

Folate intake, MTHFR C677T polymorphism, alcohol consumption, and risk for sporadic colorectal adenoma (United States)

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Abstract

Objective: The purpose of this study was to investigate whether folate intake is associated with risk for incident sporadic colorectal adenoma, and whether the association differs according to methylenetetrahydrofolate reductase (MTHFR) genotypes or is modified by intakes of alcohol or other micronutrients in the folate metabolism pathway.

Methods: The authors analyzed data from a colonoscopy-based case-control study (n = 177 cases, 228 controls) conducted in North Carolina between 1995 and 1997.

Results: The multivariate-adjusted odds ratio (OR) comparing the highest to lowest tertile of total folate intake was 0.61 (95% confidence interval [CI] 0.35–1.05); for MTHFR C677T polymorphism CT and TT genotypes relative to the CC genotype they were, respectively, 1.09 (CI: 0.71–1.66) and 0.68 (CI: 0.29–1.61); and for heavy drinkers (>3 drinks/week) compared to non-drinkers it was 1.67 (CI: 1.00–2.81). The multivariate-adjusted ORs comparing the highest to lowest tertile of total folate intake according to those with the MTHFR CC, CT, and TT genotypes, were, respectively, 0.65 (CI: 0.30–1.39), 0.57 (CI: 0.23–1.44), and 0.22 (CI: 0.02–3.19). For those in the lowest tertile of folate intake who drank more than three drinks a week compared to those who were in the highest tertile of folate intake and did not drink alcohol the OR was 6.54 (CI: 1.96–21.80). There was no substantial evidence for interactions of folate with intakes of methionine, vitamins B2, B6, or B12.

Conclusions: These data are consistent with hypotheses and previous findings that higher folate intake may reduce risk for colorectal neoplasms, perhaps especially among those who consume more alcohol.

Introduction

Many, but not all, epidemiological studies have found diets high in fruits and vegetables to be associated with a decreased risk for colon cancer [1]. Specific micronutrients found in fruits and vegetables are thought to

contribute to the observed inverse associations. In particular, fruits and vegetables are a major source of folate. Folate is a key component in nucleic acid synthesis, methionine regeneration, and phases of amino acid metabolism [2]. Folate deficiency can cause DNA damage in the form of uracil misincorporation and impaired DNA repair [3–5]. The majority of prospective epidemiological studies have found that high folate intake is associated with reduced risk both for colon cancer [6–8] and colorectal adenoma [9].

Several polymorphisms in critical enzymes involved in folate metabolism have recently been identified.

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Methylenetetrahydrofolate reductase (MTHFR), one such enzyme, determines the balance between DNA methylation and DNA synthesis by catalyzing a uni-directional one carbon transfer [10]. The 677C→T MTHFR polymorphism results in the replacement of valine for alanine, impaired *in vitro* stability, and reduced activity [11]. In addition to the 677C→T MTHFR polymorphism, other dietary factors related to methyl availability may influence the association between folate and risk for colon carcinogenesis. Specifically, alcohol, a known folate antagonist, affects the dietary methyl supply along with the nutrients methionine, vitamin B2, vitamin B6, and vitamin B12, which are important co-factors in the folate metabolism pathway [12, 13].

Of five previous epidemiological studies [14–18] investigating the association between MTHFR C677T polymorphism and risk for colorectal cancer, all but one [18] reported decreased risk for colorectal cancer associated with the homozygous variant genotype among those with a ‘low-risk’ (high folate and low alcohol) methyl diet. Although several studies have investigated the joint effects of MTHFR with nutrients involved in the folate metabolism pathway, very few have comprehensively reported on the joint effects of folate with other nutrients, alcohol, and MTHFR in relation to colonic neoplasms. To further investigate associations between folate intake, alcohol intake, other nutrients involved in the folate metabolism pathway, the MTHFR C677T polymorphism, and risk for sporadic colorectal adenomatous polyps, we analyzed data from a colonoscopy-based case-control study in a predominately Caucasian North Carolina population.

Materials and methods

Study population

Participants for this case-control study were recruited from all English speaking patients between 30 and 74 years old who were scheduled for an elective, outpatient colonoscopy over a 12-month period by four gastroenterology practices in the Piedmont region of North Carolina. Other eligibility criteria included no previous colorectal adenoma or individual history of any cancer (other than non-melanoma skin cancer), resident of the Winston-Salem or Charlotte, NC metro areas, no known genetic syndromes associated with colonic neoplasia (familial polyposis, Gardner’s syndrome), and no history of ulcerative colitis or Crohn’s disease. Since the primary purpose of this study was to investigate the potential validity of specific colon tissue biomarkers on risk for colorectal neoplasms, and

biopsies of normal appearing mucosa were part of the study, participants at risk for bleeding (bleeding disorder, took warfarin, or could not be off of aspirin for 7 days) were ineligible. Only participants with adequate visualization of the entire colon to the cecum on colonoscopy were eligible for final analyses.

All self-report information, including medical history and dietary histories, was obtained before the qualifying colonoscopy and case-control status determination. Each polyp removed at colonoscopy was examined by an index pathologist using diagnostic criteria adopted from the National Polyp Study [19]. Information regarding location, size, shape, histologic type, and degree of atypia was recorded. Patients who had concurrent adenomas and hyperplastic polyps were included as cases, and patients who had only hyperplastic polyps were included as controls.

The participation rate among all colonoscoped patients was 63%. Among the participants, 184 had adenomatous polyps, and 236 subjects were adenoma free at colonoscopy. Of these, fifteen (n = 7 cases, n = 8 controls) were excluded either for: (a) implausible total energy intake (<500 kcal/day or greater than 6000 kcal/day) on the food frequency questionnaire (n = 2 cases, n = 6 controls), (b) more than 15 blank items on the food frequency questionnaire (n = 3 cases, n = 1 control), or (c) incomplete medical history (n = 2 cases, n = 1 control). Thus, a total of 177 cases and 228 control participants were included in this analysis.

Data collection

An adaptation of the Willett semi-quantitative food frequency questionnaire (153 items), expanded to include additional vegetables, fruit, and low-fat foods, was administered to assess dietary history. Previous studies have examined the validity of the Willett food frequency questionnaire [20–22]. Study participants also provided information on medical history, smoking habits, alcohol intake, physical activity (via a modified Paffenbarger questionnaire), reproductive history (women), family history of cancer, and anthropometrics.

Average daily nutrient intake from dietary (*versus* supplemental) sources in the food frequency questionnaire was assessed as the average daily intake over the past 12 months. Supplemental use was assessed by specific questions in the food frequency questionnaire regarding multivitamin use (type and frequency) as well as use of specific vitamin and mineral supplements including folate, vitamin B2, vitamin B6, and vitamin B12 supplements (dose and frequency). Total vitamin intake was calculated as the sum of dietary and

supplemental intakes. Study participants provided specific information on whether they regularly (defined as once a week or more) took non-steroidal anti-inflammatory drugs (NSAIDs), and, if so, the frequency and duration. Similar information was also collected separately for aspirin.

Genotyping

Blood samples were drawn, processed, and stored as nuclear pellets for subsequent DNA extraction to determine genotype status. PCR was used to amplify the 198-bp MTHFR polymorphic site fragment using primers described by Frosst *et al.* (1995) [11]. The PCR product was digested with *HinfI* for 1 h at 37 °C, then separated on a 3% NuSieve agarose gel. Restriction enzyme digestion resulted in two fragments, 23 and 175-bp, when the *HinfI* site was present. The resulting alleles were designated C (restriction site absent) or T (restriction site present), producing three possible genotypes: CC, CT, and TT. Standard laboratory quality control protocol was followed. Known MTHFR genotype samples were included on each 96-well microtitre plate. If the results for these samples of known genotype were ambiguous or incorrect, the entire plate was retyped. In addition, a 10% retyping of all samples was performed. If the results of any two pairs were incongruent, both plates from which the samples were drawn were re-analyzed.

Statistical methods

Standard techniques for case-control studies were used. Age- and sex-adjusted mean baseline characteristics of cases and controls were computed and compared using analysis of covariance for continuous variables and χ^2 tests for categorical variables. The odds ratio was the measure of association used to relate case-control status to genotype or levels of exposures. For all odds ratios, 95% confidence intervals were calculated. Unless indicated otherwise, the intakes of folate and other nutrients were categorized into sex-specific tertiles based on food frequency questionnaire data among controls. Alcohol intake was categorized as three levels: no alcohol consumption, and among alcohol users, less than or equal to or greater than the median alcohol intake (3 drinks/week) among controls.

Potential effect modification was assessed by comparing stratum-specific odds ratios. Subsequently, potential for confounding was assessed. The basis for the assessment of confounding factors included: (a) biological plausibility, (b) whether the factor was associated

with outcome and exposure, and (c) whether inclusion of the variable in the model changed the risk estimate for the primary exposure variable of interest by 10% or more, either alone or in combination with other potential confounding variables. Potential confounders considered in this analysis were age, sex, race, education, family history of colon cancer, total energy intake, NSAID use, physical activity, smoking, alcohol use, body mass index (BMI), waist:hip ratio, practice site, and intakes of fat, red meat, vegetables and fruit, calcium, and vitamin E. Potential confounders were assessed in bivariate and in incremental multivariate combinations to determine the extent of confounding by covariates. Final covariates included in multivariate-adjusted models were age, sex, and total energy intake. Unconditional multivariate logistic regression models were used to obtain final estimates. The test for trend was based on the median of each category of nutrient intake.

Results

Selected characteristics of cases and controls are shown in Table 1. On average, compared to controls, cases were older, more likely to be male, more likely to be a current smoker, consumed more alcohol, and consumed less total calcium. Controls were more likely than cases to report a family history of colon cancer among first degree relatives. Mean total folate consumption, including dietary and supplemental folate, did not differ significantly between cases and controls. The distribution of MTHFR genotypes did not differ significantly between cases and controls. The frequency of having at least one T allele was 28.4% among cases and 28.2% among controls. The control population was drawn from four different gastroenterology practices. Within each practice, the control population was in Hardy-Weinburg equilibrium.

Characteristics of adenomas in cases included: 60% had multiple polyps, 36% had adenomas greater than 1.0 cm in greatest diameter, 92% had only tubular polyps, 49% had adenomas with moderate or severe dysplasia, 70% had sessile polyps, and 81 percent of polyps were located in the colon only (data not shown).

As seen in Table 2, the multivariate-adjusted odds ratio comparing those participants in the highest to those in the lowest tertile of total folate was 0.61 (95% confidence interval [95% CI]=0.35–1.05). Adjusted odds ratios comparing those participants with the TT genotype to those with the CC genotype indicated an approximate 30% non-significant reduction in adenoma risk. Relative to non-drinkers, heavier drinkers of

Table 1. Selected characteristics of study population, Markers for Adenomatous Polyps Case–Control Study, 1995–1997

Characteristic ^a	Adenoma cases (N = 177)	Controls (N = 228)	P ^b Adenoma cases versus controls
<i>Demographics</i>			
Age (yrs)	58.4 (8.4)	56.0 (10.0)	0.02
Female (%)	39.5	64.0	0.001
College graduate (%)	20	23.2	0.43
White race (%)	88.6	90.2	0.61
<i>Family history</i>			
1° Relative with colon cancer (%)	20.0	35.5	0.001
<i>Lifestyle/body size</i>			
Physical activity (METs/day)	50.3 (16.1)	51.5 (13.6)	0.27
Current cigarette smoker (%)	31.0	19.7	0.01
Total alcohol (drinks/week)	8.3 (10.2)	5.2 (5.8)	0.02
Body mass index (kg/m ²)	27.2 (5.3)	27.3 (5.9)	0.84
<i>Dietary intakes</i>			
Total energy intake (kcal/day)	2,040 (812)	1,999 (788)	0.66
Dietary fiber (g/day)	22.7 (9.3)	23.8 (10.9)	0.14
Total fat (g/day)	72.4 (39.6)	67.2 (33.1)	0.67
Total fruit and vegetables (servings/day)	6.2 (3.5)	6.5 (3.9)	0.55
Total methionine (g/day)	1.9 (1.1)	1.9 (0.9)	0.89
<i>Total folate (mcg/day)</i>			
Dietary folate (mcg/day)	419 (239)	439 (250)	0.16
Dietary folate (mcg/day)	330 (150)	331 (159)	0.41
Supplemental folate (mcg/day)	89 (177)	107 (181)	0.22
Total vitamin B6 (mg/day)	8.2 (25.1)	9.6 (29.2)	0.78
Total vitamin B12 (mcg/day)	12.3 (20.9)	11.8 (20.8)	0.99
<i>Total calcium (mg/day)</i>			
Dietary calcium (mg/day)	746 (405)	826 (451)	0.06
Dietary calcium (mg/day)	677 (337)	689 (328)	0.35
Supplemental calcium (mg/day)	70 (208)	136 (296)	0.04
<i>Total vitamin D (IU/day)</i>			
Dietary vitamin D (IU/day)	314 (256)	346 (300)	0.17
Dietary vitamin D (IU/day)	205 (122)	202 (115)	0.72
Supplemental vitamin D (IU/day)	109 (223)	144 (261)	0.17
Non-steroidal anti-inflammatory drugs (%)	47.5	53.9	0.20
<i>MTHFR genotype (%)</i>			
CC	50	52.0	
CT	43.2	39.6	
TT	6.8	8.4	0.37

^a Continuous variables presented as mean (SD); categorical variables as proportions in percent.

^b For continuous variables, based on analysis of covariance for age- and sex-adjusted mean differences, and for categorical variables, based on χ^2 test (exceptions: age variable adjusted only for sex, and sex variable adjusted only for age).

alcohol (>3 drinks/week) were at an increased risk for adenoma (multivariate-adjusted OR = 1.67, [95% CI: 1.00–2.81]). There was an approximate halving of risk for adenomas for participants in the highest versus lowest tertile of total vitamin B6. Intakes of methionine, vitamin B12, and vitamin B2 were not associated with risk for colorectal adenoma.

The associations between folate intake and risk for colorectal adenoma according to various nutrients involved in the folate metabolism pathway, MTHFR C677T polymorphism, and alcohol intake are shown in

Table 3. For these analyses, the folate-adenoma association was stratified according to MTHFR genotype to clearly assess level of risk according to mutually exclusive categories. For methionine, vitamin B12, vitamin B6, vitamin B2, and alcohol use, joint/combined effects were calculated with folate intake since there is a fairly wide spectrum of exposure to these nutrients in the general population. Although not statistically significant, there was a pattern consistent with an enhanced reduction in risk for adenoma with high folate intake among those with high vitamin B2 intake (OR = 0.54,

Table 2. Age-, sex-, and energy-adjusted associations of selected risk factors and risk for adenomatous polyps

Risk Factor	N		Age-, sex-, and energy-adjusted associations	
	Adenoma cases (N)	Controls ^d (N)	OR	95% Confidence interval ^a
<i>Total folate intake^b</i>				
Low	72	85	1.00	
Medium	62	71	0.99	0.59–1.66
High	42	72	0.61	0.35–1.05
<i>p</i> -trend			0.10	
<i>Total vitamin B12 intake</i>				
Low	60	83	1.00	
Medium	59	87	0.96	0.58–1.59
High	57	58	1.19	0.68–2.07
<i>p</i> -trend			0.51	
<i>Total vitamin B6 intake</i>				
Low	74	90	1.00	
Medium	54	64	0.75	0.44–1.27
High	48	74	0.54	0.31–0.93
<i>p</i> -trend			0.03	
<i>Total vitamin B2 intake</i>				
Low	85	94	1.00	
Medium	48	61	0.97	0.57–1.63
High	43	73	0.67	0.39–1.17
<i>p</i> -trend			0.18	
<i>Total methionine intake</i>				
Low	77	105	1.00	
Medium	58	63	1.03	0.62–1.73
High	40	60	0.76	0.38–1.53
<i>p</i> -trend			0.96	
<i>MTHFR genotype^c</i>				
CC	85	111	1.00	
CT	75	87	1.09	0.71–1.66
TT	9	19	0.68	0.29–1.61
CT + TT	84	106	1.02	0.68–1.53
<i>Alcohol</i>				
None	41	85	1.00	
0–3 drinks/week	37	53	1.10	0.65–1.85
>3 drinks/week	49	41	1.67	1.00–2.81
<i>p</i> -trend			0.02	

^a Odds ratio (95% confidence interval).

^b Sex-specific tertile ranges (median) for total folate intake (mcg/day) among males: Low: 0–306 (219), Medium: 307–472 (382), High: 473–1188 (680); Among females: Low: 0–247 (197), Medium: 248–536 (359), High: 537–1254 (705). Total vitamin B12 intake (mcg/day) among males: Low: 0–4.8 (3.2), Medium: 4.9–11.8 (7.7), High: 11.9–206 (17.0), among females: Low: 0–4.6 (2.9), Medium: 4.7–10.1 (6.7), High: 10.2–154 (14.4). Total vitamin B6 intake (mg/day) among males: Low: 0–2.4(1.8), Medium: 2.5–3.7 (2.9), High: 3.8–203.9 (5.4), among females: Low: 0–2.4 (1.7), Medium: 2.5–3.7(3.1), High: 3.8–162.8 (5.9). Total vitamin B2 intake (mg/day) among males: Low: 0–1.8(1.4), Medium: 1.9–2.9(2.3), High: 2.9–203.0 (3.9), among females: Low: 0–1.8 (1.3), 1.9–2.9 (2.2), High: 3.0–151.9 (3.9). Total methionine intake (g/day) among males: Low: 0–1.6 (1.2), Medium: 1.7–2.3 (2.0), High: 2.4–4.8 (2.7), among females: Low: 0–1.3 (1.1), Medium: 1.3–2.0 (1.6), High: 2.1–6.5 (2.4).

^c Methylene tetrahydrofolate reductase.

^d Missing information on variable of interest explains why n for cases and controls do not equal 177 and 228, respectively.

[95% CI: 0.29–1.02]) or high vitamin B6 intake (OR = 0.60, [95% CI: 0.32–1.12]). Those who were heavy drinkers (>3 drinks/week) and consumed low amounts of folate were at a significantly increased risk for colorectal adenoma (OR = 6.54, [95% CI: 1.96–

21.80]) compared to non-drinkers with high folate intake. There were no substantial or statistically significant differences in the association between folate intake and risk for colorectal adenoma according to MTHFR genotype, total methionine, vitamin B12, or vitamin B6

Table 3. Age-, sex-, and energy-adjusted associations of total folate intake and risk for adenomatous polyps according to selected risk factors

	Total folate intake (tertiles) ^a					
	Low		Medium		High	
	OR	95% CI ^b	OR	95% CI	OR	95% CI
<i>MTHFR</i> ^c genotype ^d						
CC	1.00 ^{ref}		1.35	0.64–2.84	0.65	0.30–1.39
CT	1.00 ^{ref}		0.70	0.29–1.69	0.57	0.23–1.44
TT	1.00 ^{ref}		0.94	0.13–6.97	0.22	0.02–3.19
<i>Total methionine</i> ^e						
Low	1.00 ^{ref}		1.07	0.50–2.26	0.77	0.35–1.71
Medium	1.58	0.71–3.52	0.95	0.42–2.15	0.84	0.36–1.97
High	0.81	0.25–2.60	1.37	0.52–3.62	0.54	0.19–1.58
<i>Total vitamin B12</i> ^e						
Low	1.00 ^{ref}		1.45	0.66–3.20	0.86	0.12–6.33
Medium	2.17	0.96–4.88	0.84	0.39–1.79	0.85	0.41–1.77
High	1.55	0.58–4.13	2.24	0.94–5.31	0.81	0.38–1.72
<i>Total vitamin B6</i> ^e						
Low	1.00 ^{ref}		1.00	0.45–2.22	–	
Medium	1.05	0.41–2.71	1.01	0.53–1.93	0.82	0.32–2.11
High	0.83	0.10–6.66	0.94	0.36–2.44	0.60	0.32–1.12
<i>Total vitamin B2</i> ^e						
Low	1.00 ^{ref}		1.25	0.61–2.58	0.47	0.03–8.04
Medium	0.95	0.32–2.81	0.81	0.42–1.57	0.72	0.29–1.76
High	0.39	0.07–2.28	0.74	0.27–2.04	0.54	0.29–1.02
<i>Alcohol (drinks/week)</i> ^e						
None	4.01	1.30–12.32	3.61	1.17–11.16	1.00 ^{ref}	
0–3 drinks/week	3.25	0.89–11.84	3.60	1.11–11.61	4.30	1.33–13.95
>3 drinks/week	6.54	1.96–21.80	4.72	1.47–15.15	6.08	1.76–21.03

^a All models adjusted for age, sex, and total energy intake.

^b Odds ratio (95% confidence interval).

^c Methylene tetrahydrofolate reductase.

^d Genotype is a stratifying variable with separate reference groups for each stratum, whereas total methionine, total vitamin B12, total B6, total B2, and alcohol use are meant to be jointly assessed with folate intake and have one reference cell.

^e *p* for interaction: Methionine: 0.54, Vitamin B12: 0.15, Vitamin B6: 0.96, Vitamin B2: 0.76, and Alcohol: 0.09.

intakes. Furthermore, there was no evidence that the folate-adenoma association differed according to adenoma characteristics (multiplicity, size, histologic type, degree of dysplasia, or shape), or that the *MTHFR*-adenoma association differed according to intakes of alcohol, vitamin B2, vitamin B6, vitamin B12, or methionine (data not shown). Findings for folate also did not substantially or statistically differ according to age, family history of colorectal cancer, reason for colonoscopy, or NSAID use (data not shown).

Discussion

These data suggest that a higher intake of folate may reduce risk for colorectal adenoma (OR = 0.61, [95% CI: 0.35–1.05]) and that higher amounts of alcohol increase risk (OR = 1.67, [95% CI: 1.00–2.81]), perhaps especially among those with a low intake of folate (OR = 6.54, [95% CI: 1.96–21.80]). The pattern in the

data was consistent with the hypothesis that higher folate intake may especially reduce risk among those with the *MTHFR* TT genotype (OR = 0.22 [95% CI: 0.02–3.19]); however, the confidence interval for the risk estimate were extremely wide, precluding a meaningful interpretation. The data also suggest that a higher total intake of vitamin B6 may reduce risk for colorectal adenoma (OR = 0.54, [95% CI: 0.31–0.93]), but provide little evidence for associations of adenoma with other factors in the folate metabolism pathway, including methionine, vitamins B2 and B12, and *MTHFR* genotype. There was also no substantial or statistically significant evidence that methionine, vitamins B2, B6, or B12 modified the folate-adenoma association.

This study has several limitations and strengths that should be considered in interpreting its results. Limitations include its small sample size, and thus low statistical power. Also, because all participants were drawn from a population who underwent colonoscopy, they may not have been representative of the general

population. In addition, in colonoscopy-based case-control studies of colorectal adenoma, as occurred in this one, it is not uncommon for a higher proportion of controls than of cases to have a family history of colon cancer, possibly introducing a family history bias. A likely reason for such a finding is that persons with a strong family history of colon cancer may be more likely to have a screening colonoscopy, and to begin doing so at an earlier age, before adenomas have formed. However, such a bias would tend to attenuate rather than exaggerate associations, suggesting that our findings may actually underestimate the influence of folate on risk for colorectal neoplasms. Furthermore, our findings did not vary substantially when stratified by reason for colonoscopy, age, or family history of colon cancer (data not shown). The study also has several important strengths including: (a) because the controls underwent colonoscopy they were documented to be adenoma negative; (b) all self-report information (including dietary questionnaire) was obtained before colonoscopy and case-control status was known – thus minimizing potential recall bias; (c) detailed information on supplemental intakes of folate and other B vitamins, including dose and brand of supplement, and cereal brand was collected; and (d) detailed information on other potential confounding or modifying factors, such as medical history and dose and frequency of NSAID use, was collected.

In vivo and *in vitro* studies provide considerable evidence in support of a biologically plausible association between folate intake and risk for adenoma. The role of folate as a methyl supplier in one carbon metabolism makes it a critical coenzyme required for DNA methylation and DNA synthesis. Folate deficiency may reduce methylation of DNA, and it has been shown that general hypomethylation of DNA is one characteristic of colon cancer [23]. Cell culture studies indicate that depletion of folate induces increased susceptibility to mutagenesis [24], possibly through the role of folate in DNA repair. In support of this hypothesis, *in vivo* studies found that folate depletion causes chromosomal abnormalities and DNA strand breaks [25, 26]. In rat models, folate deficiency enhanced development of colonic dysplasia and cancer [27], and further studies found that folate deficiency impaired DNA excision repair in rats [4]. Other factors involved in the folate metabolism pathway, such as alcohol, may act to enhance folate deficiency. For example, experimental studies conducted in non-human primates found that chronic alcoholism impairs folate absorption, decreases hepatic uptake, and increases urinary excretion of folic acid, thus contributing to long term folate deficiency [12].

A substantial number of studies have investigated the folate-adenoma association; however, relatively few have considered the joint effect of folate and alcohol. In this study, high folate intake was associated with an approximate 39% decrease in adenoma risk, although this finding was not statistically significant. However, our study population had a relatively high overall folate intake compared to other Caucasian populations. It could be that there was not enough variation in the range of folate intake to detect an overall association. In our data, high alcohol intake was independently associated with an increased risk for adenoma, and it also modified the folate-adenoma association such that those who consumed both higher amounts alcohol and lower amounts of folate were at a substantially higher risk for colorectal adenoma relative to non-drinkers with high folate intakes (an approximate sixfold difference in risk between the two groups). To our knowledge, only two previous adenoma studies have reported results on a possible alcohol/folate interaction. Baron *et al.* (1998) reported an approximate twofold higher risk for those with low folate and high alcohol intake ('high risk') compared to those at intermediate risk (mixed folate and alcohol status) among participants in an adenoma prevention clinical trial [28]. Similar risk estimates were reported for men and women with a 'high risk' diet participating in the Health Professionals Follow-up Study and the Nurses' Health Study [9].

There were no statistically significant associations between total folate intake and risk for colorectal adenoma according to MTHFR C677T polymorphism. Although the overall pattern of our results suggests a more prominent reduction in risk among those with high folate intake who were homozygous for the MTHFR C677T polymorphism, the small sample size and low statistical power resulted in wide confidence intervals around the risk estimate. MTHFR sits at the crux of a one carbon metabolism pathway involved in DNA synthesis and DNA methylation. The reaction catalyzed by MTHFR determines the relative amounts of 5,10-methylenetetrahydrofolate, which is needed for purine synthesis, and methyl-THF, which is needed for DNA methylation (for review, see Ref [29]). Of seven previous epidemiological studies investigating the MTHFR C677T polymorphism and risk for colorectal adenoma [30–36], one study [30] reported patterns of risk indicative of an interaction between MTHFR genotype and nutrients involved in the MTHFR metabolic pathway, including folate, vitamin B12, and vitamin B6; one reported a strong interaction between MTHFR and folate ($p=0.004$) as well as among MTHFR, folate, and smoking status ($p=0.002$) [31]; two indicated an interaction between MTHFR genotype

and alcohol, but not between MTHFR and folate intake [32, 33]; and one did not report any interaction [34]. A study conducted among middle-aged Japanese men reported a null association between MTHFR genotype and risk for adenoma, a possible interaction between MTHFR and folate ($p=0.10$), and no interaction with alcohol intake [35, 36]. In contrast to studies of MTHFR variants and colorectal adenoma, four [14–17] of five [14–18] case-control studies investigating the association between C677T MTHFR polymorphism and risk for colorectal cancer reported statistically significant or borderline statistically significant risk estimates that were strongly suggestive of an inverse association among individuals with the TT genotype who also consumed a low-risk methyl diet (*i.e.*, some combination of low alcohol and/or high methionine and/or high folate).

In our study, methionine and vitamin B12 were unrelated to risk for colorectal adenoma. Furthermore, neither methionine nor vitamin B12 modified the association between folate intake and adenoma risk. Methionine and vitamin B12, two co-factors in folate metabolism, are known contributors to methyl availability. Low methionine diets are generally associated with an increased risk for both colorectal adenoma and cancer [6, 8, 9, 30]. To our knowledge, only one previous study investigated the joint association between folate and methionine and colorectal adenoma risk. A combined analysis of the Nurses' Health Study and the Health Professionals Follow-up Study found that a diet low in folate and methionine was associated with an approximate twofold overall increased risk in adenoma, and that this association was stronger in men (OR = 3.56, [95% CI: 1.50–8.45]) [9].

In the present study, vitamin B2 was not associated with risk for colorectal adenoma, but vitamin B6 was inversely associated with risk; those in the highest tertile of vitamin B6 intake were at approximately half the risk for adenoma than those in the first tertile. Although not statistically significant, there was a pattern of decreased risk for adenoma among those with jointly high intakes of folate and vitamin B2 or vitamin B6 compared to those with low intakes of folate and vitamin B2 or vitamin B6. Recent studies have suggested that folate and vitamin B2 interact to lower plasma homocysteine levels, and that this association is unrelated to MTHFR genotype [37]. In addition to its role in maintaining homocysteine levels, vitamin B6 may reduce risk for colon carcinogenesis by reducing cell proliferation, oxidative stress, and angiogenesis [38]. There are no adenoma studies, to our knowledge, that have investigated the joint effect of folate with vitamin B2, vitamin B6, or vitamin B12, and only one colon cancer study to do so. In that study, there

was no pattern of association across categories of folate with B6, B12, or methionine [39].

Of further note, to place our results in context with the previous literature and recent societal changes relevant to folate intake, is that, in 1998, the FDA required fortification of cereals and other grain products with folate, thus making these staples a primary source of folate in the US diet. Results from the study presented here were based on data collected before the US Food and Drug Administration (FDA) folate fortification initiative was implemented. In light of the fact that extreme folate deficiency is now relatively rare in the United States, there are several reasons why studies of the type presented here remain relevant. First, there is epidemiological evidence to suggest that a methyl deficient diet may be a risk factor for colon cancer, and that MTHFR polymorphisms may differentially affect this potential relationship. Further, there have been relatively few studies that have investigated the association between folate, MTHFR polymorphisms, nutrients involved in the folate metabolism pathway, alcohol, and risk for colorectal adenoma, so studies of the type presented here serve to fill an existing gap in the epidemiological literature. Studies of the type reported here also remain highly relevant because most countries do not supplement their diets with folate.

In conclusion, the results of this study support what are now appearing to be consistent findings that higher folate intake may reduce risk for colorectal neoplasms, perhaps especially among those who consume more alcohol. The pattern in the data, although not statistically significant, were consistent with previous findings that higher folate intake may especially reduce risk among those who are homozygous for the MTHFR C677T polymorphism.

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References

1. Potter JD (1999) Colorectal cancer: molecules and populations. *J Natl Cancer Inst* **91**: 916–932.
2. Bailey LB, Gregory JF (1999) Folate metabolism and requirements. *J Nutr* **129**: 779–782.
3. Blount BC, Mack MM, Wehr CM, *et al.* (1997) Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci* **94**: 3290–3295.
4. Choi SW, Kim YI, Weitzel JN, *et al.* (1998) Folate depletion impairs DNA excision repair in the colon of the rat. *Gut* **43**: 93–99.

5. Wei Q, Shen H, Wang LE, *et al.* (2003) Association between low dietary folate intake and suboptimal cellular DNA repair capacity. *Cancer Epidemiol Biomarkers Prev* **12**: 963–969.
6. Giovannucci E, Rimm E, Ascherio A, *et al.* (1995) Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst* **87**(4): 265–273.
7. Giovannucci E, Stampfer M, Colditz G, *et al.* Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study (1998). *Ann Intern Med* **129**: 517–524.
8. Su LJ, Arab L (2001) Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. *Ann Epidemiol* **11**: 65–72.
9. Giovannucci E, Stampfer M, Colditz GA, *et al.* (1993) Folate, methionine, and alcohol intake and risk of colorectal adenoma. *J Natl Cancer Inst* **85**(11): 875–884.
10. Bailey LB, Gregory JF (1999) Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. *J Nutr* **129**: 919–922.
11. Frosst P, Blom HJ, Milos R, *et al.* (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* **10**: 111–113.
12. Halsted CH, Villanueva JA, Devlin AM, *et al.* (2002) Metabolic interactions of alcohol and folate. *J Nutr* **132**: 2367S–2372S.
13. Bailey LB. (2003) Folate, methyl-related nutrients, alcohol, and the MTHFR 677C->T polymorphism affect cancer risk: intake recommendations. *J Nutr* **133**: 3748S–3753S.
14. Chen J, Giovannucci E, Kelsey K, *et al.* (1996) A Methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* **56**: 4862–4864.
15. Ma J, Stampfer M, Giovannucci E, *et al.* (1997) Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res* **57**: 1098–1102.
16. Slattery M, Potter J, Samowitz W, *et al.* (1999) Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* **8**: 513–518.
17. Le Marchand L, Donlon T, Hankin JH, *et al.* (2002) B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). *Cancer Causes Control* **13**: 239–248.
18. Keku T, Millikan R, Worley K, *et al.* (2002) 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and Whites. *Cancer Epidemiol Biomarkers Prev* **11**: 1611–1621.
19. O'Brien MJ, Winawer SJ, Zauber AG, *et al.* (1990) The National Polyp Study. Patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. *Gastroenterology* **98**: 371–379.
20. Willett WC, Sampson L, Stampfer MJ, *et al.* (1985) Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* **122**: 51–65.
21. Munger RG, Folsom AR, Kushi LH, *et al.* (1992) Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiol* **136**: 192–200.
22. Rimm EB, Giovannucci EL, Stampfer MJ, *et al.* (1992) Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* **135**: 1114–1126 (discussion 1127–1136).
23. Laird P, Jackson-Grusby L, Fazeli A, *et al.* (1995) Suppression of intestinal neoplasia by DNA hypomethylation. *Cell* **81**: 197–205.
24. Branda R, Blickensderfer F. (1993) Folate deficiency increases genetic damage caused by alkylating agents and γ -irradiation in Chinese hamster ovary cells. *Cancer Res* **53**: 5401–5408.
25. Libbus B, Borman L, Ventrone CH, *et al.* (1990) Nutritional folate deficiency in Chinese hamster ovary cells. *Cancer Genet Cytogenet* **46**: 231–42.
26. James SJ, Miller BJ, Cross DR, *et al.* (1993) The essentiality of folate for the maintenance of deoxyribonucleotide precursor pools, DNA synthesis, and cell cycle progression in PHA-stimulated lymphocytes. *Environ Health Perspect* **101**(suppl 5): 173–178.
27. Cravo ML, Mason JB, Dayal Y, *et al.* (1992) Folate deficiency enhances the development of colonic neoplasia in dimethylhydrazine-treated rats. *Cancer Res* **52**: 5002–5006.
28. Baron JA, Sandler RS, Haile RW, *et al.* (1998) Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. *JNCI* **90**: 57–62.
29. Choi S-W, Mason JB. (2002) Folate status: effects on pathways of colorectal carcinogenesis. *J Nutr* **132**: 2413S–2418S.
30. Ulrich C, Kampman E, Bigler J, *et al.* (1999) Colorectal adenomas and the C667T MTHFR polymorphism: evidence for a gene-environment interaction? *Cancer Epidemiol Biomarkers and Prev* **8**: 659–668.
31. Ulvik A, Evensen T, Lien EA, *et al.* (2001) Smoking, folate and methylenetetrahydrofolate reductase status as interactive determinants of adenomatous and hyperplastic polyps of colorectum. *Am J Med Genet* **101**: 246–254.
32. Levine AJ, Siegmund KD, Ervin CM, *et al.* (2000) The methylenetetrahydrofolate reductase 677C->T polymorphism and distal colorectal adenoma risk. *Cancer Epidemiol Biomarkers and Prev* **9**: 657–663.
33. Giovannucci E, Chen J, Smith-Warner SA, *et al.* (2003) Methylenetetrahydrofolate reductase, alcohol dehydrogenase, diet, and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers and Prev* **12**: 970–979.
34. Chen J, Giovannucci E, Hankinson S, *et al.* (1998) A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. *Carcinogenesis* **9**(12): 2129–2132.
35. Marugame T, Tsuji E, Inoue H, *et al.* (2000) Methylenetetrahydrofolate reductase polymorphism and risk of colorectal adenomas. *Cancer Lett* **151**: 181–186.
36. Marugame T, Tsuji E, Kiyohara C, *et al.* (2003) Relation of plasma folate and methylenetetrahydrofolate reductase C677T polymorphism to colorectal adenomas. *Int J Epidemiol* **32**: 64–66.
37. Moat SJ, Ashfield-Watt PA, Powers HJ, *et al.* (2003) Effect of riboflavin status on the homocysteine-lowering effect of folate in relation to the MTHFR (C677T) genotype. *Clin Chem* **49**: 295–302.
38. Matsubara K, Komatsu S, Oka T, *et al.* (2003) Vitamin B6-mediated suppression of colon tumorigenesis, cell proliferation, and angiogenesis (review). *J Nutr Biochem* **14**: 246–250.
39. Harnack L, Jacobs DR, Nicodemus K, *et al.* (2002) Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. *Nutr Cancer* **43**: 152–158.