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Associations of Fruit and Vegetable Intake with Incident, Sporadic, Colorectal Adenoma

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Environmental Health and Epidemiology

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# Associations of Fruit and Vegetable Intake with Incident, Sporadic, Colorectal Adenoma

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## Abstract

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By Elizabeth A. Burkhardt

Although associations of fruit and vegetable (F/V) intake with colorectal neoplasms have been studied extensively, the findings have been inconsistent, perhaps due to differential distributions of potential effect modifying variables across study populations. We used data from three case-control studies conducted between 1991 and 2002 to assess associations of total fruit, total vegetables, and total fruit plus vegetable intakes with incident, sporadic, colorectal adenoma according to potential effect modifying risk factors (age, sex, nonsteroidal anti-inflammatory drug [NSAID] use, smoking, chromosome 8q24 region single nucleotide polymorphisms) and adenoma characteristics among 792 cases and 985 colonoscopy-negative controls in a pooled analysis, and among 565 cases and 536 community controls in a separate analysis. In comparisons of the highest to lowest quartiles of F/V intakes, risk estimates were close to the null overall and in the stratified analyses. The multivariable-adjusted risk estimates that differed the most from the null included those for fruit intake with adenomas overall in the analysis involving community controls (odds ratio [OR] 0.78, 95% confidence interval [CI] 0.52-1.16), among ever smokers in the pooled and community controls analyses (ORs 0.64 [CI 0.42-0.99] and 0.68 [CI 0.41-1.13], respectively), and among those who did not regularly take an NSAID in the pooled and community control analyses (ORs 0.76 [CI 0.53-1.11] and 0.70 [CI 0.46-1.08)], respectively). The fruit-adenoma inverse association tended to be stronger for multiple and distal adenomas and those with more advanced characteristics; for example, the OR for adenomas with some villous histology was 0.56 (CI 0.32-0.98). However, the OR for the association of vegetable intake with adenomas among those with the rs7837328 low risk GG genotype was 2.37 (CI 1.09-5.16). These results provide little to no support for inverse associations of fruit and vegetable intake with risk for incident, sporadic adenoma, but do provide some support for further investigations into whether 1) increased fruit intake may reduce risk for advanced adenomas, especially among persons with higher oxidative stress/inflammation from smoking or not taking an anti-inflammatory drug, and 2) chromosome 8q24 region genotypes may modify associations of vegetable intake with adenomas.

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## Table of Contents

## Chapter 1: Background

In the United States, colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second most common cause of cancer mortality. Lifetime risk is about 1 in 20, with 141,210 diagnoses and 49,380 deaths expected in 2011 [1, 2]. Incidence among U.S. men in 2008 was 50.39 per 100,000, while in women it was slightly lower, at 39.22 per 100,000 [3]. Ninety percent of all CRC occurs in individuals over age 50. African Americans have the highest rates among different racial groups in the U.S. (62.88 per 100,000 for men and 46.41 per 100,000 for women), and Ashkenazi Jews have one of the highest rates worldwide [1].

Research has suggested that a combination of environmental and genetic factors contribute to the development of CRC. Migration studies have shown that environmental and life style factors play an important role. Populations that have historically had low risk of CRC include those in India, Japan, China, Italy, and undeveloped countries. When populations migrate to higher-risk, developed countries such as the U.S., their risks increase, becoming greater than among those who remain in their home countries [4, 5].

Factors that have been established as increasing risk for CRC include older age, genetic colorectal cancer syndromes (familial adenomatous polyposis [FAP] or Gardner's Syndrome, Hereditary Non-polyposis Colon Cancer [HNPCC] or Lynch Syndrome), history of adenomatous polyps or previous colorectal cancer, a history of colorectal cancer in a first degree relative, a history of inflammatory bowel disease (Crohn's disease or ulcerative colitis), and being tall. Other factors for which there has been fairly

consistent evidence include acromegaly, excess alcohol intake, being a male, low folate intake in combination with high alcohol intake, high body mass index (BMI), high circulating concentration of insulin-like growth factor type I (IGF-I), uterosigmoidostomy, intake of red and processed meats, and non-insulin dependent diabetes mellitus [4, 6, 7]. Characteristics of adenomas that are associated with increased CRC risk include adenoma size greater than 1 cm, villous histology, multiplicity, and higher degree of dysplasia [4]. Also, tobacco use has been associated with increased risk of adenoma, and very long term smoking may increase the risk for rectal cancer [4].

Other potential risk factors for which findings have been less consistent include higher intakes of energy, protein, fat, carbohydrates, and heavily-browned meat; lower intakes of fruits, vegetables and fiber; removal of the gall bladder; family history of ovarian, endometrial, and breast cancer; and urbanization/industrialization. Other factors that have been investigated have included exposure to pesticides and asbestos; country of residence; and workplace exposures of painters, printers, railway workers, woodworkers, automotive industry workers, and metal workers [4, 7].

Factors consistently associated with lower risk for CRC include non-steroidal anti-inflammatory drug (NSAID) use, higher intake of calcium, hormone replacement therapy among women, and higher levels of physical activity. Evidence is growing that higher serum 25-OH-vitamin D levels may be associated with lower risk. Among those with a history of colorectal adenoma, early detection and removal of the adenomas sharply reduces risk. Research has also suggested weak inverse associations for intake of fruits, vegetables, fiber, low-fat dairy, fish, poultry, and methionine [8, 9].

Colorectal cancer begins in the large intestine, which is a five and one-half foot tube comprised of the colon and the rectum. Most CRC develops from adenomatous colorectal polyps, which form in cells that normally make up glands that produce mucus for lubrication of the large intestine. Colorectal cancer can occur anywhere along the length of the large intestine, from the cecum to the rectum. It always originates in the innermost tissue in the wall of the structure, and it can also grow into the outer layers [1].

Molecular events are very important in the progression from normal tissue to cancer. The molecular basis for CRC involves the DNA that programs growth, division, and death in human cells. DNA can become damaged through various means. Normally, when damaged, DNA is repaired in the cell, otherwise the cell undergoes apoptosis. When these steps do not occur, the abnormal cells can grow uncontrollably, and the result is cancer. Unlike normal cells, cancer can also metastasize, invading other tissues in the body [1].

In 1990, Fearon and Vogelstein suggested a model that describes CRC development as a stepwise process. Each stage of the process may incorporate mutation/activation of oncogenes, which can increase the speed of cell division or prolong cell life (*ras* mutations). Steps may also include mutations of tumor suppressor genes, which normally inhibit cell division and encourage cell death. More than one mutation is necessary for CRC to develop [4, 10].

In the process of colorectal carcinogenesis, one of the first steps is hyperproliferation of normal epithelial cells in the colon, which progress to cells with less ordered growth, often followed by progression to small adenomas. At least 95% of colorectal cancers

begin as adenomatous polyps. As adenomas grow, they develop greater dysplasia and progress from early, to intermediate, to late stages, eventually progressing to cancer and possibly metastasis [1, 4].

Mutation of a tumor suppressor gene, the adenomatous polyposis coli (APC) gene, through allelic loss is often an early step in cancer development. Eighty percent of individuals with sporadic CRC carry a mutation in the APC gene [11]. Transcription of DNA or gene activation may be affected later in the process through hypomethylation of DNA [4, 6]. Other important steps may include mutation of the K-*ras* protooncogene; loss of DNA in the deleted in colorectal cancer (*DCC*) gene; and mutation of the p53 tumor-suppressor gene. An additional mechanism that may also be involved is microsatellite instability, which accounts for about 15% of sporadic CRC [12] and occurs more frequently in proximal CRC. This mechanism may involve mutations in the BAX gene (which promotes apoptosis) and TGF-β type II receptor gene (a tumor suppressor gene). CRC can also involve abnormalities in mismatch repair genes, which can negatively affect DNA repair. [4, 7]. Effects of mutations in mismatch repair may be found in up to 15% of sporadic CRC cases. Hypermethylation of mismatch repair gene hMLH1, which inhibits gene transcription, is present in many CRC cases in which microsatellite instability is involved [4].

Another mechanism for colorectal carcinogenesis may involve an APC-β-catenin-Tcf-MYC pathway [4]. The Wnt/ β-catenin signaling pathway is affected by mutations of the APC gene. Altered regulation of the APC protein, which is encoded by the APC gene, causes high concentrations of β-catenin. Adhesion of β-catenin to the T-cell factor (Tcf4) affects the oncogene c-myc. Research has also shown that mutations in the β-catenin gene

are found in some CRC where no mutation in the APC gene is found [4, 11]. Furthermore, Wnt signaling, which is involved in regulation of cell proliferation, may be affected by genetic variation at the 8q24 locus [13]. This locus has also been associated with increased risk for CRC in genome-wide association studies (GWAS), and is now known to be important in CRC development [8, 14-16]. Other genetic factors that are associated with increased CRC risk include a fast NAT2 phenotype and mutations in other tumor suppressor genes, including SMAD2, SMAD4, which affect inhibition of cell growth through transforming growth factor β (TGF β), thereby affecting tumor progression [4].

Other mechanisms can also be involved in colorectal carcinogenesis. The glutathione/ glutathione-S transferase system has an important function in the colon. It detoxifies chemicals, making them less biologically active, more water soluble and easier to excrete [17]. Furthermore, chronic inflammation is a condition that can result in genetic changes (p53 mutation) advancing to dysplasia and eventually to cancer [4].

While research has not yet shown that molecular changes always occur in a specific order [6], some changes have been linked to specific timing of cancer development. For example, mutations in the APC β-catenin gene occur early in the progression, whereas P53 mutations usually occur late [4].

An important consideration in CRC research is the finding that biological mechanisms may differ according to location of the cancer, due to differences in blood and nerve supplies, microflora, enzyme concentrations, fecal profile, bile acid metabolism, molecular characteristics, and stool transit time [4, 18-20]. Chromosomal instability may

be more of a factor in distal colon cancers than in proximal cancers, whereas microsatellite instability may be a greater factor in proximal colon cancer. Diet may also be more important in distal colon cancer than in proximal colon cancer [21]. Concerning other factors that may be related to CRC subsite, risk of CRC according to subsite may differ according to gender, alcohol use, calcium intake, history of cholecystectomy, physical activity, or mutations in P53 or chromosomal regions 5q, 17q, or 18q [4].

Dietary factors have been of particular interest in CRC research. Red and processed meats have been consistently, positively associated with CRC, while an inverse association has been suggested for intake of low-fat dairy, poultry, and fish. Researchers have been particularly interested in consumption of fruits and vegetables (F/V) because they contain constituents that are believed to be anti-carcinogenic. These compounds may affect some of the steps in the disease progression described above.

The hypothesized protective effects of fruits and vegetables are supported by a number of biologically plausible mechanisms that involve effects on disease progression. Fruits and vegetables contain many bioactive compounds, which may work together to produce anti-carcinogenic effects, or their actions may overlap [21, 22]. They also contain vitamins and minerals, thereby protecting against nutritional deficiencies and reducing risk of cancer [9]. Furthermore, their bioactive constituents can be beneficial in maintenance of a healthy body weight, which is important in CRC prevention [4, 23].

Compounds in fruits and vegetables that have been hypothesized to be protective include fiber, antioxidants, antioxidant-enzyme-associated micronutrients, stilbenes, reservatrol, lignans, isothiocyanates, isothyianates, thiocyanates, short-chain fatty acids, polyamine

inhibitors, limonoids, folate, calcium, dithiolthiones, glucosinolates, indoles, coumarins, flavanoids, phenols, polyphenols, carotenoids, protease inhibitors, plant sterols, isoflavones, saponins, inositol hexaphosphate, allium compounds, limonene, and other phytochemicals. Specific foods and botanical groups which have shown particularly strong inverse associations with colorectal neoplasms include cruciferous vegetables (which contain dithiolthiones, glucosinolates, indoles, isothyocyanates, thyocyanates), citrus fruits (which contain liminoids), onions, and garlic [5, 22].

At least fifteen studies have reported associations between intake of cruciferous vegetables and CRC risk. Most of these have been inverse associations, and may be due to the presence of glucolsinolates, which are bioactive secondary metabolites. Two studies have shown that the association between intake of cruciferous vegetables and CRC may differ according to genetic differences in how individuals break down glucosinolate products. Future research on F/V intake and CRC may benefit from controlling for genetic differences in metabolism of F/V [24].

Antioxidants, which are found in all fruits and vegetables, may also be beneficial, protecting against CRC by reducing oxidative stress, therefore reducing damage to colon epithelium and reducing inflammation [23, 25]. Other compounds may be protective because of their effects on enzyme activity, or because they affect DNA methylation. Additionally, phenols found in fruits and vegetables may inhibit N-Nitrosamines reactions. Other compounds, such as dithiolthinoes found in cruciferous vegetables, contribute to glutathione activity and other anticarcinogenic effects, including blockage of electrophilic cancer-causing agents with macromolecules. Sulforaphane, also found in cruciferous vegetables, has shown promising activity against polyp and aberrant crypt

development in rodents. Lupeol, found in a number of fruits and vegetables, has been shown in *in vivo* and *in vitro* studies to fight against inflammation, DNA damage, and mutagenic activity [22]. Retinoids, which result from vitamin A metabolism, have been shown to decrease Wnt/B-catenin signaling activity [11]. Lycopene has been shown to decrease cancer activity for some human cancers. Deguelin, found in legumes, induces apoptosis and has shown promise against colon cancer. Additionally, plant sterols have beneficial effects on cell membranes; isoflavones provide weak estrogenic effects; saponins bind bile acids, which can otherwise be harmful in the large intestine; saponins and caratenoids can limit or reduce colonic epithelial cell proliferation; inositol hexaphosphate can decrease lipid peroxidation; allium compounds may inhibit the conversion of nitrate by bacteria; and folate, found in green, leafy vegetables, may increase glutathione activity and protect against hypomethylation of DNA and its resulting effects on transcription of DNA or gene activation [22]. Folate also affects alcohol metabolism, and it plays a role in alcohol's effect on CRC development [4]. Folate deficiency can lead to an increased numbers of chromosomal breaks [23]. Resveratrol, a polyphenol, has anti-oxidant and anti-inflammatory properties, and has been shown to reduce levels of B-catenin in colon cancer cells. It has also shown anti-carcinogenic properties by inducing apoptosis, aiding in liver metabolism, activating p53, assisting in DNA repair, increasing glutathione levels, scavenging free radicals, decreasing hyper proliferation, and down-regulating the Wnt signaling pathway [11, 26, 27].

Some of the hypothesized effects of fruits and vegetables have also been attributed to fiber, but research in the past decade has found many null associations between fiber

intake and CRC [28]. Hypotheses suggest that fiber could potentially reduce CRC risk for several reasons. It may permit less contact of carcinogens with colonic tissues due to its ability to decrease fecal transit time and increase stool bulk, thereby diluting the contents in the colon. Fiber may also reduce the toxic effects of bile acids in the colon by binding or diluting them. It also binds carcinogens, and it changes the composition of the microflora in the gut, which in turn affect metabolism. Additionally, fiber ferments, leading to the release of bound calcium as well as formation of volatile fatty acids which can lead to decreased carcinogenic activity [22]. Fiber may decrease transit time for stool in the colon, but it may have little effect on storage time in the rectum, so it may affect colon cancer and rectal cancer differently [23].

Different types of fiber are present in different fruits and vegetables. Cellulose, an insoluble fiber found in root vegetables, legumes, and leafy green vegetables, has been shown to reduce concentrations of fecal bile acids. Many insoluble fibers also decrease fecal transit times and increase bulk of stool. Fiber found in fruit has less effect on transit times and stool bulk [22], but has been shown in some studies to be more protective than vegetable fiber [29-31].

Cancer subsites may also be affected differently by compounds in F/V other than fiber, due to the differing biological mechanisms for tumors in different subsites [18]. The associations of F/V intake with different cancer subsites has been explored minimally, and is worthy of further study. Van Duijnhoven et al. studied different cancer subsites, finding an inverse association of F/V with CRC overall and with colon cancer but not with rectal cancer in an adjusted survival analysis model [32]. Koushik et al. also examined distal versus proximal colon cancer and found that total fruit and vegetable

intake, total fruits, and total vegetables may be inversely associated with distal colon cancer. Koushik et al. also note that incidence rates of distal colon cancer have been decreasing over the last several decades, while proximal cancers have been increasing. These changes in rates may have affected estimates in past studies [21].

A great deal of evidence supports the biological plausibility for an inverse association between F/V intake and CRC, and research from case-control studies in the 1980s and early 1990s found convincing inverse associations between fruit and vegetable intake and CRC/adenoma risk. Later studies, however, particularly prospective cohort studies, have been less consistent. Inconsistencies may be due to many factors, including the details of the F/V intake, differences in co-existing risk factors among specific populations, differences in study designs, recall bias, selection bias, or dietary exposure misclassification [9, 19, 21, 32, 33]. Another possible reason for inconsistencies among studies is the possibility of bias or attenuation of overall effects due to effect modification that has not been addressed. Family history of CRC is one factor that may have produced attenuated estimation of effects in case-control studies that used colonoscopy-negative individuals as the control group. Colonoscopy-negative individuals have often undergone colonoscopy as a result of physician recommendation due to family history of CRC. These individuals are often symptom-free and polyp-free, but likely shared environmental exposures, such as low intake of fruits and vegetables, with those in their families who had cancer or polyps. These controls, although they are free of colorectal neoplasm at the time of colonoscopy, may have clinically-detectable neoplasm within several years. Although they are eating few F/V, they appear to be low risk because they do not currently have cancer or polyps, but they may, in fact, be high risk, and soon to be

diagnosed with colorectal neoplasm [25]. This possibility can be explored by accounting carefully for family history, in part by considering effect modification.

Another possible reason for inconsistencies in past studies may be the effect of recall bias in case-control studies, due to cases remembering past events differently from controls. Case-control studies can also be subject to bias due to controls coming from a "health conscious" segment of the population, rather than being representative of the entire population from which the cases emerged [9]. Cases and controls might also have participated at different rates, which could have led to biased estimates [21]. For these reasons, we might expect prospective studies to be freer of bias.

Improper attention to confounding and effect modification may also have biased previous studies. F/V intake is often associated with other lifestyle factors, such as body mass index (BMI), physical activity level, smoking status, alcohol intake, and intake of red meat [23]. Although some of these have been accounted for in past studies, residual confounding may have been present [23], and other factors may be unobservable or un-measureable.

In addition, not all studies have reported considering factors such as smoking history, NSAID use, insulin resistance, genetics, or history of weight change as confounders or effect modifiers [23]. When these factors are considered, proper classification is also important. Misclassification of covariates may bias results towards the null [34]. Relying on self-reports for factors such as body mass index (BMI) and diabetes may also be problematic. The National Institutes of Health estimates that 27% or more of individuals with diabetes may not know of their condition. Additionally, because the

CRC disease process takes many years, evaluating confounding factors earlier in life might be appropriate [19, 24]. Furthermore, even in prospective studies, the inability to classify factors such as smoking and alcohol accurately may affect the ability to detect an association. These classifications are important because factors such as smoking may be important effect modifiers. In at least two studies, smoking modified the association of fruit and vegetable consumption with CRC [9, 32], and in at least two other studies, smoking modified the association of beta-carotene with CRC [32]. Van Duijnhoven et el. found that positive associations between F/V consumption and CRC were suggested in current smokers. Their study also found that alcohol, red meat intake, and BMI modified some associations of F/V with CRC, and that the effects were different according to subsite of the cancer [32]. Proper classification of these factors is therefore important.

Additionally, assessing dietary exposures is challenging for several reasons. Measurement error is known to be a problem in assessing dietary intakes. Studies that assess dietary intake through questionnaires may produce random misclassification, and the effect estimates produced by these studies tend to be attenuated [9]. Problems with dietary assessments also exist due to the variety of types of F/V eaten, as well as conditions for storage, preparation, growing, and nutrient content, which may be very different for different individuals, populations, and countries, producing inconsistent results. We must also consider the possibility that studies have yet to determine the exact constituents or combinations of F/V that might produce an inverse association between F/V consumption and CRC. Furthermore, inverse associations between specific compounds and CRC may have been overlooked in examining total F/V or in looking at other constituents [9], or synergistic effects may not have been considered appropriately.

Many studies assessing F/V intake and CRC were not able to use repeated measures or correct effect estimates for measurement error, nor were they able to include biomarkers for F/V intake in the analyses [23]. Few studies measured what a person ate over more than one decade, so relevant exposures during the development period for CRC might not have been assessed ideally in many studies. Dietary intake in early life may be more predictive of risk than intake in later life [34], which was the timeframe for measurement in most studies [19, 21]. Prospective cohort studies that evaluate diet and covariates over the course of time may provide appropriate assessments of F/V as risk factors and might reduce some recall bias.

Research has also shown that certain nutrients may be beneficial at early stages of cancer development. Folate has been shown to be most protective against CRC when intake occurs before the disease process begins or early in the process. Accounting for the timing of the exposure relative to stage of disease progression will be important in determining which factors/foods are beneficial for which types of cancers at what stage of disease [35].

Further reasons for inconsistencies in past studies might be due to invalid assumptions. The assumption that dose-response models should be linear or that the reference category of intake was not high enough to reduce risk in already well-nourished populations might need to be reconsidered [9, 23]. Aune et al. found a non-linear dose-response association and suggested that a possible reason for null findings in earlier meta-analyses were due to the assumption that a dose-response pattern should fit a linear model. Non-linear models might be more appropriate, suggesting that the greatest benefit of F/V intake in terms of reduction of CRC risk may occur at relatively low threshold levels of intake. Increasing

intake of F/V beyond what is normal in a reasonably balanced diet may not decrease cancer risk substantially, and only individuals with unusually low intake will benefit substantially by increasing intake [35]. The findings of Koushik et al. were similar. They found that increasing F/V intake generally was not associated with CRC, but that intakes at the lowest levels were positively associated with CRC [21].

Geographical location has also been a factor in assessing associations of F/V intake and CRC [36]. Aune et al. found that F/V intake had stronger inverse associations with CRC in European populations than in U.S. populations. These results could be explained by measurement differences between different geographic locations, genetic differences, environmental differences, or differences in the foods being consumed. The lowest level of intake (the reference level) in European countries also tended to be lower than in the U.S. or Asia. In the U.S. and Asia, the reference level of intake might already have been beyond a threshold level necessary to protect against cancer [35].

Many factors contribute to colorectal carcinogenesis. Our understanding of how all of these factors contribute to risk for colorectal neoplasms will continue to evolve as results from future studies are reported. Future studies which have the benefits of large sample size and long-term, prospective study designs are needed. Follow-up studies that include assessment of F/V consumption in earlier life and young adulthood will be beneficial so that the associations of these factors with CRC can be determined [34].

We must also determine which biological pathways are relevant at which specific subsites in the colon and rectum so that future research can be focused. Studies of relevant fruits and vegetables, studied at relevant levels of exposure [9, 19, 21], with

proper attention to confounding and effect modification are all important. Recommendations should encourage individuals to eat sufficient amounts of F/V, and also to focus on those factors known to be inversely associated with CRC, including maintenance of a healthy body mass index, getting sufficient physical activity, and moderating intake of red and processed meat and alcohol [9].

# Chapter 2: Manuscript

## Abstract

Although associations of fruit and vegetable (F/V) intake with colorectal neoplasms have been studied extensively, the findings have been inconsistent, perhaps due to differential distributions of potential effect modifying variables across study populations. We used data from three case-control studies conducted between 1991 and 2002 to assess associations of total fruit, total vegetables, and total fruit plus vegetable intakes with incident, sporadic, colorectal adenoma according to potential effect modifying risk factors (age, sex, nonsteroidal anti-inflammatory drug [NSAID] use, smoking, chromosome 8q24 region single nucleotide polymorphisms) and adenoma characteristics among 792 cases and 985 colonoscopy-negative controls in a pooled analysis, and among 565 cases and 536 community controls in a separate analysis. In comparisons of the highest to lowest quartiles of F/V intakes, risk estimates were close to the null overall and in the stratified analyses. The multivariable-adjusted risk estimates that differed the most from the null included those for fruit intake with adenomas overall in the analysis involving community controls (odds ratio [OR] 0.78, 95% confidence interval [CI] 0.52-1.16), among ever smokers in the pooled and community controls analyses (ORs 0.64 [CI 0.42-0.99] and 0.68 [CI 0.41-1.13], respectively), and among those who did not regularly take an NSAID in the pooled and community control analyses (ORs 0.76 [CI 0.53-1.11] and 0.70 [CI 0.46-1.08)], respectively). The fruit-adenoma inverse association tended to be stronger for multiple and distal adenomas and those with more advanced characteristics; for example, the OR for adenomas with some villous histology was 0.56 (CI 0.32-0.98). However, the OR for the association of vegetable intake with adenomas among those with the rs7837328 low risk GG genotype was 2.37 (CI 1.09-5.16). These results provide little to no support for inverse associations of fruit and vegetable intake with risk for incident, sporadic adenoma, but do provide some support for further investigations into whether 1) increased fruit intake may reduce risk for advanced adenomas, especially among persons with higher oxidative stress/inflammation from smoking or not taking an anti-inflammatory drug, and 2) chromosome 8q24 region genotypes may modify associations of vegetable intake with adenomas.

## Introduction

In the United States, colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second most common cause of cancer mortality. Lifetime risk is about one in 20, with 141,210 diagnoses and 49,380 deaths expected in 2011 [1, 2] . Understanding risk factors for adenomatous polyps, which are precursors of CRC [37], is an important consideration in controlling CRC rates.

Research has suggested that colorectal neoplasms result from a combination of environmental and genetic factors [4]. The associations of dietary exposures with CRC have been studied extensively in observational studies, with fruits and vegetables (F/V) being of particular interest. F/V contain high levels of anti-carcinogenic constituents that may synergistically target the many mechanisms of action that are involved in the development of CRC [22, 27, 38]. Early case-control studies found consistent, inverse associations between fruit and vegetable intake and CRC risk [39], but more recent, prospective cohort studies have found less evidence for consistent associations [36, 40, 41]. Given the strong biological plausibility for inverse associations, the reasons for the inconsistencies in observational studies are unclear. Researchers have attempted to understand the associations by conducting additional follow-up studies and meta-analyses [23, 36] and by addressing such issues as specific F/V consumed [19, 21, 33, 40], specific constituents of F/V [26, 29, 35, 42-44], subsites of the cancers/adenomas [19, 21, 40], time periods of dietary exposures [34], genetic factors [12, 45], confounders, and the possibility of non-linear dose-response effects [23], but evidence of consistent associations has continued to be limited. We hypothesized that possible differential

associations according to potential effect modifiers, or by stage of colon carcinogenesis, are possible reasons for the inconsistencies in past studies. In the absence of appropriate analyses on these factors, the results of past studies may have been attenuated.

Evidence in the literature and biological plausibility suggest that potential effect modifiers may include age, sex, and smoking status [32], due to the effects of age-related DNA methylation, hormonal differences, and differences in exposure to oxidative stress, respectively. These factors have been considered frequently as possible effect modifiers in past studies.

Potential effect modifiers that have been studied less frequently include family history of CRC, nonsteroidal anti-inflammatory drug (NSAID) use [46], and genetic variants in the 8q24 chromosomal region [13, 45]. NSAID use is an important consideration because of the important role that inflammation plays in CRC development [4, 46]. The increased use of NSAIDs in western nations in recent decades may have masked the associations between F/V intake and CRC. Additionally, early case-control studies frequently used colonoscopy-negative controls, possibly introducing a bias due to family history of CRC. Although both family history of CRC and NSAID use have been considered possible confounders in several studies, few reported considering them as potential effect modifiers [21, 23, 47, 48]. Finally, to our knowledge, the only study of colorectal neoplasms that has considered possible interaction of F/V intake with a genetic variant at the 8q24 locus was a study by Hutter et al., in which CRC, but not adenoma status, was considered. Hutter et al. analyzed associations with only one of the three SNPs considered in the present study [45].

In addition, the estimated associations of F/V intake with colorectal adenomas in different subsites of the colon have been inconsistent in previous studies [19, 21, 40, 48]. Biological mechanisms may differ according to the location of a neoplasm due to differences in blood and nerve supplies, microflora, enzyme concentrations, fecal profile, bile acid metabolism, and molecular characteristics [4, 18-20]. Diet may be more important in the etiology of distal than in proximal colon cancer [4, 21].

Finally, because of a paucity of data concerning the associations of F/V intake with adenoma size, shape, subtype, degree of atypia, and multiplicity [23, 48], we have included these characteristics in our analyses. These characteristics may represent the likelihood of an adenoma progressing to cancer, and are therefore important in considering how F/V intake may affect colorectal carcinogenesis.

## Methods

For the present study, we utilized data from three case-control studies of incident, sporadic, colorectal adenoma: the Minnesota Cancer Prevention Research Unit study (MCPRU), which recruited participants from a large gastroenterology practice in the Minneapolis/St. Paul metropolitan area between 1991 and 1994; the Markers of Adenomatous Polyps I study (MAP I), which recruited from among four gastroenterology practices in the Winston-Salem and Charlotte, NC metropolitan areas between 1994 and 1998; and the Markers of Adenomatous Polyps II study (MAP II), which recruited from a large gastroenterology practice in Columbia, S.C. between 2000 and 2003 [33, 49-52].

We performed two separate analyses. The first compared cases from all three studies with colonoscopy-negative controls from all three studies who were recruited as described below and pooled into a single analysis. The second analysis compared cases

from only the MCPRU study with community controls, recruited separately for the MCPRU study, as described below.

The outcome of interest for all studies was incident, sporadic, colorectal adenomatous polyps. Participants for the pooled analysis were recruited during routine scheduling of elective outpatient colonoscopies. All participants were required to be residents of the metropolitan areas in which the respective studies took place. They spoke English, were aged 30-74, and were of any race. Participants had no history of adenomatous colorectal polyps, inflammatory bowel disease (Crohn's disease or ulcerative colitis), genetic colorectal cancer syndromes (familial adenomatous polyposis or Gardner's Syndrome), or cancer other than non-melanoma skin cancer [33, 49-52].

Potential participants whose colonoscopies did not reach the cecum were not eligible. Polyps found at colonoscopy were removed, and the number, location, size, and shape recorded. Size was measured *in vivo* by using fully-opened endoscopic forceps as a reference. The study index pathologist examined the tissue according to criteria used by the National Polyp Study and recorded the polyp type (adenomatous, hyperplastic, mixed, other), subtype if adenomatous (tubular, villous, tubulovillous), and degree of dysplasia. Cases were defined as having at least one sporadic, pathology-confirmed, first incident adenomatous polyp, either singly or concurrently with hyperplastic polyps or other adenomatous polyps. Colonoscopy-negative controls had no adenomatous or hyperplasic polyps. Patients with new diagnoses of cancer or inflammatory bowel disease were ineligible for the studies [33, 49-52]. In the MAPI and MAPII studies, potential participants unable to use a polyethylene glycol bowel preparation or who were at risk for bleeding were excluded [49, 52] .

In the MCPRU study, in addition to the colonoscopy-negative controls, community controls were randomly selected and recruited from the Minnesota Drivers' Registry. The eligibility requirements were the same as for participants recruited during colonoscopy scheduling. Community controls were frequency matched to MCPRU cases according to 5-year age group, sex, and zip code. They were not required to undergo a colonoscopy, and their current polyp status was not confirmed [33].

For all studies, self-reported information on medical history, anthropometrics (height, weight, and waist and hip circumferences), history of cancer in first degree relatives, gynecological and reproductive history (women only), demographics, education, lifestyle, diet, physical activity, alcohol use, smoking history, NSAID use, and reason for the colonoscopy was collected through questionnaires before colonoscopy and prior to knowledge of case-control status. Questionnaires and venous blood samples were collected at the colonoscopy visit [33, 50-52]. MCPRU community controls, who did not undergo colonoscopy, returned their questionnaires by prepaid mail, but blood samples were not collected from them [33]. All participants gave signed, informed consent [50, 51].

A modified version of the semi-quantitative Willett Food Frequency Questionnaire (FFQ) was used to assess diet, fat/oil intake, and dietary supplement use during the previous year [33, 49, 51, 52]. The Willett FFQ, which has been validated in several studies [53-55], was expanded for the MCPRU, MAPI, and MAPII studies to include additional fruits, vegetables, and low fat items. For all food items, participants were asked to specify how often they ate a serving of the food by choosing a category of frequency of consumption. Servings were defined as one-half cup for most fruits and vegetables and

one cup for raw salad greens [33]. Nutrient intake was estimated using a nutrient database and analysis program developed by Dr. Walter Willett at the Channing Laboratory at Harvard University [33, 50]. Values for combined fruit and/or vegetable consumption in this data were calculated by using the midpoint of each category of intake for each specific food item and summing these values to determine the number of servings per week [25].

Blood samples were genotyped for 8q24 variants using blood drawn at colonoscopy visits and stored for later use [25]. Three SNPs from each blood sample were genotyped for all cases and controls who provided samples. Genotyping was performed at the University of Minnesota's Biomedical Genomics Center. The iPLEX Sequenom genotyping platform was used to genotype rs10808555 and rs7837328, and a Taqman genotyping platform was used to genotype rs6983267. Blinded duplicate samples were found to correlate at 98%, 98%, and 97%, respectively, for the three SNPs [15]. Since blood was not drawn from the MCPRU study community controls, they were not included in analyses involving genotype [25] .

Among all colonoscopy patients initially eligible for the MCPRU study, 68% agreed to participate. Among these, 707 were free of adenoma at colonoscopy and 574 were diagnosed with incident adenoma. Among 846 possible community controls, the participation rate was 65% (n=550) [33]. Our MCPRU analysis included these 574 adenoma cases and 550 community controls. Our complete pooled data set included these 574 MCPRU adenoma cases and 707 MCPRU colonoscopy-negative controls, in addition to the cases and controls pooled from the MAPI and MAPII studies.

Among all patients undergoing colonoscopy in the MAP I study, 63% agreed to participate. Of these, 184 had adenomatous polyps, and 236 were free of adenoma [49]. The pooled data for the present study included 184 cases and 187 colonoscopy-negative controls from the MAP I study. In the MAP II study, 351 patients were identified. Among the 232 patients (86.6%) who agreed to participate and gave informed consent, 203 (87.5%) met all of the eligibility requirements. The final sample size was 49 cases and 154 colonoscopy-negative controls [50]. The pooled data for the present study included 49 cases and 125 controls from the MAP II study. Our complete pooled data set included cases and colonoscopy-negative controls recruited as described above through colonoscopy scheduling for all three studies, for a total of 809 cases and 1,019 colonoscopy-negative controls.

For the present study, participants were excluded from the analysis if they were missing values for fruit and/or vegetable intake (nine cases and 14 controls in the MCPRU study and an additional three cases and 27 controls in the pooled study). Participants were also excluded if their total energy intake was less than 600 kilocalories (kcal) per day or greater than 6,000 kcal/day (no additional cases or controls in the MCPRU data and an additional five cases and seven controls in the pooled data). The final data set included 792 cases and 985 colonoscopy-negative controls for the pooled analyses and 565 cases and 536 community controls for the MCPRU analyses.

## Statistical Analysis

SAS version 9.3 was used for all data cleaning and analyses. Continuous variables for total fruits, total vegetables, and total fruits and vegetables were categorized into study- and sex-specific quartiles according to the distribution of exposure among the controls.

The study populations were described according to case/control status using t-tests for continuous variables and chi-square tests for categorical variables. Continuous variables that were not normally distributed were transformed as appropriate to improve normality prior to inference testing.

Odds ratios with 95% confidence intervals (CIs) to estimate associations of fruit and vegetable intake with adenomas were calculated using unconditional logistic regression. The reference category was considered the lowest quartile of intake for each analysis. P-values for trend were found by assigning ordinal variables for categories of intake and treating these variables as continuous.

Risk factors considered for possible inclusion as covariates in logistic regression models included age, sex, race, education, family history of CRC, physical activity, body mass index, non-steroidal anti-inflammatory drug (NSAID) use, hormone replacement therapy among women, alcohol intake, smoking status, multivitamin use, serum 25-OH vitamin $D_3$ level, and intakes of total calcium, total folate, total fat, dietary fiber, red and processed meat, total energy, total vegetables (for the fruit models), and total fruits (for the vegetable models). Criteria used to assess these factors as potential confounders

included biological plausibility, evidence in the literature, association of each factor with fruit or vegetable intake and adenoma, a change of the odds ratio for the primary exposure variable by at least 10%, and absence of collinearity or high correlation with other variables. The variables included in the final models were age, sex, total energy intake, family history of CRC, regular NSAID use, smoking status, and total folate intake.

Analyses were also stratified by age (≥ 56 years and < 56 years), sex, smoking status (ever versus never), family history of colorectal cancer in a first degree relative, regular (≥ once/week) nonsteroidal anti-inflammatory drug use, and 8q24 region genotypes (for the pooled analysis only). In addition, associations of fruit and vegetable intake with adenomas with different characteristics were estimated for the pooled analysis. We specified advanced adenomas as those that were multiple (≥ 3), sessile, or had size ≥ 1 cm (the *in vivo* measurement of the maximum diameter of the largest adenoma), distal location, moderate or severe dysplasia, or a villous component. Less advanced adenoma characteristics were specified as fewer than three adenomas, size < 1 cm, proximal location, mild dysplasia, tubular histology, and pedunculated shape.

## Results

Selected characteristics of cases and controls by study are shown in Table 1. Study participants in the pooled and MCPRU studies were primarily white (97%) with a mean age of 55 years. Compared with controls, cases tended to be older and more likely to be male, have a history of smoking, drink more alcohol, have a higher body mass index, have lower total folate intakes, have higher total energy intakes, consume more total fat,

and consume fewer weekly servings of fruits and total fruits and vegetables. Cases were also less likely to be high school graduates (or beyond), take NSAIDs regularly (≥ once/week), take hormone replacement therapy (if female), or take multivitamins. The primary difference in characteristics between the pooled and MCPRU studies was that, compared with cases, colonoscopy-negative controls in the pooled study were more likely to have a family history of CRC in a first degree relative, whereas community controls in the MCPRU study were less likely to have a family history of CRC.

Odds ratios to estimate crude and adjusted associations of F/V intake with adenomas (Table 2) were primarily close to null and not statistically significant, without clear patterns. The possible exception was in our comparison involving the MCPRU community controls, in which there was a statistically significant trend for decreasing risk with higher intake of fruit in the crude analysis (p-trend = 0.04). With multivariate adjustment, the inverse pattern persisted, with a 22% lower risk for those in the highest quartile of fruit intake compared to the lowest quartile, but neither the point estimates nor the test for trend across quartiles were statistically significant.

In general, there was little evidence for strong or statistically significant associations of F/V intake with adenomas according to levels of other risk factors (Table 3). Possible exceptions were for associations of total fruit intake with adenomas among those who had ever smoked and among those who did not regularly take an NSAID. These subgroups had risk estimates that were relatively strong, consistent across the comparisons involving the two control groups, and nearly statistically significant, with an approximately 32% lower risk among those who had ever smoked and an approximately

24% lower risk among those who did not take NSAIDs when comparing the highest to lowest quartiles of fruit intake.

Estimated associations according to 8q24 region genotypes (Table 4) suggested overall decreased risk of adenomas with increased F/V intake among those with the higher-risk genotypes and overall increased risk of adenomas with increased F/V intake among those with the lower-risk genotypes, but the associations were primarily nonsignificant with wide confidence intervals. Statistically significant positive associations among those with the GG (low-risk) genotype for the rs7837328 SNP suggested a greater than two-fold risk of adenomas with increased vegetable and total F/V intake. Sample sizes were generally small for genotype analyses.

Restricting analyses to cases with only certain adenoma characteristics (Table 5) suggested a pattern of generally stronger inverse associations for fruit intake with adenomas that were distal, sessile, or had advanced characteristics (including those that were multiple [≥ 3 adenomas] or had larger size, greater dysplasia, or some villous histology) than with adenomas having less advanced characteristics. Comparing the highest to lowest quartiles of fruit intake suggested statistically significant 36% lower risk for sessile adenomas and 44% lower risk for adenomas with a villous component. On the other hand, there was also a statistically significant 63% higher risk of distal adenomas among those with the highest compared to lowest quartile of total F/V intake.

## Discussion

Overall, our findings provide little support for inverse associations of fruit and vegetable intake with risk for incident, sporadic, colorectal adenoma. Although our findings were

generally null, we did find suggestions of possible inverse associations of fruit intake with adenoma among those who have ever smoked, do not regularly take an NSAID, and have higher risk 8q24 region genotypes, as well as with multiple, distal, and sessile adenomas and adenomas with generally more advanced characteristics. We also found positive associations of vegetable intake with adenoma among those who had non-risk 8q24 genotypes.

The reasons for our findings that suggest the possibility of inverse associations of fruit, but not vegetable intake, with adenomas are not clear, but past studies offer possible clues. Several studies found no association of vegetable fiber intake with colorectal neoplasms (CN), but found inverse associations of the highest compared to lowest fruit fiber intake groups with colorectal neoplasms. Two of these studies found statistically significant inverse associations [29, 31], and one was borderline statistically significant [30]. Fruits also contain a number of phytochemicals, including antioxidants such as vitamin C, carotenoids, and flavonoids, which can have many anti-carcinogenic effects, including anti-inflammatory effects [36]. Additive and synergistic activity among the many phytochemicals in fruits may be responsible for their potential anti-carcinogenic effects [22].

Past studies have been inconsistent concerning association of fruit intake with CN. Of 20 cohort studies on associations of fruit intake with CRC, 13 found lower risk for CRC with higher consumption. The inverse associations found in two of these studies were statistically significant, but the direct associations found in 11 of the studies were not. A meta-analysis of eight of these studies suggested that women may benefit more from fruit (risk ratio 0.81, 95% confidence interval [CI] 0.85-0.98) than men [36]. A panel from the

American Institute for Cancer Research concluded in its 2007 report that evidence that fruit may protect against CN is substantial, but limited and inconsistent [36]. Further research on specific fruits and compounds in fruit could provide additional insight into possible effects of fruit intake against colorectal neoplasms.

Our findings suggested that fruit intake may be modestly inversely associated with adenomas among those who have ever smoked. The estimated associations of the highest relative to lowest quartiles of fruit intakes in this subgroup in the pooled analysis (odds ratio [OR] 0.64, CI 0.42-0.99) and the MCPRU analysis involving community controls (OR 0.68 [CI 0.41-1.13]) were similar. Such an inverse association involving antioxidant/anti-inflammatory compound-containing fruits might be more apparent in smokers because of their higher levels of oxidative stress.

Past studies of associations of F/V intakes with colorectal neoplasms according to smoking status have yielded inconsistent findings. Among five large cohort studies that assessed associations of F/V intake with CN according to smoking status, as in our study, two found inverse associations of fruit intake with adenoma to be stronger among those who had ever smoked than among those who had never smoked [32, 48], although one finding was for colon cancer but not rectal cancer [32]. Two of the five studies found no differential associations according to smoking status [21, 41], and one found that inverse associations of fruit, vegetable, and total F/V intakes were stronger among those who had never smoked [56]. Our findings, in context with these past findings, indicate that further study of the associations of F/V intake with CN according to smoking status are warranted in order to address past inconsistencies and determine true associations.

In our study, we also found some relative consistency of associations of fruit intakes with adenomas among those who did not regularly take NSAIDs, with risk estimates that were not statistically significant but, relative to our other findings, strong and consistent across the comparisons involving the two control groups. The OR for the highest to lowest intakes of fruit in the pooled analysis was 0.76 (CI 0.53-1.11), and it was 0.70 (CI 0.46, 1.08) in the MCPRU community controls analysis.

The biological plausibility for differential associations according to NSAID use is strong, based on the importance of inflammation in CRC development [4]. F/V contain many anti-inflammatory constituents, but it is possible that increased use of NSAIDs in recent decades has masked most of the anti-inflammatory effects of F/V among regular NSAID users. Among those who do not regularly take an NSAID, the anti-inflammatory effects of F/V might be found more readily than among those who regularly take an NSAID.

We found no previous studies that reported finding evidence of effect modification of the associations of F/V with CN according to NSAID use [48]. Taking into consideration our findings and the strong biological plausibility for differential associations according to NSAID use, however, this topic warrants future investigation with a larger sample so that associations according to NSAID use can be better assessed.

Our analyses according to 8q24 region genotypes produced primarily non-significant results with wide confidence intervals, likely due to insufficient sample sizes. These analyses also involved multiple comparisons, so our few significant findings (as with all of our other findings) may be due to chance. There is, however, some plausibility for effect modification by 8q24 region genotypes. SNP rs6983267 has been shown to affect

Wnt signaling, which regulates cell proliferation and is a factor in colorectal carcinogenesis [13, 57]. Because other SNPs in the same region are in moderately strong linkage disequilibrium, the two other 8q24 region SNPs that we assessed (rs7837328 and rs10808555) may have similar effects. Certain constituents in F/V may also affect Wnt signaling, including retinoids, which are produced from vitamin A, and resveratrol, a polyphenol found in the skin of red grapes and other fruits [11].

In our literature search, we found only one study that considered possible interactions of F/V intake with an 8q24 genotype. This study, by Hutter et al., found statistically non-significant evidence that higher risk of CRC associated with 8q24 region rs6983267 risk alleles was less among those with higher vegetable consumption and higher fruit consumption. Hutter et al. also found that, among all 12 of the risk factors considered in their study, only the findings for vegetable intake were statistically significant. In their study, the magnitude of the association of rs16892766 (located at region 8q23.3) with CRC increased with increased levels of vegetable intake, suggesting that vegetable intake may be among the strongest modifiers of certain genetic risk(s) for CRC, and that it may actually increase risk among those with certain genotypes [45].

In our analyses according to 8q24 genotypes, our findings of lower risk of adenoma with increased F/V intakes among those with high-risk genotypes and higher risk among those with lower-risk genotypes were primarily not statistically significant, but were relatively consistent for all three of the SNPs investigated. This pattern was most evident for rs6983267 and rs7837328. Inverse associations according to genotype were strongest for associations of fruit intake with adenoma, and positive associations according to genotype were strongest for associations of vegetable intake with adenoma.

A particularly unexpected result of our study was the positive association of F/V intake, particularly of vegetable intake, with adenoma among those with the rs7837328 GG (non-risk) genotype. Our sample sizes were small, and the confidence intervals were wide, but these results were stronger and more consistent than many of our findings. The strongest finding by Hutter et al. also suggested an interaction of vegetable intake with genotype.

Not accounting for genotypes, other studies have also occasionally found positive associations of F/V intake with CRC. Among 20 risk estimates from 17 cohort studies of the association of non-starchy vegetable intake with CRC, eight suggested statistically non-significant increased risk [36]. We have no biologically plausible explanation for positive associations of vegetable intake with CN. However, future investigations may be worthwhile to determine whether these positive associations may be due to chance or perhaps to biological mechanisms not previously considered.

Overall, our findings according to 8q24 region genotypes suggest that CRC research could benefit from further study of genotypes as potential effect modifiers of associations of F/V with CRC. The possibilities that individuals with high risk genotypes might benefit from increased intakes of F/V, but that individuals with other genotypes might be harmed by vegetable intake, are considerations worthy of further investigation.

In comparing associations of F/V intake with distal adenomas to associations of F/V intake with proximal adenomas, we had statistically non-significant findings that hinted at stronger inverse associations of fruit intake with distal adenomas than with proximal adenomas. Comparing the highest to lowest quartiles of fruit intake, our findings for

distal adenomas (OR 0.78 [CI 0.54-1.13]) and for proximal adenomas (OR 0.91 [CI 0.52-1.57]) are in agreement with previous findings that suggest that inverse associations of F/V with CRC are stronger for distal than for proximal neoplasms [19, 21, 40]. The biological basis for differential associations includes differences in the blood supply, nerve supply, microflora, enzyme concentrations, fecal profile, and bile-acid metabolism between these two locations. Mechanisms of action also differ for distal and proximal cancers, with microsatellite instability being more common in the proximal colon and chromosomal instability being more common for distal tumors [4, 18, 20]. Different dietary risk factors may have differential effects on the location of neoplasms [21, 40].

Studies as far back as the 1980s reported that both environmental and genetic factors may have differential effects on risk for colorectal neoplasms according to location of the neoplasm [4, 18]. More recent studies have found differing results. Among the seven studies we found that assessed associations of F/V intakes according to the locations of the CNs, four found no evidence of differential associations [32, 33, 58, 59], while three studies found some evidence that associations were stronger for distal than for proximal neoplasms [19, 21, 40]. Our study does not contradict the possibility that evidence may be accumulating to suggest a stronger association of fruit intake with distal colorectal adenomas than with proximal adenomas. Further research to confirm these associations is warranted [18, 20].

Our restriction of analyses to cases with only certain adenoma characteristics weakly suggests a pattern of generally stronger inverse associations for fruit intake with adenomas that were multiple ($\geq 3$), sessile, or had advanced characteristics (including those with larger size, greater dysplasia, or some villous histology) than with adenomas

having less advanced characteristics. Although most adenomas do not become cancerous, adenomas with advanced characteristics are associated with higher rates of malignancy [60]. In spite of the different levels of risk associated with specific adenoma characteristics, these characteristics have not been taken into account as frequently as adenoma location in studies of associations of F/V intake with colorectal neoplasms. Of the five studies we found that assessed associations of F/V with adenoma characteristics, two reported that only cruciferous vegetables were inversely associated with larger adenomas [61, 62]. One of the five studies reported that fruit intake was inversely associated with polyp size, and that vegetable intake was inversely associated only with polyp recurrence [63]. Another of the five studies reported that there was no association of fruit intake with the size of distal adenomas [56], and another reported that F/V consumption was not associated with adenoma multiplicity or histology [48]. These findings are inconsistent, although the biological plausibility discussed previously supports the possibility that fruits might protect against the neoplastic progression of adenomas. Our study's weak suggestions of inverse associations of fruit intake with advanced polyp characteristics offer support for further study of these associations.

Our study had several limitations, including a sample size that was limited for stratified analyses, especially considering the multiple comparisons. In addition, assessing dietary intakes is always complex, with misclassification and measurement error being common [60]. Our dietary assessment required participants to estimate F/V intakes only for the 12 months immediately prior to the study, a time period that may not have captured the relevant exposure period for CRC risk [34].

Additional limitations concerning assessment of F/V intake included possible misclassification of participants' exposures due to social desirability in responses [33]. Also, our grouping of F/V into only three broad groups (total fruits, total vegetables, and total fruits and vegetables) did not account for the diversity of the types of F/V or the effects of specific F/V consumed. An assessment of individual F/V and of F/V groupings would be a valuable addition to this study. Our groupings, however, were straightforward, and may have accounted for synergism among a large variety of fruits and vegetables in a manner that other groupings would not.

Limitations in selection of controls were also present. Although the colonoscopy-negative controls likely had a greater frequency of family history than the source population, current polyp status among the community controls' was not confirmed. Our inclusion of both control groups and our comparison of the findings may have offset these issues. In addition, our participants were 97% white, so our findings cannot be assumed to apply to other racial groups.

Our study also had several strengths, including that our pooled analysis combined samples from three different geographic locations. Our stratification on risk factors was also a strength; levels of NSAID use and genotypes have seldom been considered as potential effect modifiers in previous studies on associations of F/V with adenoma. To our knowledge, region 8q24 genotypes for rs10808555 and rs7837328 have not been considered before in studies of associations of F/V intake with colorectal neoplasms.

In conclusion, we found patterns of associations that suggest that future CRC research may benefit from further evaluation of associations of F/V intake with colorectal

adenoma risk according to smoking status, NSAID use, and 8q24 region genotypes as well as according to adenoma characteristics. Further research on associations of fruit intake with colorectal neoplasms according to these factors may be particularly valuable. Studies in which these factors are assessed using the benefits of large sample size and long-term, prospective study designs are needed.

# Chapter 3: Conclusions and Recommendations

## Public Health Implications

The results of our study were primarily null, with weak suggestions for possible differential associations in certain subgroups or individuals. Individuals and populations will likely benefit from larger prospective studies that can more definitively address the specific associations suggested by our study, and the public health implications will depend on the outcomes of these studies. Public health policy and recommendations will evolve as the results of additional studies confirm or refute our findings.

It is known that consumption of fruits and vegetables has many health benefits. Regardless of the true associations of F/V intake with colorectal neoplasms (CN), most individuals will likely benefit by consuming sufficient amounts of F/V. More specific recommendations might be appropriate in some situations, such as for individuals at high risk for CN. These individuals will benefit by being advised of the latest findings concerning the associations of F/V intake with CN. If specific F/V, or specific levels of consumption, are found to increase or decrease risk, individuals will benefit by being advised appropriately. In the case of our study, the results weakly suggested that increased fruit intake may be more inversely associated with colorectal adenomas and adenoma progression than is vegetable intake. If future studies confirm this finding, then individuals will likely benefit from this knowledge.

Another situation for which specific recommendations might be advised is in the event that differential associations truly do indicate substantial, differential risks. In our study and the Hutter et al. study, findings suggested that individuals with specific genotypes

might benefit from eating F/V, while those with other genotypes might actually be at greater risk with increased intake [45]. If these associations are found to be true, then knowledge of them will be crucial to individuals who will benefit from knowing their risks according to genetic susceptibility and environmental exposures. Further studies assessing risk according to specific genotypes will benefit individuals, as will individuals' knowledge of their own genotypes. Although our results were inconsistent concerning associations according to age, sex, and family history, and our results were weak concerning other patterns of association that were suggested, the results do encourage further research. As results of future research are known, individuals can be advised accordingly.

Future studies and adequate education of the public concerning these associations will require sufficient support. Case-control and prospective studies need to be properly supported so that the true associations of F/V intake with CN can be determined. Further support will then be needed to educate the public about the findings and offer adequate accessibility to information and nutritious foods so that our various populations can follow guidelines easily.

## Future directions

Although a great deal of evidence supports the biological plausibility for an inverse association of F/V intake with colorectal neoplasms (CN), the results of studies concerning these associations have been inconsistent. Case-control studies from the 1980s and early 1990s resulted in primarily inverse associations of F/V intake with CN [64], while more recent prospective studies have been inconsistent and have often

produced weak or null results. [9, 23, 58]. The inconsistencies among the many studies are likely attributable to a number of factors which can be addressed in future studies.

Although our pooled case-control study and its comparison MCPRU study had inconsistencies even within themselves, our results suggested that future CRC research may benefit from further evaluation of associations of F/V intake with colorectal adenoma risk according to 8q24 region genotypes and according to characteristics of adenoma. These factors showed suggestions of differential associations in our analyses yet have rarely been studied. Future studies in which these factors are assessed using the benefits of large sample size and long-term, prospective study designs are needed.

Although prospective cohort studies are costly and have continued to produce many inconsistent findings concerning the associations of F/V intake with CN, the prospective cohort study is likely the most useful study design for assessing an outcome such as CRC, which often develops over decades. Such a design that assesses diet and other potential risk factors with repeated measures from childhood onward would provide data on diet and potential covariates that would be useful in determining the true, relevant exposure periods for CRC [34]. Recall bias and temporality/cause-effect issues could be addressed and minimized.

If, in some situations, case-control studies are the best alternative to prospective studies, these studies will benefit from attention to issues that can otherwise be problematic. Efforts will be required to avoid recall bias, minimize family history bias in selection of controls, prevent outcome misclassification among controls due to lack of confirmation

of CN status, and avoid bias due to differing participation rates of cases and controls. These issues may have contributed to bias in past case-control studies.

Whether any future study is prospective or retrospective, proper assessments of confounding and effect modification will also be important. Future studies will benefit from ensuring that data are available for all important confounders and effect modifiers. Data collection on all relevant factors will be important, as well as collection of blood and tissue samples to ensure the retrospective ability to measure factors that are later suspected or established as risk factors. Correct classification of covariates is also important in avoiding attenuation of results [34]; studies will benefit by using data collection methods that have been validated. Additionally, proper assessment of factors such as BMI and diabetes status is important because self-reporting may contribute to misclassification of these covariates [19, 24].

Other issues of bias also need to be avoided in future studies. With any study, it is possible that a "health conscious" segment of the population may be more likely than less health conscious individuals to consent to participation [9]. Recruitment must proceed carefully in order to avoid this issue. Large sample sizes are also needed for sufficient power to detect differential associations according to different levels of multiple factors.

We must also determine which biological pathways are relevant at which specific subsites in the colon and rectum so that future research can be focused. Other important goals of future studies include determining which fruits and vegetables are relevant, in what combinations, at what levels of exposure, and according to which methods of food preparation and cooking [9, 19, 21]. Attainment of these goals will contribute to our

understanding of diet and CRC in a number of ways, including clarification of results of studies from differing geographic locations, where specific foods consumed can be vastly different [23]. Consideration of the possibility of a threshold effect or a non-linear dose-response association between F/V intake and CRC was also found to be important in a study by Aune et el., and would be valuable to consider in future studies [23].

Other issues might also be contributors to the fact that studies from the 1980s and early 1990s resulted in primarily inverse associations, while more recent studies have often produced null results. One possible reason for the discrepancy is that modern-day produce is not as nutritious as produce grown decades ago due to modern-day agricultural practices and depletion of soil nutrients [65]. Populations would benefit from agricultural research that aims to produce crops that have maximum nutritional value.

Another possible reason for the difference in older versus newer studies is that the incidence rates of distal colon cancer have been decreasing over the last several decades, while proximal cancers have been increasing. [18, 20, 21]. Because diet may be more inversely associated with distal than with proximal CN, these changes in rates may have affected results of studies that did not differentiate between distal and colon cancers. Future studies would benefit from reporting associations according to location of the neoplasms.

While research on the associations of F/V intake with CN continues, we can be reminded that F/V intake has many beneficial impacts on areas of health and wellness other than colorectal health. Until more is known about the associations of F/V with CN, individuals might benefit from being encouraged to eat sufficient amounts of F/V for

other health benefits. For CRC prevention, individuals might be encouraged to focus on other factors known to be inversely associated with CRC, including getting sufficient amounts of physical activity, maintaining a healthy BMI, and moderating intakes of alcohol, red meat, and processed meat [9].

## References


1. American Cancer Society, *Colorectal Cancer*: Atlanta: American Cancer Society, 2011.
2. American Cancer Society, *Colorectal Cancer Facts and Figures 2011-2013*: Atlanta: American Cancer Society, 2011.
3. National Cancer Institute, *Colon and Rectum Statistics.* SEER Cancer Statistics Review 1975-2008, 2011.
4. Giovannucci, E. and K. Wu, *Cancers of the Colon and Rectum*, in *Cancer Epidemiology and Prevention*, D. Schottenfeld and J. J. Fraumeni, Editors. 2006, Oxford University Press: New York.
5. Forte, A., et al., *Dietary chemoprevention of colorectal cancer.* Annali Italiani di Chirurgia, 2008. **79**(4): p. 261-7.
6. Mayer, R., *Gastrointestinal Tract Cancer*, in *Harrison's Principles of Internal Medicine*, D.K. DL Longo, SL Hauser, JL Jameson, J Loscalzo, AS Fauci, Editor 2011, McGraw-Hill: New York.
7. Potter, J.D., *Colorectal cancer: molecules and populations.* Journal of the National Cancer Institute, 1999. **91**(11): p. 916-32.
8. Tomlinson, I., et al., *A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21.* Nature Genetics, 2007. **39**(8): p. 984-8.
9. Key, T.J., *Fruit and vegetables and cancer risk.* British Journal of Cancer, 2011. **104**(1): p. 6-11.
10. Fearon, E.R. and B. Vogelstein, *A genetic model for colorectal tumorigenesis.* Cell, 1990. **61**(5): p. 759-67.
11. Tarapore, R.S., I.A. Siddiqui, and H. Mukhtar, *Modulation of Wnt/beta-catenin signaling pathway by bioactive food components.* Carcinogenesis, 2011.
12. Gay, L.J., et al., *MLH1 promoter methylation, diet, and lifestyle factors in mismatch repair deficient colorectal cancer patients from EPIC-Norfolk.* Nutrition and Cancer, 2011. **63**(7): p. 1000-10.
13. Tuupanen, S., et al., *The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling.* Nature Genetics, 2009. **41**(8): p. 885-90.
14. Zanke, B.W., et al., *Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24.* Nature Genetics, 2007. **39**(8): p. 989-94.
15. Yang, B., et al., *Genetic variants at chromosome 8q24, colorectal epithelial cell proliferation, and risk for incident, sporadic colorectal adenomas.* 2011.
16. Berndt, S.I., et al., *Pooled analysis of genetic variation at chromosome 8q24 and colorectal neoplasia risk.* Human Molecular Genetics, 2008. **17**(17): p. 2665-2672.
17. Grubben, M.J., et al., *Low colonic glutathione detoxification capacity in patients at risk for colon cancer.* European Journal of Clinical Investigation, 2006. **36**(3): p. 188-92.
18. Iacopetta, B., *Are there two sides to colorectal cancer?* International Journal of Cancer, 2002. **101**(5): p. 403-8.

19. Annema, N., et al., *Fruit and vegetable consumption and the risk of proximal colon, distal colon, and rectal cancers in a case-control study in Western australia.* Journal of the American Dietetic Association, 2011. **111**(10): p. 1479-90.
20. Gervaz, P., P. Bucher, and P. Morel, *Two colons-two cancers: paradigm shift and clinical implications.* Journal of Surgical Oncology, 2004. **88**(4): p. 261-6.
21. Koushik, A., et al., *Fruits, vegetables, and colon cancer risk in a pooled analysis of 14 cohort studies.* Journal of the National Cancer Institute, 2007. **99**(19): p. 1471-83.
22. Bostick, R.M., *Diet and Nutrition in the Etiology and Primary Prevention of Colon Cancer*, in *Preventive Nutrition: The Comprehensive Guide for Health Professionals*, A. Bendich and R.J. Deckelbaum, Editors. 2001, Humana Press, Inc.: Totowa, NJ.
23. Aune, D., et al., *Nonlinear reduction in risk for colorectal cancer by fruit and vegetable intake based on meta-analysis of prospective studies.* Gastroenterology, 2011. **141**(1): p. 106-18.
24. Pusatcioglu, C.K. and C. Braunschweig, *Moving beyond diet and colorectal cancer.* Journal of the American Dietetic Association, 2011. **111**(10): p. 1476-8.
25. Bostick, R., *Personal communication*, 2011: Atlanta, GA.
26. Rimando, A.M. and N. Suh, *Biological/chemopreventive activity of stilbenes and their effect on colon cancer.* Planta Medica, 2008. **74**(13): p. 1635-43.
27. Araujo, J.R., P. Goncalves, and F. Martel, *Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines.* Nutr Res, 2011. **31**(2): p. 77-87.
28. Turati, F., et al., *Adherence to the European food safety authority's dietary recommendations and colorectal cancer risk.* European Journal of Clinical Nutrition, 2012.
29. Bingham, S.A., et al., *Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study.* Lancet, 2003. **361**(9368): p. 1496-501.
30. Michels, K.B., et al., *Fiber intake and incidence of colorectal cancer among 76,947 women and 47,279 men.* Cancer Epidemiology, Biomarkers and Prevention, 2005. **14**(4): p. 842-9.
31. Peters, U., et al., *Dietary fibre and colorectal adenoma in a colorectal cancer early detection programme.* Lancet, 2003. **361**(9368): p. 1491-5.
32. van Duijnhoven, F.J., et al., *Fruit, vegetables, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition.* American Journal of Clinical Nutrition, 2009. **89**(5): p. 1441-52.
33. Smith-Warner, S.A., et al., *Fruits, vegetables, and adenomatous polyps: the Minnesota Cancer Prevention Research Unit case-control study.* Am J Epidemiol, 2002. **155**(12): p. 1104-13.
34. Ruder, E.H., et al., *Adolescent and mid-life diet: risk of colorectal cancer in the NIH-AARP Diet and Health Study.* American Journal of Clinical Nutrition, 2011. **94**(6): p. 1607-19.
35. Lee, J.E. and A.T. Chan, *Fruit, vegetables, and folate: cultivating the evidence for cancer prevention.* Gastroenterology, 2011. **141**(1): p. 16-20.

36. World Cancer Research Fund/American Institute for Cancer Research. *Food, Nutrition, and Physical Activity and the Prevention of Cancer: a Global Perspective*. 2007. Washington, D.C.
37. Livstone, E.M. *Polyps of the Colon and Rectum*. The Merck Manuals:The Merck Manual for Healthcare Professionals 2007 December, 2007 [cited 2011 September 24]; Available from: http://www.merckmanuals.com/professional/gastrointestinal_disorders/tumors_of_the_gi_tract/polyps_of_the_colon_and_rectum.html?qt=&sc=&alt=.
38. Ricciardiello, L., F. Bazzoli, and V. Fogliano, *Phytochemicals and colorectal cancer prevention--myth or reality?* Nat Rev Gastroenterol Hepatol, 2011. **8**(10): p. 592-6.
39. Steinmetz, K.A., et al., *Vegetables, fruit, and colon cancer in the Iowa Women's Health Study.* American Journal of Epidemiology, 1994. **139**(1): p. 1-15.
40. Voorrips, L.E., et al., *Vegetable and fruit consumption and risks of colon and rectal cancer in a prospective cohort study: The Netherlands Cohort Study on Diet and Cancer.* American Journal of Epidemiology, 2000. **152**(11): p. 1081-92.
41. Michels, K.B., et al., *Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers.* Journal of the National Cancer Institute, 2000. **92**(21): p. 1740-52.
42. Kennedy, D.A., et al., *Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis.* Cancer Epidemiol, 2011. **35**(1): p. 2-10.
43. Bingham, S.A., et al., *Is the association with fiber from foods in colorectal cancer confounded by folate intake?* Cancer Epidemiology, Biomarkers and Prevention, 2005. **14**(6): p. 1552-6.
44. Papas, M.A., E. Giovannucci, and E.A. Platz, *Fiber from fruit and colorectal neoplasia.* Cancer Epidemiology, Biomarkers and Prevention, 2004. **13**(8): p. 1267-70.
45. Hutter, C.M., et al., *Characterization of gene-environment interactions for colorectal cancer susceptibility loci.* Cancer Research, 2012.
46. Klampfer, L., *Cytokines, inflammation and colon cancer.* Curr Cancer Drug Targets, 2011. **11**(4): p. 451-64.
47. Miller, P.E., et al., *Dietary patterns and colorectal adenoma and cancer risk: a review of the epidemiological evidence.* Nutr Cancer, 2010. **62**(4): p. 413-24.
48. Millen, A.E., et al., *Fruit and vegetable intake and prevalence of colorectal adenoma in a cancer screening trial.* American Journal of Clinical Nutrition, 2007. **86**(6): p. 1754-64.
49. Boyapati, S.M., et al., *Folate intake, MTHFR C677T polymorphism, alcohol consumption, and risk for sporadic colorectal adenoma (United States).* Cancer Causes and Control, 2004. **15**(5): p. 493-501.
50. Daniel, C.R., et al., *TGF-alpha expression as a potential biomarker of risk within the normal-appearing colorectal mucosa of patients with and without incident sporadic adenoma.* Cancer Epidemiology, Biomarkers and Prevention, 2009. **18**(1): p. 65-73.
51. Fedirko, V., et al., *Blood 25-hydroxyvitamin D3 concentrations and incident sporadic colorectal adenoma risk: a pooled case-control study.* American Journal of Epidemiology, 2010. **172**(5): p. 489-500.

52. Sidelnikov, E., et al., *Colorectal mucosal expression of MSH2 as a potential biomarker of risk for colorectal neoplasms.* Cancer Epidemiology, Biomarkers and Prevention, 2009. **18**(11): p. 2965-73.
53. Munger, R.G., et al., *Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews.* American Journal of Epidemiology, 1992. **136**(2): p. 192-200.
54. Rimm, E.B., et al., *Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals.* American Journal of Epidemiology, 1992. **135**(10): p. 1114-26; discussion 1127-36.
55. Willett, W.C., et al., *Reproducibility and validity of a semiquantitative food frequency questionnaire.* American Journal of Epidemiology, 1985. **122**(1): p. 51-65.
56. Michels, K.B., et al., *Fruit and vegetable consumption and colorectal adenomas in the Nurses' Health Study.* Cancer Research, 2006. **66**(7): p. 3942-53.
57. Yang, B., et al., *Genetic variants at chromosome 8q24, colorectal epithelial cell proliferation, and risk for incident, sporadic colorectal adenomas*, 2011.
58. Lanza, E., et al., *The polyp prevention trial continued follow-up study: no effect of a low-fat, high-fiber, high-fruit, and -vegetable diet on adenoma recurrence eight years after randomization.* Cancer Epidemiology, Biomarkers and Prevention, 2007. **16**(9): p. 1745-52.
59. Magalhaes, B., B. Peleteiro, and N. Lunet, *Dietary patterns and colorectal cancer: systematic review and meta-analysis.* European Journal of Cancer Prevention, 2012. **21**(1): p. 15-23.
60. Peipins, L.A. and R.S. Sandler, *Epidemiology of colorectal adenomas.* Epidemiologic Reviews, 1994. **16**(2): p. 273-97.
61. Faivre, J., et al., *Environmental and familial risk factors in relation to the colorectal adenoma--carcinoma sequence: results of a case-control study in Burgundy (France).* European Journal of Cancer Prevention, 1997. **6**(2): p. 127-31.
62. Hoff, G., et al., *Colorectal adenomas and food. A prospective study of change in volume and total mass of adenomas in man.* Scandinavian Journal of Gastroenterology, 1988. **23**(10): p. 1253-8.
63. Almendingen, K., B. Hofstad, and M.H. Vatn, *Dietary habits and growth and recurrence of colorectal adenomas: results from a three-year endoscopic follow-up study.* Nutrition and Cancer, 2004. **49**(2): p. 131-8.
64. Giovannucci, E., et al., *Relationship of diet to risk of colorectal adenoma in men.* Journal of the National Cancer Institute, 1992. **84**(2): p. 91-8.
65. Juroszek, P., et al., *Fruit Quality and Bioactive Compounds with Antioxidant Activity of Tomatoes Grown On-Farm: Comparison of Organic and Conventional Management Systems* J. Agric. Food Chem., 2009. **57**: p. 1188-1194.

**Table 1. Selected characteristics of incident sporadic colorectal adenoma cases and controls in pooled[a] and Minnesota Cancer Prevention Unit (MCPRU)[b] case-control studies**

| Characteristic[c] | Pooled cases[d] | Pooled Controls[e] | p[f] | MCPRU cases[d] | MCPRU community controls[g] | p[f] |
|---|---|---|---|---|---|---|
| | n=792 | n=985 | | n=565 | n=536 | |
| Age (years) | 58.1 (9.3) | 53.7 (10.6) | <0.0001 | 58.1 (9.7) | 57.7 (10.4) | 0.59 |
| Male (%) | 61.1 | 37.2 | <0.0001 | 61.6 | 55.2 | 0.03 |
| White race (%) | 96.5 | 96.6 | 1.00 | 97.7 | 97.2 | 0.47 |
| High school graduate or beyond (%) | 88.3 | 91.8 | 0.02 | 89.7 | 92.9 | 0.06 |
| Family history of CRC[h] (%) | 15.6 | 30.2 | <0.0001 | 16.4 | 6.9 | <0.0001 |
| Ever smoked (%) | 69.5 | 53.0 | <0.0001 | 67.4 | 55.8 | <0.0001 |
| Alcohol intake (g/day) | 9.3 (16.1) | 5.8 (12.4) | <0.0001 | 10.1 (16.5) | 8.1 (15.5) | 0.01 |
| NSAID use[j] (%) | 14.9 | 25.2 | <0.0001 | 11.7 | 17.0 | 0.01 |
| Hormone replacement[k] (%) | 48.2 | 54.8 | 0.06 | 39.5 | 44.7 | 0.27 |
| Physical activity (METS/week)[i] | 241 (246) | 224 (197) | 0.36 | 262 (276) | 267 (274) | 0.58 |
| Body mass index ($kg/m^2$) | 27.5 (5.2) | 27.2 (5.4) | 0.09 | 27.4 (4.7) | 26.8 (4.5) | 0.05 |
| Multivitamin use (%) | 24.9 | 33.9 | <0.0001 | 22.6 | 30.7 | 0.003 |
| Total energy intake (kcal/d)[o] | 2069.5 (780.2) | 1961.3 (729.3) | 0.003 | 2089.2 (775.8) | 2052.4 (720.3) | 0.50 |
| Total calcium[l] (mg/d) | 910 (509) | 950 (512) | 0.07 | 959 (531) | 987 (552) | 0.37 |
| Total folate[m] (µg/d) | 411 (239) | 431 (251) | 0.19 | 399 (237) | 429 (250) | 0.02 |
| Total fat (g/d) | 73.1 (35.6) | 67.5 (31.3) | 0.001 | 73.1 (34.3) | 70.2 (31.3) | 0.21 |
| Dietary fiber (g/d) | 21.7 (9.5) | 21.3 (9.9) | 0.22 | 21.8 (9.6) | 22.2 (10.0) | 0.42 |
| Red, processed meat (servings/week)[n] | 7.6 (6.7) | 6.7 (5.4) | 0.07 | 7.3 (6.1) | 6.9 (5.6) | 0.41 |
| Serum 25-OH vitamin $D_3$ (ng/ml) | 24.2 (10.2) | 25.1 (10.8) | 0.14 | -- | -- | -- |

Table continues

**Table 1. Continued**

| Characteristic[c] | Pooled cases[d] | Pooled Controls[e] | p[f] | MCPRU cases[d] | MCPRU community controls[g] | p[f] |
|---|---|---|---|---|---|---|
| | n=792 | n=985 | | n=565 | n=536 | |
| Dietary fiber (g/d) | 21.7 (9.5) | 21.3 (9.9) | 0.22 | 21.8 (9.6) | 22.2 (10.0) | 0.42 |
| Red, processed meat (servings/week)[n] | 7.6 (6.7) | 6.7 (5.4) | 0.07 | 7.3 (6.1) | 6.9 (5.6) | 0.41 |
| Total fruits and vegetables (servings/week) | 41.8 (23.8) | 42.3 (25.9) | 0.95 | 42.3 (23.8) | 44.5 (23.5) | 0.03 |
| Total fruits (servings/week) | 16.1 (12.3) | 16.9 (12.7) | 0.09 | 16.8 (12.4) | 18.5 (12.9) | 0.009 |
| Total vegetables (servings/week) | 25.8 (16.2) | 25.3 (17.2) | 0.34 | 25.5 (15.7) | 26.0 (14.8) | 0.22 |
| 8q24 genotypes | | | | | | |
| rs6983267 | | | | | | |
| GG | 151 (35.4) | 142 (26.1) | | | | |
| GT | 194 (45.4) | 270 (49.5) | | -- | -- | -- |
| TT | 82 (19.2) | 133 (24.4) | 0.005 | | | |
| rs10808555 | | | | | | |
| GG | 78 (15.1) | 55 (8.9) | | | | |
| AG | 222 (43.0) | 274 (44.1) | | -- | -- | -- |
| AA | 216 (41.9) | 292 (47.0) | 0.004 | | | |
| rs7837328 | | | | | | |
| AA | 119 (23.1) | 102 (16.5) | | | | |
| AG | 262 (50.9) | 302 (48.7) | | -- | -- | -- |
| GG | 134 (26.0) | 216 (34.8) | 0.001 | | | |

[a] Pooled from three case-control studies: Minnesota Cancer Prevention Research Unit (MCPRU) study, 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003.
[b] Minnesota Cancer Prevention Research Unit (MCPRU) study collected data for both colonoscopy-negative controls and community controls. Only colonoscopy-negative controls were included in pooled analyses. Only community controls were included in all MCPRU analyses.
[c] Mean and standard deviation reported unless otherwise noted.
[d] Pooled and MCPRU cases had at least one sporadic, first incident adenomatous colorectal polyp at colonoscopy.
[e] Pooled controls were colonoscopy-negative, free of adenomatous and hyperplastic colorectal polyps at colonoscopy.
[f] Chi-square test for dichotomous variables; t-test of normalized variables for continuous non-normally distributed variables.
[g] MCPRU community controls were frequency matched to MCPRU cases on age, sex, and zip code.
[h] First degree relative with colorectal cancer.
[I] Metabolic equivalents of vigorous and moderate physical activity per week.
[j] Nonsteroidal anti-inflammatory drug use at least once a week.
[k] Hormone replacement therapy among women only.
[l] Total calcium intake from diet and supplements.
[m] Includes folate from natural sources, supplements, and fortified foods.
[n] Sum of red meat and processed meat servings per week.
[o] kilocalories per day.

**Table 2. Associations of fruit and vegetable intakes with incident sporadic colorectal adenoma in pooled[a] and MCPRU[b] case-control studies**

| Food group quartile | Pooled | | MCPRU | |
|---|---|---|---|---|
| | Crude | Fully adjusted | Crude | Fully adjusted |
| | OR (95% CI) | OR[c] (95% CI) | OR (95% CI) | OR[c] (95% CI) |
| Total fruits and vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.02 (0.79, 1.32) | 1.19 (0.89, 1.58) | 0.90 (0.65, 1.25) | 0.85 (0.61, 1.20) |
| 3 | 0.99 (0.76, 1.30) | 1.04 (0.76, 1.43) | 0.74 (0.53, 1.03) | 0.73 (0.51, 1.05) |
| 4 | 1.08 (0.83, 1.41) | 1.08 (0.77, 1.53) | 0.78 (0.56, 1.09) | 0.79 (0.53, 1.18) |
| *p trend* | *0.63* | *0.79* | *0.08* | *0.16* |
| Total fruits | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.97 (0.75, 1.26) | 0.96 (0.72, 1.28) | 0.95 (0.68, 1.31) | 0.98 (0.69, 1.38) |
| 3 | 0.97 (0.75, 1.26) | 0.98 (0.72, 1.32) | 0.87 (0.63, 1.20) | 0.93 (0.65, 1.33) |
| 4 | 0.91 (0.69, 1.19) | 0.83 (0.59, 1.17) | 0.71 (0.51, 1.00) | 0.78 (0.52, 1.16) |
| *p trend* | *0.52* | *0.37* | *0.04* | *0.23* |
| Total vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.87 (0.67, 1.14) | 1.03 (0.77, 1.38) | 0.77 (0.56, 1.08) | 0.81 (0.57, 1.15) |
| 3 | 1.29 (0.99, 1.67) | 1.37 (1.01, 1.85) | 0.91 (0.66, 1.27) | 0.86 (0.61, 1.22) |
| 4 | 1.10 (0.85, 1.42) | 1.20 (0.86, 1.66) | 0.84 (0.60, 1.17) | 0.85 (0.58, 1.24) |
| *p trend* | *0.13* | *0.11* | *0.45* | *0.44* |

[a] Pooled from three case-control studies: Minnesota Cancer Prevention Research Unit study (MCPRU), 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003. Cases had at least one incident sporadic colorectal adenoma. Controls had no adenomatous or hyperplastic colorectal polyps at colonoscopy.
[b] Minnesota Cancer Prevention Unit (MCPRU) analyses compare incident sporadic colorectal adenoma cases with community controls frequency matched on age, sex, and zip code.
[c] Adjusted for age, sex, hormone replacement therapy (among women), total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ once/wk.) nonsteroidal anti-inflammatory drug use, smoking status, and total folate intake.

**Table 3. Associations of fruit and vegetable intakes with incident, sporadic colorectal adenoma according to levels of other risk factors for colorectal neoplasms in pooled[a] and MCPRU[b] case-control studies; only odds ratios for highest relative to lowest quartiles of intakes shown**

| Risk Factor | Total fruits and vegetables | | Total fruits | | Total vegetables | |
|---|---|---|---|---|---|---|
| | Pooled OR[c] (95% CI) | MCPRU OR[c] (95% CI) | Pooled OR[c] (95% CI) | MCPRU OR[c] (95% CI) | Pooled OR[c] (95% CI) | MCPRU OR[c] (95% CI) |
| *Age* | | | | | | |
| ≥ 56 | 1.02 (0.64, 1.62) | 0.68 (0.41, 1.12) | 0.70 (0.44, 1.09) | 0.76 (0.46, 1.24) | 1.32 (0.85, 2.06) | 0.81 (0.50, 1.33) |
| < 56 | 1.19 (0.70, 2.01) | 0.90 (0.46, 1.78) | 1.13 (0.66, 1.93) | 0.78 (0.39, 1.55) | 1.08 (0.65, 1.78) | 0.83 (0.44, 1.56) |
| *Sex*[d] | | | | | | |
| Male | 0.88 (0.55, 1.40) | 0.68 (0.41, 1.14) | 0.78 (0.50, 1.24) | 0.92 (0.55, 1.53) | 1.06 (0.67, 1.66) | 0.74 (0.45, 1.22) |
| Female | 1.43 (0.85, 2.41) | 1.07 (0.57, 2.04) | 0.89 (0.53, 1.48) | 0.59 (0.32, 1.11) | 1.39 (0.85, 2.28) | 1.10 (0.60, 2.04) |
| *Family history of CRC*[e] | | | | | | |
| Yes | 1.35 (0.61, 3.01) | 0.57 (0.12, 2.84) | 0.58 (0.27, 1.25) | 1.23 (0.30, 5.09) | 1.93 (0.92, 4.07) | 0.67 (0.16, 2.78) |
| No | 1.04 (0.71, 1.52) | 0.81 (0.53, 1.23) | 0.91 (0.62, 1.33) | 0.78 (0.51, 1.18) | 1.08 (0.75, 1.57) | 0.88 (0.59, 1.31) |
| *Smoking status*[f] | | | | | | |
| Never | 1.09 (0.60, 1.95) | 0.74 (0.38, 1.42) | 1.12 (0.64, 1.96) | 0.83 (0.43, 1.60) | 1.06 (0.61, 1.85) | 0.80 (0.43, 1.47) |
| Ever | 0.97 (0.64, 1.48) | 0.78 (0.47, 1.29) | 0.64 (0.42, 0.99) | 0.68 (0.41, 1.13) | 1.20 (0.79, 1.80) | 0.87 (0.53, 1.42) |
| *No regular NSAID use*[g] | | | | | | |
| | 0.92 (0.63, 1.34) | 0.75 (0.48, 1.15) | 0.76 (0.53, 1.11) | 0.70 (0.46, 1.08) | 1.12 (0.78, 1.62) | 0.82 (0.54, 1.25) |

Abbreviations: OR, odds ratio; CI, confidence interval; CRC, colorectal cancer; NSAID, nonsteroidal anti-inflammatory drug.

[a] Data pooled from three case-control studies: Minnesota Cancer Prevention Research Unit study (MCPRU), 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003. Cases had at least one incident sporadic colorectal adenoma. Controls had no adenomatous or hyperplastic colorectal polyps at colonoscopy.

[b] MCPRU, Minnesota Cancer Prevention Unit study. Compares first incident sporadic colorectal adenoma cases with community controls frequency matched on age, sex, and zip code.

[c] Reference group is the lowest quartile of intake for each analysis. Except where indicated, odds ratios are adjusted for age, sex, hormone replacement therapy (among women), total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ one/wk.) nonsteroidal anti-inflammatory drug use, smoking status, and total folate intake.

[d] ORs not adjusted for sex.

[e] First degree relative with history of colorectal cancer. ORs not adjusted for family history.

[f] ORs not adjusted for smoking status.

[g] OR values for regular NSAID use not shown due to small sample sizes. ORs for no regular NSAID use are not adjusted for NSAID use.

**Table 4. Associations[a] of fruit and vegetable intakes with incident, sporadic colorectal adenoma according to chromosome 8q24 region genotypes in a pooled case-control study[b]**

| Food group quartile | rs6983267 GG 139/135[c] | rs6983267 GT 187/255[c] | rs6983267 TT 79/128[b] | rs10808555 GG 208/279[b] | rs10808555 AG 212/261[c] | rs10808555 AA 73/52[c] | rs7837328 AA 112/97[c] | rs7837328 AG 251/284[c] | rs7837328 GG 129/210[c] |
|---|---|---|---|---|---|---|---|---|---|
| Fruits and vegetables | | | | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.68 (0.33,1.38) | 1.13 (0.63, 2.03) | 1.87 (0.74, 4.72) | 0.89 (0.28, 2.87) | 0.96 (0.56, 1.66) | 1.68 (0.99, 2.86) | 0.51 (0.22, 1.17) | 1.32 (0.80, 2.20) | 1.90 (0.96, 3.78) |
| 3 | 0.66 (0.29, 1.48) | 1.22 (0.66, 2.25) | 2.29 (0.88, 5.97) | 1.26 (0.35, 4.50) | 0.73 (0.40, 1.33) | 1.57 (0.87, 2.82) | 0.85 (0.33, 2.23) | 0.93 (0.53, 1.61) | 1.77 (0.85, 3.70) |
| 4 | 0.56 (0.23, 1.33) | 1.86 (0.94, 3.70) | 2.04 (0.68, 6.08) | 0.76 (0.17, 3.37) | 0.70 (0.36, 1.38) | 1.61 (0.84, 3.11) | 0.52 (0.17, 1.57) | 0.88 (0.48, 1.63) | 2.43 (1.07, 5.53) |
| Fruits | | | | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.79 (0.39, 1.61) | 0.73 (0.40, 1.34) | 2.22 (0.91, 5.41) | 0.48 (0.15, 1.53) | 0.92 (0.52, 1.61) | 1.29 (0.76, 2.19) | 0.86 (0.38, 1.93) | 0.83 (0.50, 1.39) | 1.69 (0.86, 3.34) |
| 3 | 0.66 (0.31, 1.42) | 1.05 (0.58, 1.90) | 2.09 (0.84, 5.23) | 0.49 (0.13, 1.87) | 1.12 (0.64, 1.96) | 0.94 (0.54, 1.62) | 1.09 (0.45, 2.67) | 0.72 (0.42, 1.22) | 1.50 (0.77, 2.91) |
| 4 | 0.58 (0.25, 1.36) | 1.01 (0.51, 2.02) | 1.35 (0.47, 3.86) | 0.59 (0.16, 2.23) | 0.73 (0.38, 1.39) | 0.59 (0.30, 1.14) | 0.67 (0.25, 1.82) | 0.61 (0.33, 1.10) | 0.84 (0.36, 1.93) |
| Vegetables | | | | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.93 (0.46, 1.91) | 1.06 (0.58, 1.91) | 1.27 (0.50, 3.25) | 0.83 (0.27, 2.53) | 0.71 (0.41, 1.24) | 1.72 (0.98, 3.01) | 0.69 (0.30, 1.57) | 0.81 (0.48, 1.36) | 2.42 (1.19, 4.92) |
| 3 | 1.05 (0.50, 2.18) | 1.65 (0.89, 3.08) | 2.43 (0.95, 6.18) | 1.74 (0.48, 6.28) | 1.06 (0.59, 1.92) | 1.97 (1.13, 3.43) | 1.08 (0.44, 2.65) | 1.30 (0.76, 2.21) | 2.46 (1.20, 5.06) |
| 4 | 1.10 (0.47, 2.57) | 1.56 (0.82, 3.00) | 1.53 (0.56, 4.15) | 1.26 (0.28, 5.68) | 0.98 (0.52, 1.87) | 1.69 (0.92, 3.10) | 0.81 (0.27, 2.40) | 1.18 (0.66, 2.12) | 2.37 (1.09, 5.16) |

[a] Odds ratios and 95% confidence intervals; adjusted for age, sex, hormone replacement therapy (among women), total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ once/wk.) nonsteroidal anti-inflammatory drug use, smoking status, and total folate intake.
[b] Pooled from 3 case-control studies: Minnesota Cancer Prevention Research Unit study (MCPRU), 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003. Cases had ≥ 1 incident, sporadic, colorectal adenoma. Controls had no adenomatous or hyperplastic polyps at colonoscopy.
[c] n for cases/controls.

**Table 5. Associations of fruit and vegetable intakes with incident, sporadic, colorectal adenoma characteristics in a pooled case-control study[a]; only odds ratios for highest relative to lowest quartiles of intakes shown**

| Characteristic of largest adenoma | Total fruits and vegetables | | Total fruits | | Total vegetables | |
|---|---|---|---|---|---|---|
| | OR[b] | 95% CI | OR[b] | 95% CI | OR[b] | 95% CI |
| *Size*[b] | | | | | | |
| ≥ 1 cm | 0.89 | (0.53, 1.48) | 0.62 | (0.37, 1.04) | 1.10 | (0.67, 1.80) |
| < 1 cm | 1.15 | (0.78, 1.69) | 0.93 | (0.64, 1.36) | 1.24 | (0.85, 1.79) |
| *Location* | | | | | | |
| Distal[d] | 1.63 | (1.18, 2.27) | 0.78 | (0.54, 1.13 | 1.31 | (0.91, 1.87) |
| Proximal[e] | 0.84 | (0.47, 1.51) | 0.91 | (0.52, 1.57) | 0.86 | (0.49, 1.52) |
| *Degree of dysplasia* | | | | | | |
| Moderate/severe | 1.01 | (0.66, 1.55) | 0.77 | (0.51, 1.17) | 1.08 | (0.72, 1.62) |
| Mild | 1.12 | (0.72, 1.72) | 0.89 | (0.58, 1.37) | 1.32 | (0.87, 2.00) |
| *Subtype* | | | | | | |
| Tubulovillous/ villous | 0.71 | (0.41, 1.25) | 0.56[f] | (0.32, 0.98) | 1.06 | (0.62, 1.80) |
| Tubular | 1.23 | (0.84, 1.80) | 0.92 | (0.63, 1.33) | 1.29 | (0.89, 1.85) |
| *Shape* | | | | | | |
| Sessile | 1.05 | (0.70, 1.58) | 0.64 | (0.43, 0.96) | 1.33 | (0.90, 1.97) |
| Pedunculated | 1.01 | (0.56, 1.81) | 0.82 | (0.47, 1.45) | 1.18 | (0.68, 2.04) |
| *Total number of adenomas* | | | | | | |
| ≥ 3 | 0.87 | (0.36, 2.08) | 0.73 | (0.33, 1.60) | 1.17 | (0.51, 2.72) |
| < 3 | 1.09 | (0.77, 1.55) | 0.83 | (0.59, 1.18) | 1.19 | (0.85, 1.67) |

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Data pooled from three case-control studies: Minnesota Cancer Prevention Research Unit study (MCPRU), 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003. Cases had at least one confirmed, incident sporadic colorectal adenoma. Controls had no adenomatous or hyperplastic colorectal polyps at colonoscopy.

[b] *In vivo* measurement of maximum diameter of largest adenoma.

[c] Reference group is lowest quartile of intake for each analysis. ORs adjusted for age, sex, hormone replacement therapy, total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ once/wk.) non-steroidal anti-inflammatory drug use, smoking status, and total folate intake.

[d] Splenic fixture, descending colon, sigmoid colon, rectum.

[e] Cecum, ascending colon, hepatic fixture, transverse colon.

[f] p-trend = 0.05

# Appendix

**Supplemental Table 2. Associations of fruit and vegetable intakes with incident sporadic colorectal adenoma in pooled[a] case-control study**

| Food group quartile | Crude odd ratio | Minimally adjusted odds ratio[b] | Fully adjusted odds ratio[c] |
|---|---|---|---|
| | OR (95% CI[d]) | OR (95% CI[d]) | OR (95% CI[d]) |
| Fruits and vegetables | | | |
| 1 | 1.00 | 1.00 | 1.00 |
| 2 | 1.02 (0.79, 1.32) | 1.03 (0.78, 1.36) | 1.19 (0.89, 1.58) |
| 3 | 0.99 (0.76, 1.30) | 0.85 (0.63, 1.15) | 1.04 (0.76, 1.43) |
| 4 | 1.08 (0.83, 1.41) | 0.83 (0.61, 1.14) | 1.08 (0.77, 1.53) |
| *p trend* | 0.63 | 0.16 | 0.79 |
| Total fruits | | | |
| 1 | 1.00 | 1.00 | 1.00 |
| 2 | 0.97 (0.75, 1.26) | 0.86 (0.65, 1.14) | 0.96 (0.72, 1.28) |
| 3 | 0.97 (0.75, 1.26) | 0.79 (0.59, 1.04) | 0.98 (0.72, 1.32) |
| 4 | 0.91 (0.69, 1.19) | 0.61 (0.45, 0.83) | 0.83 (0.59, 1.17) |
| *p trend* | 0.52 | 0.002 | 0.37 |
| Total vegetables | | | |
| 1 | 1.00 | 1.00 | 1.00 |
| 2 | 0.87 (0.67, 1.14) | 0.91 (0.69, 1.21) | 1.03 (0.77, 1.38) |
| 3 | 1.29 (0.99, 1.67) | 1.25 (0.94, 1.67) | 1.37 (1.01, 1.85) |
| 4 | 1.10 (0.85, 1.42) | 0.97 (0.71, 1.32) | 1.20 (0.86, 1.66) |
| *p trend* | 0.13 | 0.64 | 0.11 |

[a] Pooled from three case-control studies: Minnesota Cancer Prevention Research Unit study (MCPRU), 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003. Cases had at least one incident sporadic colorectal adenoma. Controls had no adenomatous or hyperplastic colorectal polyps at colonoscopy.

[b] Adjusted for age, sex, hormone replacement therapy (among women), and total energy intake (kilocalories)

[c] Adjusted for age, sex, hormone replacement therapy (among women), total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ once/wk.) nonsteroidal anti-inflammatory drug use, smoking status, and total folate intake.

[d] CI, confidence interval

**Supplemental Table 3. Associations[a] of fruit and vegetable intakes with incident, sporadic colorectal adenoma according to levels of other risk factors for colorectal neoplasms in pooled[b]case-control study**

| Food group quartile | Age | | Sex[c] | | Smoking status[d] | |
|---|---|---|---|---|---|---|
| | ≥ 56 | < 56 | Male | Female | Ever | Never |
| | 480 cases/ 410 controls | 278/ 528 | 471/358 | 287/580 | 526/498 | 232/440 |
| Fruits and vegetables | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.01 (0.68, 1.52) | 1.42 (0.94, 2.15) | 1.02 (0.68, 1.53) | 1.45 (0.95, 2.19) | 1.19 (0.83, 1.70) | 1.13 (0.70, 1.82) |
| 3 | 1.05 (0.68, 1.61) | 1.02 (0.63, 1.66) | 0.95 (0.62, 1.47) | 1.17 (0.73, 1.88) | 0.95 (0.65, 1.40) | 1.04 (0.60, 1.79) |
| 4 | 1.02 (0.64, 1.62) | 1.19 (0.70, 2.01) | 0.88 (0.55, 1.40) | 1.43 (0.85, 2.41) | 0.97 (0.64, 1.48) | 1.09 (0.60, 1.95) |
| *p trend* | *0.91* | *0.71* | *0.58* | *0.31* | *0.72* | *0.86* |
| Total fruits | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.80 (0.53, 1.21) | 1.13 (0.75, 1.69) | 0.97 (0.65, 1.45) | 0.92 (0.61, 1.39) | 0.79 (0.56, 1.12) | 1.25 (0.76, 2.06) |
| 3 | 1.00 (0.66, 1.51) | 0.88 (0.56, 1.39) | 0.93 (0.61, 1.42) | 1.03 (0.67, 1.58) | 0.77 (0.53, 1.11) | 1.28 (0.77, 2.12) |
| 4 | 0.70 (0.44, 1.09) | 1.13 (0.66, 1.93) | 0.78 (0.50, 1.24) | 0.89 (0.53, 1.48) | 0.64 (0.42, 0.99) | 1.12 (0.64, 1.96) |
| p *trend* | *0.25* | *0.97* | *0.31* | *0.81* | *0.04* | *0.66* |
| Total vegetables | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.32 (0.88, 1.98) | 0.79 (0.51, 1.22) | 0.96 (0.64, 1.45) | 1.16 (0.76, 1.77) | 1.03 (0.71, 1.51) | 0.95 (0.60, 1.52) |
| 3 | 1.43 (0.95, 2.15) | 1.34 (0.86, 2.08) | 1.06 (0.70, 1.60) | 1.88 (1.21, 2.93) | 1.31 (0.91, 1.89) | 1.34 (0.80, , 2.25) |
| 4 | 1.32 (0.85, 2.06) | 1.08 (0.65, 1.78) | 1.06 (0.67, 1.66) | 1.39 (0.85, 2.28) | 1.20 (0.79, 1.80) | 1.06 (0.61, 1.85) |
| p *trend* | *0.18* | *0.36* | *0.74* | *0.05* | *0.23* | *0.55* |

**Table continues**

**Supplemental Table 3. Continued**

| Food group quartile | Family history of colorectal cancer[e] | | NSAID use ≥ 1 time per week[f] | |
|---|---|---|---|---|
| | Yes | No | Yes | No |
| | 115 cases/282 controls | 643/656 | 110/233 | 648/705 |
| Fruits and vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.51 (0.78, 2.93) | 1.12 (0.81, 1.54) | 2.03 (0.99, 4.15) | 1.07 (0.78, 1.46) |
| 3 | 1.02 (0.49, 2.12) | 1.05 (0.74, 1.49) | 2.01 (0.92, 4.37) | 0.91 (0.64, 1.29) |
| 4 | 1.35 (0.61, 3.01) | 1.04 (0.71, 1.52) | 2.40 (1.01, 5.66) | 0.92 (0.63, 1.34) |
| *p trend* | *0.69* | *0.90* | *0.06* | *0.53* |
| Total fruits | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.66 (0.34, 1.28) | 1.06 (0.77, 1.46) | 0.89 (0.45, 1.79) | 0.96 (0.70, 1.32) |
| 3 | 0.74 (0.37, 1.47) | 1.07 (0.77, 1.50) | 1.16 (0.57, 2.33) | 0.93 (0.67, 1.30) |
| 4 | 0.58 (0.27, 1.25) | 0.91 (0.62, 1.33) | 1.15 (0.50, 2.62) | 0.76 (0.53, 1.11) |
| *p trend* | *0.20* | *0.75* | *0.65* | *0.20* |
| Total vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.07 (0.52, 2.22) | 1.02 (0.74, 1.41) | 1.20 (0.58, 2.49) | 1.01 (0.73, 1.40) |
| 3 | 1.66 (0.83, 3.32) | 1.29 (0.92, 1.81) | 2.75 (1.28, 5.93) | 1.20 (0.87, 1.67) |
| 4 | 1.93 (0.92, 4.07) | 1.08 (0.75, 1.57) | 1.72 (0.75, 3.96) | 1.12 (0.78, 1.62) |
| *p trend* | *0.05* | *0.39* | *0.06* | *0.36* |

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug.

[a] Odds ratios (ORs) and 95% confidence intervals are given. Reference group is the lowest quartile of intake for each analysis. Except where indicated, odds ratios are adjusted for age, sex, hormone replacement therapy (among women), total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ one/wk.) nonsteroidal anti-inflammatory drug use, smoking status, and total folate intake.

[b] Data pooled from three case-control studies: Minnesota Cancer Prevention Research Unit study (MCPRU), 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003. Cases had at least one incident sporadic colorectal adenoma. Controls had no adenomatous or hyperplastic colorectal polyps at colonoscopy.

[c] ORs not adjusted for sex.

[d] ORs not adjusted for smoking status.

[e] First degree relative with history of colorectal cancer. ORs not adjusted for family history.

[f] NSAID, nonsteroidal anti-inflammatory drug. ORs not adjusted for NSAID use.

**Supplemental Table 3b. Associations[a] of fruit and vegetable intakes with incident, sporadic colorectal adenoma according to levels of other risk factors for colorectal neoplasms in the Minnesota Cancer Prevention Research Unit study (MCPRU)[b] (1991-1994)**

| Food group quartile | Age | | Sex[c] | | Smoking status[d] | |
|---|---|---|---|---|---|---|
| | ≥ 56 | < 56 | Male | Female | Ever | Never |
| | 351 cases 337 controls) | 203 cases 195 controls | 345 cases 296 controls | 214 cases 239 controls | 374 cases 296 controls | 180 cases 236 controls |
| Total fruits and vegetables | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.68 (0.43, 1.06) | 1.03 (0.60, 1.79) | 0.75 (0.48, 1.17) | 1.09 (0.63, 1.87) | 0.73 (0.48, 1.12) | 1.01 (0.57, 1.80) |
| 3 | 0.66 (0.41, 1.04) | 0.75 (0.40, 1.40) | 0.74 (0.46, 1.17) | 0.77 (0.42, 1.40) | 0.79 (0.50, 1.24) | 0.61 (0.33, 1.13) |
| 4 | 0.68 (0.41, 1.12) | 0.90 (0.46, 1.78) | 0.68 (0.41, 1.14) | 1.07 (0.57, 2.04) | 0.78 (0.47, 1.29) | 0.74 (0.38, 1.42) |
| *p trend* | *0.15* | *0.57* | *0.15* | *0.89* | *0.34* | *0.19* |
| Total fruits | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.84 (0.53, 1.33) | 1.26 (0.73, 2.18) | 1.16 (0.74, 1.81) | 0.77 (0.44, 1.33) | 0.92 (0.60, 1.39) | 1.03 (0.56, 1.89) |
| 3 | 0.90 (0.57, 1.41) | 0.91 (0.49, 1.70) | 1.01 (0.63, 1.60) | 0.84 (0.47, 1.47) | 1.04 (0.66, 1.63) | 0.76 (0.41, 1.39) |
| 4 | 0.76 (0.46, 1.24) | 0.78 (0.39, 1.55) | 0.92 (0.55, 1.53) | 0.59 (0.32, 1.11) | 0.68 (0.41, 1.13) | 0.83 (0.43, 1.60) |
| *p trend* | *0.35* | *0.42* | *0.66* | *0.15* | *0.27* | *0.40* |
| Total vegetables | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.83 (0.53, 1.29) | 0.70 (0.39, 1.25) | 0.76 (0.48 1.19) | 0.88 (0.51, 1.54) | 0.75 (0.48, 1.17) | 0.90 (0.52, 1.58) |
| 3 | 0.75 (0.48, 1.17) | 1.14 (0.62, 2.07) | 0.72 (0.45, 1.13) | 1.21 (0.69, 2.12) | 0.87 (0.56, 1.36) | 0.77 (0.42, 1.41) |
| 4 | 0.81 (0.50, 1.33) | 0.83 (0.44, 1.56) | 0.74 (0.45, 1.22) | 1.10 (0.60, 2.04) | 0.87 (0.53, 1.42) | 0.80 (0.43, 1.47) |
| *p trend* | *0.34* | *0.86* | *0.21* | *0.54* | *0.68* | *0.40* |

**Table continues**

**Supplemental Table 3b. Continued**

| Food group quartile | Family history of colorectal cancer[e] | | NSAID use ≥ 1 time per week[f] | |
|---|---|---|---|---|
| | Yes | No | Yes | No |
| | 89 cases<br>37 controls) | 465 cases<br>495 controls | 64 cases<br>89 controls | 490 cases<br>443 controls |
| Total fruits and vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.62 (0.15, 2.51) | 0.83 (0.58, 1.19) | 2.29 (0.86, 6.09) | 0.74 (0.51, 1.06) |
| 3 | 0.33 (0.08, 1.36) | 0.77 (0.53, 1.13) | 1.93 (0.69, 5.38) | 0.64 (0.43, 0.95) |
| 4 | 0.57 (0.12, 2.84) | 0.81 (0.53, 1.23) | 1.30 (0.44, 3.88) | 0.75 (0.48, 1.15) |
| *p trend* | *0.36* | *0.26* | *0.75* | *0.11* |
| Total fruits | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.98 (0.54, 7.26) | 0.95 (0.66, 1.37) | 1.38 (0.54, 3.53) | 0.92 (0.63, 1.33) |
| 3 | 1.09 (0.30, 4.00) | 0.94 (0.64, 1.36) | 2.01 (0.77, 5.29) | 0.83 (0.56, 1.23) |
| 4 | 1.23 (0.30, 5.09) | 0.78 (0.51, 1.18) | 1.53 (0.53, 4.40) | 0.70 (0.46, 1.08) |
| *p trend* | *0.88* | *0.27* | *0.30* | *0.10* |
| Total vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.51 (0.12, 2.12) | 0.82 (0.57, 1.18) | 3.83 (1.28, 11.47) | 0.66 (0.45, 0.95) |
| 3 | 0.55 (0.15, 2.03) | 0.87 (0.60, 1.26) | 2.47 (0.86, 7.10) | 0.74 (0.51, 1.09) |
| 4 | 0.67 (0.16, 2.78) | 0.88 (0.59, 1.31) | 1.53 (0.47, 4.94) | 0.82 (0.54, 1.25) |
| *p trend* | *0.63* | *0.57* | *0.89* | *0.39* |

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug.
[a] Odds ratios (ORs) and 95% confidence are given. Reference group is the lowest quartile of intake for each analysis. Except where indicated, odds ratios are adjusted for age, sex, hormone replacement therapy (among women), total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ one/wk.) nonsteroidal anti-inflammatory drug use, smoking status, and total folate intake.
[b] MCPRU, Minnesota Cancer Prevention Unit study. Compares first incident sporadic colorectal adenoma cases with community controls frequency matched on age, sex, and zip code.
[c] ORs not adjusted for sex.
[d] ORs not adjusted for smoking status.
[e] First degree relative with history of colorectal cancer. ORs not adjusted for family history.
[f] Regular (≥ once/week) use of nonsteroidal anti-inflammatory drugs. ORs not adjusted for NSAID use.

**Supplemental Table 5. Associations of fruit and vegetable intakes with incident, sporadic colorectal adenoma characteristics in a pooled case-control study[a]**

| Food group quartile | ≥ 1cm[b] (239/938)[c] | < 1 cm[b] (519/938)[c] | Distal[d] (571/938)[c] | Proximal[e] (179/938)[c] |
|---|---|---|---|---|
| | OR[f] (95% CI) | OR[f] (95% CI) | OR[f] (95% CI) | OR[f] (95% CI) |
| Total fruits and vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.00 (0.66, 1.51) | 1.23(0.90, 1.70) | 1.03 (0.92, 1.17) | 1.14 (0.71, 1.83) |
| 3 | 0.89 (0.56, 1.41) | 1.13 (0.79, 1.60) | 2.80 (2.08, 3.76) | 1.01 (0.61, 1.70) |
| 4 | 0.89 (0.53, 1.48) | 1.15 (0.78, 1.69) | 1.63 (1.18, 2.27) | 0.84 (0.47, 1.51) |
| *p trend* | *0.58* | *0.57* | *0.60* | *0.54* |
| Total fruits | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.90 (0.60, 1.36) | 0.90 (0.72, 1.37) | 0.96 (0.71, 1.31) | 0.95 (0.59, 1.55) |
| 3 | 0.78 (0.50, 1.22) | 1.09 (0.78, 1.52) | 0.98 (0.71, 1.36) | 1.02 (0.62, 1.68) |
| 4 | 0.62 (0.37, 1.04) | 0.93 (0.64, 1.36) | 0.78 (0.54, 1.13) | 0.91 (0.52, 1.57) |
| *p trend* | *0.07* | *0.90* | *0.28* | *0.81* |
| Total vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.89 (0.57, 1.38) | 1.11 (0.80, 1.53) | 1.02 (0.74, 1.41) | 1.00 (0.62, 1.63) |
| 3 | 1.32 (0.86, 2.03) | 1.37 (0.98, 1.91) | 1.41 (1.02, 1.95) | 1.19 (0.73, 1.93) |
| 4 | 1.10 (0.67, 1.80) | 1.24 (0.85, 1.79) | 1.31 (0.91, 1.87) | 0.86 (0.49, 1.52) |
| *p trend* | *0.40* | *0.14* | *0.05* | *0.86* |

**Table continues**

**Supplemental Table 5. Continued**

| Food group quartile | Moderate/severe dysplasia[g] (384/938)[d] | Mild dysplasia[g] (370/938)[d] | Villous/ Tubulovillous[h] (199/938)[d] | Tubular[h] (552/938)[d] |
|---|---|---|---|---|
| | OR[f] (95% CI) | OR[f] (95% CI) | OR[f] (95% CI) | OR[f] (95% CI) |
| Total fruits and vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.27 (0.90, 1.80) | 1.01 (0.70, 1.46) | 0.89 (0.57, 1.40) | 1.23 (0.90, 1.69) |
| 3 | 0.92 (0.62, 1.37) | 1.15 (0.78, 1.71) | 0.94 (0.58, 1.53) | 1.08 (0.76, 1.53) |
| 4 | 1.01 (0.66, 1.55) | 1.12 (0.72, 1.72) | 0.71 (0.41, 1.25) | 1.23 (0.84, 1.80) |
| *p trend* | *0.76* | *0.52* | *0.31* | *0.40* |
| Total fruits | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.96 (0.68, 1.36) | 0.93 (0.65, 1.34) | 0.92 (0.59, 1.45) | 0.95 (0.69, 1.30) |
| 3 | 0.87 (0.60, 1.27) | 1.11 (0.77, 1.61) | 0.82 (0.51, 1.33) | 1.05 (0.76, 1.45) |
| 4 | 0.77 (0.51, 1.17) | 0.89 (0.58, 1.37) | 0.56 (0.32, 0.98) | 0.92 (0.63, 1.33) |
| *p trend* | *0.20* | *0.87* | *0.05* | *0.82* |
| Total vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.91 (0.63, 1.31) | 1.15 (0.80, 1.66) | 0.98 (0.61, 1.56) | 1.06 (0.77, 1.46) |
| 3 | 1.33 (0.92, 1.91) | 1.36 (0.93, 1.98) | 1.25 (0.78, 2.01) | 1.41 (1.02, 1.95) |
| 4 | 1.08 (0.72, 1.62) | 1.32 (0.87, 2.00) | 1.06 (0.62, 1.80) | 1.29 (0.89, 1.85) |
| *p trend* | *0.34* | *0.13* | *0.61* | *0.07* |

**Table continues**

**Supplemental Table 5. Continued**

| Food group quartile | Sessile[i] (433/938)[c] | Pedunculated[i] (190/938)[c] | ≥ 3 adenomas[j] (82/938)[c] | < 3 adenomas[j] (672/938)[c] |
|---|---|---|---|---|
| | OR[f] (95% CI) | OR[f] (95% CI) | OR[f] (95% CI) | OR[f] (95% CI) |
| Total fruits and vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.18 (0.84, 1.65) | 1.19 (0.75, 1.46) | 0.76 (0.37, 1.56) | 1.19 (0.89, 1.60) |
| 3 | 0.92 (0.63, 1.34) | 1.21 (0.73, 1.99) | 1.18 (0.57, 2.47) | 1.02 (0.74, 1.42) |
| 4 | 1.05 (0.70, 1.58) | 1.01 (0.56, 1.81) | 0.87 (0.36, 2.08) | 1.09 (0.77, 1.55) |
| *p trend* | *0.93* | *0.87* | *0.99* | *0.82* |
| Total fruits | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.84 (0.60, 1.18) | 1.07 (0.68, 1.68) | 0.53 (0.26, 1.11) | 0.99 (0.74, 1.32) |
| 3 | 0.90 (0.63, 1.27) | 0.92 (0.56, 1.51) | 0.72 (0.34, 1.51) | 1.01 (0.74, 1.37) |
| 4 | 0.64 (0.43, 0.96) | 0.82(0.47, 1.45) | 0.73 (0.33, 1.60) | 0.83 (0.59, 1.18) |
| *p trend* | *0.06* | *0.46* | *0.52* | *0.41* |
| Total vegetables | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 1.00 (0.70, 1.43) | 0.93 (0.57, 1.49) | 1.08 (0.53, 2.21) | 1.02 (0.75, 1.37) |
| 3 | 1.38 (0.97, 1.97) | 1.37 (0.85, 2.21) | 1.23 (0.60, 2.52) | 1.38 (1.01, 1.87) |
| 4 | 1.33 (0.90, 1.97) | 1.18 (0.68, 2.04) | 1.17 (0.51, 2.72) | 1.19 (0.85, 1.67) |
| *p trend* | *0.06* | *0.31* | *0.63* | *0.11* |

Abbreviations: OR, odds ratio; CI, confidence interval.
[a] Data pooled from three case-control studies: Minnesota Cancer Prevention Research Unit study (MCPRU), 1991-1994; Markers for Adenomatous Polyps I, 1994-1998; Markers for Adenomatous Polyps II, 2000-2003. Cases had at least one confirmed, incident sporadic colorectal adenoma. Controls had no adenomatous or hyperplastic colorectal polyps at colonoscopy.
[b] *In vivo* measurement of maximum diameter of largest adenoma.
[c] n for cases/controls
[d] Splenic fixture, descending colon, sigmoid colon, rectum.
[e] Cecum, ascending colon, hepatic fixture, transverse colon.
[f] Reference group is lowest quartile of intake for each analysis. ORs adjusted for age, sex, hormone replacement therapy, total energy intake, family history of colorectal cancer in a first degree relative, regular (≥ once/wk.) non-steroidal anti-inflammatory drug use, smoking status, and total folate intake.
[g] Degree of dysplasia for the largest adenoma (i*n vivo* measurement of maximum diameter)
[h] Subtype of largest adenoma (i*n vivo* measurement of maximum diameter)
[i] Shape of the largest adenoma (i*n vivo* measurement of maximum diameter)
[j] Total number of adenomas