyamines in a Randomized, Double-Blinded Trial for Colon Cancer Prevention. Journal of the National Cancer Institute 1998;90(16):1212-1218.

75. Raj KP, Zell, JA, Rock, CI, McLaren, EC, Zoumas-Morse, C., Gerner, EW, Meysken, FL. Role of dietary polyamines in a phase III clinical trial of DFMO and sulindac for prevention of metachronous colorectal adenomas: a potential target for colon cancer prevention. Journal of Clinical Oncology 2010;28(15s):(suppl; abstr 1523).

76. O'Brien MJ WS, Zauber, AG, Gottlieb, LS, Sternberg, SS, Diaz, B, Dickersin, GR, Erwin, S, Geller, S, Kasimian, D. The National Polyp Study. Patient and

polyp characteristics associated with high-grade dysplasia in colorectal adenomas. Gastroenterology 1990;28(2):371 - 379.

77. Farriol M, Segovia-Silvestre T, Venereo Y, Orta X. Antioxidant effect of polyamines on erythrocyte cell membrane lipoperoxidation after free-radical damage. Phytotherapy Research 2003;17(1):44-47.

78. Hauret KG, Bostick RM, Matthews CE, Hussey JR, Fina MF, Geisinger KR, et al. Physical Activity and Reduced Risk of Incident Sporadic Colorectal Adenomas: Observational Support for Mechanisms Involving Energy Balance and Inflammation Modulation. American Journal of Epidemiology 2004;159(10):983-992.

79. Aitken JF, Bain, C.J., Ward, M., Siskind, V., MacLennan, R. Risk of colorectal adenomas in patients with a family history of colorectal cancer: some implications for screening programmes. Gut 1996;39(1):105-108.

80. Linsalata M, Russo F. Nutritional factors and polyamine metabolism in colorectal cancer. Nutrition 2008;24(4):382-389.

81. Bardocz S, Grant, G., Brown, D.D., Ralph, A., Pusztai, A. Polyamines in food-implications for growth and health. European Journal of Nutrition 1993;4:66-71.

82. Bardocz S. The role of dietary polyamines. European Journal of Nutrition 1993;47:683-690.

83. Cade JE, Burley VJ, Warm DL, Thompson RL, Margetts BM. Food-frequency questionnaires: a review of their design, validation and utilisation. Nutrition Research Reviews 2004;17(01):5-22.

84. Gordis L. Epidemiology. Philadelphia: Saunders; 2000.

85. Rosario AS, Wellmann Jr, Heid IM, Wichmann H-E. Radon Epidemiology: Continuous and Categorical Trend Estimators When the Exposure Distribution is Skewed and Outliers May Be Present. Journal of Toxicology and Environmental Health, Part A: Current Issues 2006;69(7):681 - 700.

86. Kumanyika SK. Diet and nutrition as influences on the morbiditymortality gap. Annals of Epidemiology 1993;3(2):154-158.

Tables of Results

Table 1. Selected characteristics of study population, Cancer Prevention Research Unit Case-Control Study, 1991 - 1994

Characteristics	Cases (N⁋=564)*	Colonoscopy-negative controls (N⁋=684)*	Community-based controls (N⁋=535)*	P-value[a]‡	P-value[b]‡
Demographics					
Age (years)	58.1(0.4)	52.8 (0.4)	57.7 (0.4)	0.0001	0.46
Men (%)	61.7	37.6	55.1	0.0001	0.03
Education > 12 years (%)	62.8	67.5	63.2	0.25	0.41
Family History of CRC in 1° relative (%)	20.0	34.2	9.4	0.0001	0.0001
Cigarette smoke Pack-years for ever smokers (%)					
0	1.1	1.4	1.7	0.002**	0.26**
≤ 22	45.3	56.4	48.6		
> 22	53.6	42.2	49.7		
Alcohol Use Daily use (gm/day)	0.7 (0.05)	0.5 (0.05)	0.6 (0.06)	0.0001	0.005
Physical activity Moderate-vigorous MET hours/wk (%)					
0	2.0	1.8	1.5	0.89**	0.62**
≤25	49.3	50.2	48.6		
>25	48.8	48.1	49.9		
Dietary intake Total energy intake (kcal/day)	2,090.7 (32.7)	2,017.2 (27.6)	2,054.5 (31.1)	0.08	0.42
Total fat intake (gm/day)	73.1 (1.4)	68.9 (1.2)	70.2 (1.4)	0.05	0.23
Dietary Fiber (gm/day)	21.8 (0.4)	21.7 (0.4)	22.2 (0.4)	0.90	0.40
Total fruits and vegetables (servings/wk)	42.3 (1.0)	43.9 (1.0)	44.5 (1.0)	0.28	0.03

Red meat intake (servings/wk)	4.7 (0.2)	4.6 (0.1)	4.4 (0.1)	0.87	0.25
Total calcium (mg/day)	959.4 (22.4)	985.2 (20.1)	987.7 (23.9)	0.27	0.37
25-OH serum vitamin D levels (IU)	23.8 (0.4)	24.7 (0.4)	-	0.14	-
Dietary vitamin (IU/day)	325.8 (10.8)	-	354.7 (11.4)	-	0.05
Hormone replacement therapy (females)					
Ever use (%)	38.8	49.7	44.17	0.009	0.24
Years used (no.)	5.8 (0.9)	5.9 (0.5)	5.9 (0.7)	0.93	0.89
Anthropometrics					
Height (m)	1.7 (0.0)	1.7 (0.0)	1.7 (0.0)	0.0001	0.19
Weight (kg)	81.7 (0.7)	76.8 (0.6)	79.3 (0.7)	0.0001	0.02
Body mass index (kg/m^2)	27.4 (0.2)	26.9 (0.2)	26.8 (0.2)	0.07	0.05
Waist/hip ratio (%)					
< 0.925	45.4	66.8	51.0	0.0001	0.061
≥ 0.925	54.6	33.2	49.0		
Regular NSAID use (%)	2.0	5.1	5.1	0.003	0.005
Oxidative balance score (OBS) (%)					
≤ 2	27.8	21.6	19.4	0.01**	0.0007**
3 to 6	67.0	72.2	73.3		
≥ 7	5.1	6.1	7.3		
Polyamine intake					
Daily Polyamine intake (nmol/day)	335,191.5 (8,814.7)	357,233.34 (8123.7)	354,521.1 (9,115.6)	0.06	0.17

* Values are mean (standard error) unless otherwise noted

a refers to p-value comparing cases vs. colonoscopy-negative controls

b refers to p-value comparing cases vs. community controls

‡ ANOVA used for continuous variables and Chi-square test used for categorical variables

ᵽ Numbers may not sum up to total study sample due to missing data

** P-value for trend test reported.

Table 2. Crude, age-, sex-, and total-energy intake-adjusted, and multivariate-adjusted[a] associations of polyamine intake level with incident, sporadic colorectal adenomas.

Exposure	Crude ORs (95% CI)		Age-, sex-, and total energy intake-adjusted ORs (95% CI)		Multivariate-adjusted[a/b] ORs (95% CI)	
Polyamine quartiles[c]	Cases vs. colonoscopy-negative controls	Cases vs. Community controls	Cases vs. colonoscopy-negative controls	Cases vs. Community controls	Cases vs. colonoscopy-negative controls[a]	Cases vs. Community controls[b]
1	1.00*ref*	1.00*ref*	1.00*ref*	1.00*ref*	1.00*ref*	1.00*ref*
2	0.79 (0.58 - 1.07)	0.93 (0.67 - 1.29)	0.76 (0.55 - 1.05)	0.89 (0.64 - 1.23)	0.56 (0.35 - 0.90)	0.90 (0.57 - 1.40)
3	0.88 (0.63 - 1.22)	0.73 (0.52 - 1.02)	0.73 (0.51 - 1.05)	0.68 (0.48 - 0.96)	0.52 (0.30 - 0.90)	0.68 (0.40 - 1.15)
4	0.72 (0.53 - 0.99)	0.78 (0.56 - 1.09)	0.59 (0.41 - 0.85)	0.69 (0.47 - 0.99)	0.57 (0.33 - 1.00)	0.76 (0.43 - 1.33)
p-trend	*0.19*	*0.22*	*0.0001*	*0.08*	*0.0001*	*0.04*

[a]Model adjusted for age, sex, total energy intake, pack-years of smoking, physical activity, NSAID use, dietary fiber, calcium intake, and serum 25-OH vitamin D.

[b]Model adjusted for age, sex, total energy intake, pack-years of smoking, physical activity, NSAID use, dietary fiber, and calcium intake.

[c]Polyamine intake level categorized according to quartile cut points. 1: <= 202287.56 nmol/day, 2: 202,287.6 nmol/day to 317,071.6 nmol/day, 3: 317,071.6 nmol/day to 451,683.1 nmol/day, 4: > 451,683.1 nmol/day

Table 3. Multivariate-adjusted[a/b] associations of polyamine intake level and risk for incident, sporadic colorectal adenomas according to levels of the risk factors for colorectal neoplasms.

		Cases	*vs.* Colonoscopy-negative controls[a]			*vs.* Community controls[b]		
		N	N	OR	95% CI	N	OR	95% CI
	Oxidative balance score (OBS)[c]							
	Low OBS (<4)							
Polyamine quartiles[d]	1	101	104	1.00*ref*		80	1.00*ref*	
	2	83	95	0.69	0.38 - 1.24	54	1.03	0.58 - 1.81
	3	40	45	0.58	0.27 - 1.24	35	0.92	0.45 - 1.90
	4	37	38	0.60	0.26 - 1.38	29	0.88	0.40 - 1.93
	p-trend			*0.0001*			*0.25*	
	High OBS (≥4)							
Polyamine quartiles[d]	1	62	62	1.00*ref*		53	1.00*ref*	
	2	71	104	0.40	0.17 - 0.92	81	0.62	0.30 - 1.30
	3	79	93	0.47	0.20 - 1.09	98	0.36	0.17 - 0.77
	4	91	143	0.51	0.22 - 1.20	105	0.39	0.18 - 0.84
	p-trend			*0.0003*			*0.04*	
	Waist-to-hip ratio							
	Low waist-to-hip (< 0.925)							
Polyamine quartiles[d]	1	79	109	1.00*ref*		77	1.00*ref*	
	2	65	131	0.53	0.27 - 1.03	68	0.75	0.39 - 1.44
	3	60	98	0.64	0.30 - 1.35	70	0.64	0.31 - 1.32
	4	52	119	0.64	0.28 - 1.44	58	0.53	0.23 - 1.19
	p-trend			*0.001*			*0.71*	

	High waist-to-hip (≥ 0.925)							
	1	84	57	1.00[ref]		56	1.00[ref]	
Polyamine quartiles[d]	2	89	68	0.60	0.29 - 1.21	67	0.88	0.48 - 1.63
	3	59	40	0.41	0.18 - 0.96	63	0.56	0.28 - 1.12
	4	76	62	0.52	0.23 - 1.18	76	0.73	0.36 - 1.48
	p-trend			*0.0005*			*0.005*	
	Daily alcohol intake (mg/day)							
	Low alcohol intake (<0.63)							
	1	102	123	1.00[ref]		85	1.00[ref]	
Polyamine quartiles[d]	2	102	153	0.53	0.29 - 0.96	93	0.72	0.40 - 1.28
	3	78	107	0.44	0.21 - 0.91	101	0.44	0.23 - 0.87
	4	77	132	0.62	0.30 - 1.27	103	0.43	0.22 - 0.86
	p-trend			*0.0001*			*0.06*	
	High alcohol intake (≥0.63)							
	1	61	43	1.00[ref]		48	1.00[ref]	
Polyamine quartiles[d]	2	52	46	0.60	0.26 - 1.38	42	0.92	0.46 - 1.85
	3	41	31	0.56	0.26 - 1.35	32	0.75	0.35 - 1.63
	4	51	49	0.49	0.19 - 1.26	31	0.99	0.43 - 2.29
	p-trend			*0.01*			*0.72*	
	Family history of CRC							
	Yes							
	1	26	43	1.00[ref]		6	1.00[ref]	
Polyamine quartiles[d]	2	34	54	1.75	0.52 - 5.84	8	1.53	0.27 - 8.59
	3	14	40	0.69	0.16 - 3.05	12	0.65	0.08 - 5.12
	4	17	50	0.97	0.24 - 3.93	11	1.70	0.21 - 13.44
	p-trend			*0.18*			*0.62*	

	No							
	1	106	84	1.00[ref]		81	1.00[ref]	
Polyamine quartiles[d]	2	98	110	0.44	0.24 - 0.83	101	0.56	0.31 - 0.98
	3	85	67	0.54	0.26 - 1.10	92	0.52	0.29 - 0.97
	4	75	99	0.40	0.19 - 0.86	84	0.52	0.26 - 1.00
	p-trend			*0.0001*			*0.05*	
	HRT (Ever use)[e]							
	Yes							
	1	35	45	1.00[ref]		29	1.00[ref]	
Polyamine quartiles[d]	2	16	67	0.15	0.04 - 0.61	26	0.17	0.05 - 0.63
	3	16	49	0.30	0.07 - 1.32	30	0.18	0.05 - 0.71
	4	16	51	1.22	0.32 - 4.56	21	0.76	0.19 - 3.10
	p-trend			*0.01*			*0.05*	
	No							
	1	41	57	1.00[ref]		32	1.00[ref]	
Polyamine quartiles[d]	2	35	67	0.29	0.11 - 0.78	35	0.70	0.25 - 1.97
	3	29	42	0.53	0.17 - 1.66	36	0.64	0.20 - 2.10
	4	26	49	0.38	0.11 - 1.32	31	0.30	0.09 - 1.03
	p-trend			*0.07*			*0.51*	

[a]Model adjusted for age, sex, total energy intake, pack-years of smoking, physical activity, NSAID use, dietary fiber, calcium intake, and 25-OH vitamin D.
[b]Model adjusted for age, sex, total energy intake, pack-years of smoking, physical activity, NSAID use, dietary fiber, and calcium intake.
[c]High OBS (≥ 4; higher exposure to antioxidants) and low OBS (< 4; higher exposure to pro-oxidants). Dichotomized at median in community controls.
[d]Polyamine intake level categorized according to quartile cut points. 1: <= 202,287.6 nmol/day, 2: 202,287.6 nmol/day to 317,071.6 nmol/day, 3: 317,071.6 nmol/day to 451,683.1 nmol/day, 4: > 451,683.1 nmol/day.
[e]Stratification of HRT use only assessed in female population and model used to to assess stratification on HRT use did not include sex due to collinearity issues.

Table 4. Multivariate-adjusted[a/b] associations of polyamine intake level and risk for incident, sporadic colorectal adenomas according to selected adenoma characteristics.

	N (Cases/Colonoscopy-negative controls)[a]	OR (95% C.I.)				
		1	2	3	4	*p-trend*
Location						
Distal colon and rectum	377/684	1.00[ref]	0.63 (0.38 - 1.04)	0.61 (0.34 - 1.10)	0.52 (0.28 - 0.98)	0.0006
Proximal colon	183/684	1.00[ref]	0.51 (0.26 - 1.01)	0.43 (0.20 - 0.92)	0.86 (0.41 - 1.80)	0.0001
Size						
<10mm	343/684	1.00[ref]	0.58 (0.35 - 0.98)	0.61 (0.34 - 1.09)	0.54 (0.29 - 1.01)	0.0001
≥10mm	161/684	1.00[ref]	0.74 (0.41 - 1.34)	0.58 (0.28 - 1.18)	0.84 (0.41 - 1.71)	0.01
Histology						
Tubular	375/684	1.00[ref]	0.58 (0.34 - 0.98)	0.62 (0.34 - 1.13)	0.60 (0.32 - 1.16)	0.0001
Tubulovillous/ Villous	191/684	1.00[ref]	0.63 (0.34 - 1.17)	0.46 (0.22 - 0.96)	0.65 (0.31 - 1.36)	0.02
Dysplasia						
Mild-moderate	246/684	1.00[ref]	0.58 (0.31 - 1.07)	0.53 (0.27 - 1.04)	0.60 (0.29 - 1.24)	0.0002
Severe atypia	318/684	1.00[ref]	0.60 (0.35 - 1.01)	0.58 (0.31 - 1.08)	0.63 (0.33 - 1.18)	0.0001
Shape						
Pedunculated	136/684	1.00[ref]	0.68 (0.38 - 1.19)	0.71 (0.37 - 1.36)	0.53 (0.26 - 1.07)	0.001
Sessile	297/684	1.00[ref]	0.52 (0.31 - 0.90)	0.54 (0.30 -0.96)	0.64 (0.36 - 1.16)	0.0001

	N(Cases/Community-based controls)[b]	1	2	3	4	*p-trend*
Location						
Distal colon and rectum	377/535	1.00[ref]	0.81 (0.50 - 1.30)	0.60 (0.35 - 1.04)	0.50 (0.28 - 0.91)	0.03
Proximal colon	183/535	1.00[ref]	0.81 (0.44 - 1.48)	0.47 (0.23 - 0.97)	0.83 (0.41 - 1.66)	0.12
Size						
<10mm	343/535	1.00[ref]	0.74 (0.46 - 1.20)	0.52 (0.30 - 0.90)	0.47 (0.26 - 0.85)	0.01
≥10mm	161/535	1.00[ref]	1.13 (0.65 - 1.96)	0.68 (0.35 - 1.31)	0.94 (0.48 - 1.83)	0.4
Histology						
Tubular	375/535	1.00[ref]	0.77 (0.47 - 1.26)	0.61 (0.35 - 1.07)	0.58 (0.32 - 1.05)	0.07
Tubulovillous/ Villous	191/535	1.00[ref]	0.84 (0.47 - 1.49)	0.46 (0.23 - 0.92)	0.64 (0.32 - 1.38)	0.33
Dysplasia						
Mild-moderate	246/535	1.00[ref]	0.74 (0.42 - 1.31)	0.51 (0.26 - 0.98)	0.48 (0.24 - 0.97)	0.02
Severe atypia	318/535	1.00[ref]	0.83 (0.51 - 1.36)	0.60 (0.34 - 1.05)	0.68 (0.38 - 1.21)	0.27
Shape						
Pedunculated	136/684	1.00[ref]	1.06 (0.63 - 1.79)	0.78 (0.43 - 1.42)	0.60 (0.31 -1.16)	0.37
Sessile	297/684	1.00[ref]	0.67 (0.42 - 1.07)	0.47 (0.27 - 0.81)	0.55 (0.32 - 0.97)	0.02

[a] Model adjusted for age, sex, total energy intake, pack-years of smoking, physical activity, NSAID use, dietary fiber, calcium intake, and 25-OH vitamin D.

[b]Model adjusted for age, sex, total energy intake, pack-years of smoking, physical activity, NSAID use, dietary fiber, and calcium intake.

[d]Polyamine intake level categorized according to quartile cut points. 1: <= 202,287.6 nmol/day, 2: 202,287.6 nmol/day to 317,071.6 nmol/day, 3: 317,071.6 nmol/day to 451,683.1 nmol/day, 4: > 451,683.1 nmol/day.

Figures

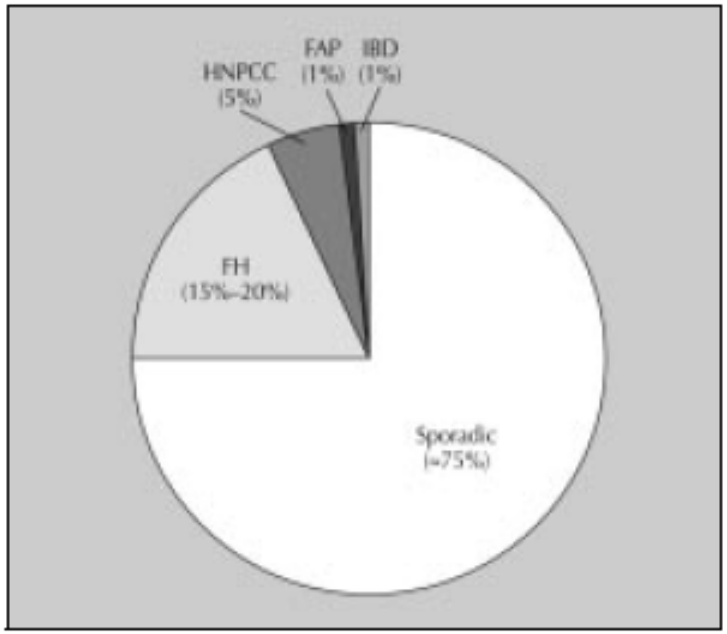

Figure 1 Breakdown of the causes of CRC. The p percentage of cases caused by each cause is indicated. 'Sporadic" cases consist of women and men aged 50 years and above with no known increased risk factors. FAP--familial adenomatous polyps; HNPCC hereditary non-polyposis colorectal cancer; IBD inflammatory bowel diseases; FH-- positive family history.

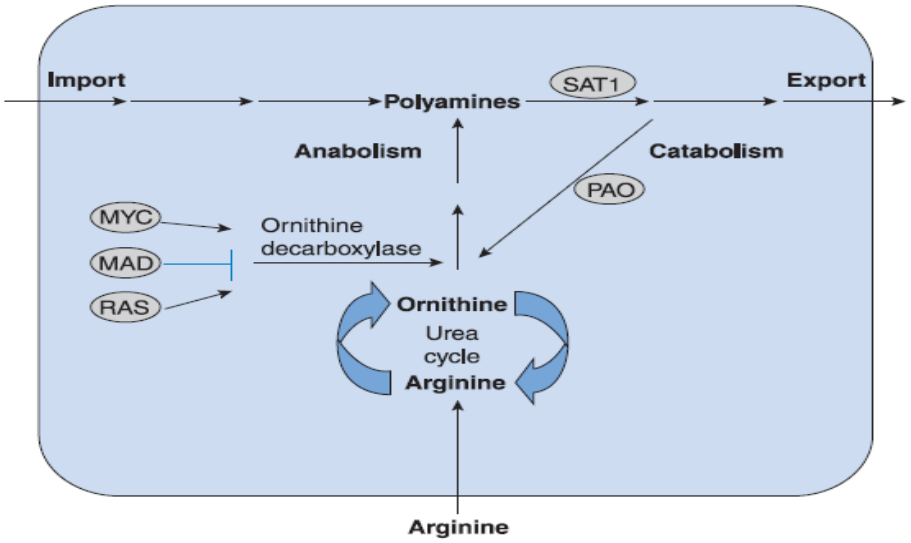

Figure 2 Schematic of polyamine transport via import, export, anabolism, and catabolism. Arginine is imported into the cell, and is then converted into ornithine via the urea cycle. Ornithine is then converted into polyamine by Ornithine decarboxylase (ODC). Decreased catabolism or export further increases polyamine pools.

CHAPTER III: SUMMARY, PUBLIC HEALTH IMPLICATIONS, AND FUTURE DIRECTIONS

The findings of this study add to the sparse literature on the association between dietary exposures and colorectal cancer. There was a statistically significant inverse association between dietary polyamines and incident, sporadic colorectal adenomas, especially for smaller, distal adenomas. The main sources of dietary polyamines are derived from fruits and vegetables (28, 66, 80); it is plausible that the inverse association observed here may be due to macronutrients present in fruits and vegetables, which have been inversely associated with colorectal adenomas (28, 33, 35, 36), but not in the case-control study population that this analyses was based on.

There are several notable implications of our findings in the context of public health. First, colorectal cancer is an avoidable disease due to the environmental nature of the etiology of most cases. Since the advent of the fast-food nation, diet has substantially contributed to both morbidity and mortality rates in the U.S.(86). However, there has been a recent change in the tide, with major campaigns advocating for healthier food choices and organic diet life styles. Given the results of this investigation, further research into possible role that dietary polyamines, particularly from fruits and vegetables sources, may play in lowering adenoma risk is needed.

On a larger scale, further confirmatory studies, in conjunction with our findings could inform policies that could lead to recommendations by appropriate agencies, on what precise range of polyamine intake is needed to reduce adenoma risk. Consequently, this could make in-roads for polyamine contents to become basic nutritional information available on food labels to allow consumers to plan for a balanced polyamine intake as

part of their adoption of healthier life styles. Furthermore, findings of interactions between several risk factors and dietary polyamines could perhaps lead to specific, targeted interventions for individuals based on their risk profile. Those found with risk factors that act synergistically on the inverse association of polyamines with adenoma risk can be encouraged and educated on how to adopt diets centered on balancing polyamine intake. Conversely, individuals with risk factors that act antagonistically on the association of polyamines with adenomas can be targeted for adoption of a polyamine-restricted diet. At the very least, the methodology illustrated here can serve as a baseline for other, larger-scale studies to further assess this association.

There are several avenues of future research needed to further investigate the association of dietary polyamines with adenoma risk. The polyamine species, spermine, spermidine, and putrescine may play somewhat different roles in carcinogenesis. Spermine and spermidine were found to be higher in polyps and tumor tissue samples compared to normal mucosa, whereas putrescine levels were not found to differ much between the three samples (60-62). These findings suggest that it would be worthwhile to investigate the association of species-specific polyamines with colorectal adenomas. This could address whether certain polyamine sub-species have stronger associations with adenoma risk compared to the others, or whether some are positively associated with adenoma risk, while others sub-species are inversely associated as was found in our study.

Also, in order to address the role of dietary polyamines on adenoma incidence, a long-term, follow-up study could be conducted measuring polyamine intake as the exposure of interest and adenomatous polyps as the outcome. This could perhaps,

elucidate what role dietary polyamines may play in the incidence of sporadic adenomas. That is, address the question of whether increased polyamine levels in tumor tissue samples were due only to the enhanced uptake by the tumors, or whether intake of dietary polyamines increased risk of tumor incidence.

Since polyamines have been established to be positively associated with colorectal neoplasms (60, 62), a randomized clinical trial may be unethical, although such a study would allow us to directly measure and comment on the effect that dietary polyamines have on risk for incident, sporadic colorectal adenomas. Overall, our findings support further research on the potential association of dietary polyamines with colorectal adenomas.

APPENDICES

Polyamine Quantification Information

Table of Polyamine content by serving size of food items as reported by Zoumas-Morse et al

Food item	Serving size	Polyamine (nmol) per serving size
Putrescine		
Corn (fresh/canned)	½ cup	560,000/902,880
Grapefruit juice	1 cup	276,640
Oranges	1 medium	174,230
Orange juice	1 cup	154,629
Grits	1 cup	99,728
Crab (canned)	½ cup	93,555
Grapefruit	½ medium	90,176
Cream of potato soup	1 cup	70,930
Tortilla chips	1 small bag	56,717
Tomato and V8[b] juice	1 cup	56,181
Spermidine		
Corn (fresh/canned)	½ cup	137,682/221,111
Green pea soup	1 cup	65,552
Pear	1 medium	60,756

Cheese enchilada	1 medium	48,770
Tempeh	3 oz	42,618
Soy burgers	1	39,616
Peas (fresh/canned)	½ cup	35,920/38,165[a]
Lentil soup	1 cup	37,117
Pasta with meat sauce	1 cup	36,059
Tofu hotdog	1	27,121
Spermine		
Green pea soup	1 cup	36,988
Chicken liver	4 oz	33,226
Chili with meat and beans	1 cup	26,441
Chicken breast (grilled/roasted)	1 large	21,560/24,420[a]
Black bean soup	1 cup	23,786
Pear, fresh	1 medium	23,572
Peas (fresh/canned)	½ cup	20,840/22,143[a]
Bean with bacon soup	1 cup	22,062
Ground turkey	3 oz	21,535
Tempeh	3 oz	20,565

Table of Polyamine content of food items by average contribution to diet as reported by Zoumas-Morse et al

Food item	Polyamine contribution (nmol/day)
Putrescine	
Orange juice and grapefruit juice	44,441
Oranges, grapefruit, and tangerines (not including juice)	17,613
Fresh tomatoes	10,042
Bananas	7,344
Beer (all types)	6,374
Corn and hominy	5,832
Cheese (eg, American, cheddar)	5,592
Potato chips, tortilla chips, corn chips, puffs, and pretzels	4,595
Burritos, tacos, tostadas, and quesadillas	4,411
Green pepper and green chilies	4,343
Spermidine	
Green peas	3,283
Cheese, such as American and cheddar	3,124
Lasagna and pasta with meat sauce	2,900
Potatoes (boiled, baked, and mashed)	2,388
Burritos, tacos, tostadas, and quesadillas	1,890
Dark breads (including dark bagels and rolls)	1,736

Green salad (lettuce or spinach)	1,535
Low- or reduced-fat cheese	1,456
Corn and hominy	2,765
Broccoli	1,347
Spermine	
Ground meat	2,186
Lunch meats (eg, ham, turkey, bologna, and salami)	1,977
Green peas	1,905
Lasagna and pasta with meat sauce	1,443
Peanut butter, peanuts, and other nuts and seeds	1,237
Rice, noodles, and other grains	1,136
Chili with meat and beans	1,027
Bean soups such as pea, lentil, and black bean	747
Cheese (eg, American, cheddar)	670
Stew, pot pie, and casseroles with meat or chicken	656

Method of Polyamine quantification

Polyamine intake calculation according to frequency of consumption of food item							
Polyamine content for which food items were reported by average contribution to diet per day by Zoumas-Morse et al							
Food Item	**N**	**Frequency of consumption**	**Food item's average polyamine contribution to diet Zoumas *et al***	**Daily intake according to frequency of consumption (servings/day)**	**Study population average daily intake (servings/day)**	**Ratio (individual intake/population average intake**	**Ratio x average polyamine contribution (nmol/day)**
Baked potato	61	Never or less than once per month	2388	0	0.143	0	0
	484	1-3 per month	2388	0.067	0.143	0.5	1114.4
	685	Once per week	2388	0.143	0.143	1.0	2388.0
	1077	2-4 times per week	2388	0.429	0.143	3.0	7164.0
	221	5-6 times per week	2388	0.786	0.143	5.5	13134.0
	101	Once per day	2388	1.000	0.143	7.0	16716.0
	7	2-3 times per day	2388	2.500	0.143	17.5	41790.0
	4	4-5 times per day	2388	4.500	0.143	31.5	75222.0
	1	6+ times per day	2388	6.000	0.143	42.0	100296.0
Banana	311	Never or less than once per month	0	0	0.143	0.0	0.0
	539	1-3 per month	7344	0.067	0.143	0.5	3427.2
	450	Once per week	7344	0.143	0.143	1.0	7344.0
	823	2-4 times per week	7344	0.429	0.143	3.0	22032.0
	176	5-6 times per week	7344	0.786	0.143	5.5	40392.0

	318	Once per day	7344	1.000	0.143	7.0	51408.0
	25	2-3 times per day	7344	2.500	0.143	17.5	128520.0
	1	6+ times per day	7344	6.000	0.143	42.0	308448.0
Beansoup	1558	Never or less than once per month	0	0.000	0.067	0.0	0.0
	826	1-3 per month	747	0.067	0.067	1.0	747.0
	198	Once per week	747	0.143	0.067	2.1	1600.7
	49	2-4 times per week	747	0.429	0.067	6.4	4802.1
	4	5-6 times per week	747	0.786	0.067	11.8	8803.9
Bologna	985	Never or less than once per month	0	0.000	0.067	0.0	0.0
	796	1-3 per month	1977	0.067	0.067	1.0	1977.0
	389	Once per week	1977	0.143	0.067	2.1	4236.4
	336	2-4 times per week	1977	0.429	0.067	6.4	12709.3
	70	5-6 times per week	1977	0.786	0.067	11.8	23300.4
	32	Once per day	1977	1.000	0.067	15.0	29655.0
	28	2-3 times per day	1977	2.500	0.067	37.5	74137.5
	5	4-5 times per day	1977	4.500	0.067	67.5	133447.5
Low-fat Cheese	848	Never or less than once per month	0	0.000	0.067	0.0	0.0
	898	1-3 per month	1456	0.067	0.067	1.0	1456.0
	489	Once per week	1456	0.143	0.067	2.1	3120.0
	301	2-4 times per week	1456	0.429	0.067	6.4	9360.0
	40	5-6 times per week	1456	0.786	0.067	11.8	17160.0
	40	Once per day	1456	1.000	0.067	15.0	21840.0

	8	2-3 times per day	1456	2.500	0.067	37.5	54600.0
	2	4-5 times per day	1456	4.500	0.067	67.5	98280.0
Broccoli	462	Never or less than once per month	0	0	0.067	0.0	0.0
	997	1-3 per month	1347	0.067	0.067	1.0	1347.0
	692	Once per week	1347	0.143	0.067	2.1	2886.4
	420	2-4 times per week	1347	0.429	0.067	6.4	8659.3
	49	5-6 times per week	1347	0.786	0.067	11.8	15875.4
	20	Once per day	1347	1.000	0.067	15.0	20205.0
	1	2-3 times per day	1347	2.500	0.067	37.5	50512.5
	1	4-5 times per day	1347	4.500	0.067	67.5	90922.5
	1	6+ times per day	1347	6.000	0.067	90.0	121230.0
Dark Bread	1152	Never or less than once per month	0	0.000	0.067	0.0	0.0
	616	1-3 per month	1736	0.067	0.067	1.0	1736.0
	353	Once per week	1736	0.143	0.067	2.1	3720.0
	295	2-4 times per week	1736	0.429	0.067	6.4	11160.0
	72	5-6 times per week	1736	0.786	0.067	11.8	20460.0
	98	Once per day	1736	1.000	0.067	15.0	26040.0
	31	2-3 times per day	1736	2.500	0.067	37.5	65100.0
	4	4-5 times per day	1736	4.500	0.067	67.5	117180.0
Green pepper	1442	Never or less than once per month	0	0	0.067	0.0	0.0
	698	1-3 per month	4343	0.067	0.067	1.0	4343.0
	297	Once per week	4343	0.143	0.067	2.1	9306.4

	150	2-4 times per week	4343	0.429	0.067	6.4	27919.3
	25	5-6 times per week	4343	0.786	0.067	11.8	51185.4
	15	Once per day	4343	1.000	0.067	15.0	65145.0
	3	2-3 times per day	4343	2.500	0.067	37.5	162862.5
Chips	974	Never or less than once per month	0	0	0.067	0.0	0.0
	843	1-3 per month	4595	0.067	0.067	1.0	4595.0
	438	Once per week	4595	0.143	0.067	2.1	9846.4
	299	2-4 times per week	4595	0.429	0.067	6.4	29539.3
	45	5-6 times per week	4595	0.786	0.067	11.8	54155.4
	32	Once per day	4595	1.000	0.067	15.0	68925.0
	12	2-3 times per day	4595	2.500	0.067	37.5	172312.5
	1	4-5 times per day	4595	4.500	0.067	67.5	310162.5
Pasta	134	Never or less than once per month	0	0	0.143	0.0	0.0
	780	1-3 per month	4343	0.067	0.143	0.5	2026.7
	978	Once per week	4343	0.143	0.143	1.0	4343.0
	657	2-4 times per week	4343	0.429	0.143	3.0	13029.0
	76	5-6 times per week	4343	0.786	0.143	5.5	23886.5
	16	Once per day	4343	1.000	0.143	7.0	30401.0
	3	2-3 times per day	4343	2.500	0.143	17.5	76002.5
	1	6+ times per day	4343	6.000	0.143	42.0	182406.0
Peanut Butter	805	Never or less than once per month	0	0	0.143	0.0	0.0
	644	1-3 per month	1237	0.067	0.143	0.5	577.3

	440	Once per week	1237	0.143	0.143	1.0	1237.0
	468	2-4 times per week	1237	0.429	0.143	3.0	3711.0
	116	5-6 times per week	1237	0.786	0.143	5.5	6803.5
	121	Once per day	1237	1.000	0.143	7.0	8659.0
	36	2-3 times per day	1237	2.500	0.143	17.5	21647.5
	7	4-5 times per day	1237	4.500	0.143	31.5	38965.5
	3	6+ times per day	1237	6.000	0.143	42.0	51954.0
Tomato	346	Never or less than once per month	0	0	0.143	0.0	0.0
	899	1-3 per month	10042	0.067	0.143	0.5	4686.3
	665	Once per week	10042	0.143	0.143	1.0	10042.0
	587	2-4 times per week	10042	0.429	0.143	3.0	30126.0
	86	5-6 times per week	10042	0.786	0.143	5.5	55231.0
	46	Once per day	10042	1.000	0.143	7.0	70294.0
	11	2-3 times per day	10042	2.500	0.143	17.5	175735.0
	1	4-5 times per day	10042	4.500	0.143	31.5	316323.0
White Rice	652	Never or less than once per month	0	0	0.067	0.0	0.0
	1282	1-3 per month	1136	0.067	0.067	1.0	1136.0
	484	Once per week	1136	0.143	0.067	2.1	2434.3
	182	2-4 times per week	1136	0.429	0.067	6.4	7302.9
	16	5-6 times per week	1136	0.786	0.067	11.8	13388.6
	11	Once per day	1136	1.000	0.067	15.0	17040.0
	4	2-3 times per day	1136	2.500	0.067	37.5	42600.0
Meat dishes	320	Never or less than once per month	0	0.000	0.143	0.0	0.0

	784	1-3 per month	656	0.067	0.143	0.5	306.1
	808	Once per week	656	0.143	0.143	1.0	656.0
	635	2-4 times per week	656	0.429	0.143	3.0	1968.0
	63	5-6 times per week	656	0.786	0.143	5.5	3608.0
	26	Once per day	656	1.000	0.143	7.0	4592.0
	4	2-3 times per day	656	2.500	0.143	17.5	11480.0
Meat dishes 2	396	Never or less than once per month	0	0.000	0.143	0.0	0.0
	932	1-3 per month	656	0.067	0.143	0.5	306.1
	810	Once per week	656	0.143	0.143	1.0	656.0
	447	2-4 times per week	656	0.429	0.143	3.0	1968.0
	39	5-6 times per week	656	0.786	0.143	5.5	3608.0
	19	Once per day	656	1.000	0.143	7.0	4592.0
	5	2-3 times per day	656	2.500	0.143	17.5	11480.0
Cheese	376	Never or less than once per month	0	0.000	0.143	0.0	0.0
	807	1-3 per month	9386	0.067	0.143	0.5	4380.1
	648	Once per week	9386	0.143	0.143	1.0	9386.0
	587	2-4 times per week	9386	0.429	0.143	3.0	28158.0
	103	5-6 times per week	9386	0.786	0.143	5.5	51623.0
	75	Once per day	9386	1.000	0.143	7.0	65702.0
	32	2-3 times per day	9386	2.500	0.143	17.5	164255.0
	1	4-5 times per day	9386	4.500	0.143	31.5	295659.0

Food items for which polyamine content was reported by serving size Zoumas-Morse et al					
Food Item	**N**	**Frequency of consumption**	**Food item's polyamine content by serving size (nmol/serving size) to diet Zoumas *et al***	**Daily intake according to frequency of consumption (servings/day)**	**Frequency x polyamine contribution (nmol/day)**
Liver	2336	Never or less than once per month	33226	0.0	0.0
	275	1-3 per month	33226	0.1	1114.4
	22	Once per week	33226	0.1	2388.0
	3	2-4 times per week	33226	0.4	7164.0
	1	Once per day	33226	1.0	13134.0
Corn	384	Never or less than once per month	902880	0.0	0.0
	1092	1-3 per month	902880	0.1	60192.0
	813	Once per week	902880	0.1	128982.9
	328	2-4 times per week	902880	0.4	386948.6
	20	5-6 times per week	902880	0.8	709405.7
	7	Once per day	902880	1.0	902880.0
	1	2-3 times per day	902880	2.5	2257200.0
Grape fruit juice	1944	Never or less than once per month	276640	0.0	0.0
	379	1-3 per month	276640	0.1	18442.7
	107	Once per week	276640	0.1	39520.0

	125	2-4 times per week	276640	0.4	118560.0
	26	5-6 times per week	276640	0.8	217360.0
	34	Once per day	276640	1.0	276640.0
	9	2-3 times per day	276640	2.5	691600.0
Grape fruit	1361	Never or less than once per month	90176	0.0	0.0
	677	1-3 per month	90176	0.1	6011.7
	257	Once per week	90176	0.1	12882.3
	236	2-4 times per week	90176	0.4	38646.9
	35	5-6 times per week	90176	0.8	70852.6
	58	Once per day	90176	1.0	90176.0
	5	2-3 times per day	90176	2.5	225440.0
	1	4-5 times per day	90176	4.5	405792.0
	1	6+ times per day	90176	6.0	541056.0
Pear	1675	Never or less than once per month	84328	0.0	0.0
	697	1-3 per month	84328	0.1	5621.9
	160	Once per week	84328	0.1	12046.9
	78	2-4 times per week	84328	0.4	36140.6
	10	5-6 times per week	84328	0.8	66257.7
	9	Once per day	84328	1.0	84328.0
Peas	559	Never or less than once per month	60308	0.0	0.0
	1063	1-3 per month	60308	0.1	4020.5
	747	Once per week	60308	0.1	8615.4
	249	2-4 times per week	60308	0.4	25846.3

	17	5-6 times per week	60308	0.8	47384.9
	7	Once per day	60308	1.0	60308.0
	1	4-5 times per day	60308	4.5	271386.0
Tofu	2472	Never or less than once per month	63183	0.0	0.0
	101	1-3 per month	63183	0.1	4212.2
	36	Once per week	63183	0.1	9026.1
	22	2-4 times per week	63183	0.4	27078.4
	2	5-6 times per week	63183	0.8	49643.8
	4	Once per day	63183	1.0	63183.0
Tomato Juice	1724	Never or less than once per month	56181	0.0	0.0
	640	1-3 per month	56181	0.1	3745.4
	171	Once per week	56181	0.1	8025.9
	84	2-4 times per week	56181	0.4	24077.6
	10	5-6 times per week	56181	0.8	44142.2
	5	Once per day	56181	1.0	56181.0
Chicken- No skin	418	Never or less than once per month	23010	0.0	0.0
	563	1-3 per month	23010	0.1	1532.7
	751	Once per week	23010	0.1	3284.3
	776	2-4 times per week	23010	0.4	9852.9
	89	5-6 times per week	23010	0.8	18063.6
	18	Once per day	23010	1.0	22990.0
	10	4-5 times per day	23010	2.5	57475.0
	1	6+ times per day	23010	6.0	137940.0