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Relationship of Folate, Vitamin B-6, Vitamin B-12, and Methionine Intake to Incidence of Colorectal Cancers

Lisa Harnack, David R. Jacobs, Jr., Kristin Nicodemus, DeAnn Lazovich, Kristin Anderson, and Aaron R. Folsom

Abstract: *It is hypothesized that diets deficient in folate, methionine, and vitamins B-6 and B-12 cause DNA hypomethylation and, as a result, increase risk of colorectal cancers. Furthermore, it is proposed that alcohol, a methyl group antagonist, increases risk of colorectal cancers among those with low intake of folate. Data from the Iowa Women's Health Study, a population-based cohort of incident cancer, were used to examine the relationship of folate, methionine, and vitamins B-6 and B-12 to occurrence of cancers of the colon (*n *= 598) and rectum (*n *= 123) over 13 yr of follow-up. There were no independent associations of folate, methionine, or vitamins B-6 and B-12 derived from a food frequency questionnaire with incidence of colon cancer. Adjusted relative risks (RRs) of rectal cancer were similar across categories of folate, vitamin B-12, and methionine intake, but RRs increased progressively with increasing intake of vitamin B-6 [*P *(for trend) = 0.03]. RRs suggested that incidence of cancer of the proximal colon was lower among those with 1) high folate and high vitamin B-12 intake [RR = 0.59, 95% confidence interval (CI) = 0.39–0.89] and 2) high folate and high vitamin B-6 intake (RR = 0.65, 95% CI = 0.50–0.84) than among those with the lowest intake of these nutrients. Incidence of cancer of the proximal colon was also somewhat lower among those with high folate and low alcohol intake (RR = 0.44, 95% CI = 0.22–0.89). Findings provide limited support for an association between dietary factors involved in DNA methylation and risk of cancers of the colon and rectum.*

Introduction

Methylation of DNA is one step in the regulation of gene activity (1,2). In colon neoplasms, global DNA hypomethylation is consistently found (3–5), which suggests that disturbances in methylation of DNA may influence colon carcinogenesis.

Several dietary factors, including folate, methionine, alcohol, and vitamins B-6 and B-12, are involved directly or indirectly in DNA methylation, leading to the hypothesis that these dietary constituents may contribute to initiation or progression of colorectal cancers. Hypomethylation may be initiated by inadequate cellular levels of the methyl donor *S*-adenosylmethionine (6), the production of which involves several dietary factors, including folate, methionine, and vitamins B-6 and B-12 (7–9). In addition, alcohol may indirectly alter DNA methylation patterns by affecting the intestinal absorption (10), hepatobiliary metabolism (11), and renal excretion (12) of folate. Thus alcohol has been hypothesized to be associated with an increased risk of colorectal cancers among those with low intake of folate.

Although biochemical findings provide a rationale for the hypothesis that diets deficient in folate, methionine, and vitamins B-6 and B-12 cause aberrations in DNA methylation and influence colon carcinogenesis, findings from epidemiological studies of the relationship of one or more of these nutrients to risk of colorectal cancers (13–30) have been equivocal. Furthermore, some studies have found the association of folate with colorectal cancers to be similar across intake levels of alcohol (16,18); others have found a low-folate and high-alcohol diet to be associated with higher risk of colorectal cancers than a high-folate and low-alcohol diet (13,14,17,19,22,29,30).

In consideration of the inconsistency of findings from previous studies of the association of nutrients involved in DNA methylation with colorectal cancer, the aim of our study was to examine the relationship of folate, methionine, and vitamins B-6 and B-12 to occurrence of cancers of the colon and rectum. Furthermore, we explored whether the association of folate with risk of colorectal cancers varied by intake of alcohol, methionine, and vitamins B-6 and B-12. We hypothesized that a low-folate and high-alcohol diet would be associated with higher risk of colorectal cancers than would a high-folate and low-alcohol diet and that low folate intake combined with low intake of other nutrients involved in DNA methylation would result in an increased risk of colorectal cancers. We used data from the Iowa Women's Health Study (IWHS), a population-based cohort of postmenopausal women in whom diet was measured before diagnosis of incident colorectal cancer.

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Subjects and Methods

Study Population

The methods used in the IWHS have been published elsewhere (31,32). Briefly, in January 1986, a questionnaire was sent to 99,826 randomly selected women, ages 55–69 yr, whose names were included on the 1985 Iowa state drivers' license list. A total of 41,836 women (42.3%) completed the questionnaire and were followed for cancer incidence and mortality. A comparison of respondents and nonrespondents disclosed that respondents were, on average, 2 mo younger and slightly more likely to live in rural, less-affluent areas than were nonrespondents. The incidence of colon cancer in respondents and nonrespondents after 5 yr of follow-up was similar (33). The average age of respondents was 61.7 yr; 99% of respondents were Caucasian.

Women with a history of cancer at baseline other than skin cancer (based on self-report) were excluded from all analyses (n = 3,830), as were those with >29 blank items on the food frequency questionnaire (FFQ) or with calculated energy intake of <500 or >5,000 kcal (n = 2,790). Those with missing covariate information were also excluded from all analyses.

Identification of Cases of Colorectal Cancers

Information on cancer diagnosis was ascertained through the State Health Registry of Iowa, which is part of the National Cancer Institute's Surveillance, Epidemiology, and End Results Program. Vital status of cohort members was determined through computer linkage of participant identifiers with Iowa death certificates, through mailed follow-up questionnaires in 1987, 1989, 1992, and 1997, and via the National Death Index for nonrespondents to the mailed follow-ups. Response rates for the follow-up questionnaires were 91%, 89%, 83%, and 79%, respectively.

Through 31 December 1998, after 13 yr of follow-up, 598 women who met inclusion criteria developed colon cancer [International Classification of Diseases, Oncology (ICD-O) codes C18.0–C18.9] and 123 developed rectal cancer (ICD-O code C20.0). Tumors from the cecum to the hepatic flexure were considered to be in the proximal colon (n = 248). Those located from the hepatic flexure to the rectosigmoid junction were considered to be in the distal colon (n = 274). The location in the colon was unknown for 76 of the women who developed colon cancer.

Exposure Assessment

Exposure was assessed by a self-administered questionnaire administered at baseline. Reported body weight and height were used to calculate body mass index (kg/m^2). Waist-to-hip ratio was derived from circumference measurements made by study participants using a paper tape measure. Physical activity index (high, medium, low) was derived from the reported frequency of engagement in moderate and vigorous leisure time activities. Diet was assessed using a semiquantitative FFQ that was almost identical to that used in the 1984 Nurses' Health Study (34). The usual intakes of 127 food items were ascertained. Women were also questioned on their use of any multi- or single vitamin or mineral supplements. Estimates of average nutrient intakes per day were calculated on the basis of the FFQ and supplement use data. Alcoholic beverages (beer, red wine, white wine, and liquor) were included on the FFQ, allowing for assessment of alcohol consumption.

The FFQ used in the present study accounted for 93% of total caloric intake in a validation study with 194 female nurses (35). In a reliability study in the Iowa population, the correlations between folate, vitamin B-6, and vitamin B-12 intake estimates derived from the FFQ and the average of five 24-h dietary recalls were 0.43, 0.70, and 0.58, respectively (36). Because of the limited number of dietary recalls collected, correlations are likely attenuated, thus providing an underestimate of the validity of the FFQ.

Data Analysis

To examine the association of folate, methionine, and vitamins B-6 and B-12 with incidence of colon and rectal cancers, nutrients were categorized by quintiles and tertiles, respectively. Proportional hazards regression was used to derive relative risks (RRs) adjusted for age and other potentially confounding variables (37). Variables considered for inclusion as covariates included height, body mass index, waist-to-hip ratio, physical activity, estrogen use, history of oral contraceptive use, pack-years of cigarette smoking, alcohol consumption, education level, and dietary intake of energy, calcium, sucrose, fiber, and vitamins A, C, D, and E. Those variables found in age-adjusted analyses to be associated with folate intake or the outcome of interest were included in the final set of adjusting variables. RR estimates for colon cancer were adjusted for age, pack-years of cigarettes, body mass index, estrogen use, and intakes of calcium, vitamin E, and energy. Analyses examining rectal cancer were adjusted for age, pack-years of cigarettes, oral contraceptive use, and intake of calcium, vitamin E, and energy. A trend test using the χ^2 statistic was performed to examine trends in risk ratios across levels of each nutrient variable (coded ordinally).

To examine the interaction of folate intake with alcohol, methionine, and vitamins B-6 and B-12, nutrient intakes were dichotomized at the medians. Alcohol consumption was also dichotomized (<20 and ≥20 g/day). Interactions were tested by including a cross-product term of the two variables of interest in the proportional hazards model.

Results

Age-adjusted RR estimates indicated an inverse association of colon cancer with folate, methionine, and vitamins B-6 and B-12 (Table 1). After adjustment for other covariates,

Table 1. RRs and 95% CI of Colon Cancer by Folate, Vitamin B-6, Vitamin B-12, Methionine, and Alcohol Intake in the IWHS, 1986–98[a]

	Cases/Person-Years	RR[b] (95% CI)	RR[c] (95% CI)
Folate, μg/day			
32.14–231.12	138/83,050	1.00	1.00
231.13–307.56	133/83,170	0.96 (0.75–1.21)	1.04 (0.80–1.35)
307.57–405.81	116/83,456	0.82 (0.64–1.05)	0.96 (0.71–1.29)
405.82–634.02	104/83,200	0.74 (0.57–0.96)	0.92 (0.65–1.29)
634.03–2555.20	107/82,716	0.76 (0.59–0.98)	1.12 (0.77–1.63)
P for trend		0.02	0.67
Vitamin B-6, mg/day			
0.19–1.58	144/82,847	1.00	1.00
1.59–2.10	123/82,779	0.85 (0.67–1.08)	0.95 (0.72–1.24)
2.11–2.85	123/84,570	0.83 (0.65–1.05)	0.94 (0.69–1.27)
2.86–4.35	108/82,879	0.74 (0.58–0.95)	0.90 (0.64–1.28)
4.36–457.12	100/82,516	0.69 (0.53–0.89)	0.95 (0.67–1.36)
P for trend		0.01	0.88
Vitamin B-12, μg/day			
0.03–5.12	135/82,608	1.00	1.00
5.13–8.55	116/83,789	0.85 (0.66–1.09)	0.98 (0.74–1.28)
8.56–13.32	119/83,233	0.87 (0.68–1.12)	0.99 (0.76–1.31)
13.33–18.35	126/83,088	0.93 (0.73–1.18)	1.09 (0.83–1.43)
18.36–1553.37	102/82,874	0.75 (0.58–0.97)	0.94 (0.69–1.27)
P for trend		0.09	0.86
Methionine, g/day			
0.18–1.27	138/82,715	1.00	1.00
1.28–1.60	111/82,710	0.81 (0.63–1.04)	0.90 (0.68–1.19)
1.61–1.92	131/83,251	0.95 (0.75–1.21)	1.07 (0.78–1.47)
1.93–2.34	121/83,172	0.88 (0.69–1.13)	0.98 (0.69–1.39)
2.35–10.57	97/83,744	0.71 (0.55–0.92)	0.72 (0.48–1.09)
P for trend		0.03	0.19
Alcohol, g/day			
<20	572/397,592	1.00	1.00
≥20	26/17,999	1.03 (0.73–1.44)	1.08 (0.72–1.62)

a: Abbreviations are as follows: RR, relative risk; CI, confidence interval; IWHS, Iowa Women's Health Study.
b: Adjusted for age only.
c: Adjusted for age, pack-years of cigarettes (none, 1–19, 20–39, or ≥40), body mass index (quintiles), estrogen use (current, former, never), and intakes of calcium, vitamin E, and energy (quintiles).

however, there was no independent association of folate, methionine, or vitamin B-6 or B-12 with incidence of colon cancer. Covariates were added to the model in stages to examine the substantial confounding more closely. With the addition of pack-years of cigarette smoking, body mass index, estrogen use, and energy, the associations of folate, vitamin B-6, vitamin B-12, and methionine with colon cancer were greatly attenuated and no longer statistically significant. The associations were further attenuated with the addition of calcium and vitamin E. Analyses stratified by location (distal and proximal) were conducted and produced results similar to those seen in the unstratified analyses (data not shown). Adjusted RRs of rectal cancer were similar across categories of folate, vitamin B-12, methionine, and alcohol intake (Table 2). In contrast, incidence of rectal cancer rose progressively with increasing intake of vitamin B-6 [*P* (trend) = 0.03].

Analyses examining the associations of folate, vitamin B-6, and vitamin B-12 with colon and rectal cancers were repeated with nutrient contribution from foods separated from nutrient contribution from supplements. Results were unchanged, except the positive association of vitamin B-6 and rectal cancer was limited to supplemental sources {multivariate RR from low to high tertile = 1.00, 1.23 [95% confidence interval (CI) = 0.67–2.25], and 1.86 (95% CI = 1.01–3.41)} and not food sources [multivariate RR from low to high tertile = 1.00, 0.97 (95% CI = 0.62–1.52), and 1.08 (95% CI = 0.66–1.78)].

For most analyses, the association of folate with colon and rectal cancers was similar across intake levels of alcohol, methionine, and vitamins B-6 and B-12 (Tables 3 and 4). In some analyses, however, the association appeared to vary. RR estimates suggested that incidence of cancer of the proximal colon was lower ($P < 0.05$) among those with 1) both high folate and high vitamin B-6 intake (RR = 0.65, 95% CI = 0.50–0.84) and 2) both high folate and high vitamin B-12 intake (RR = 0.59, 95% CI = 0.39–0.89) than among those with the lowest intake of these nutrients. In addition, incidence of cancer of the proximal colon was somewhat lower among those with high folate and low alcohol

Table 2. RRs of Rectal Cancer by Folate, Vitamin B-6, Vitamin B-12, Methionine, and Alcohol Intake in the IWHS, 1986–98

	Cases/Person-Years	RR[a] (95% CI)	RR[b] (95% CI)
Folate, μg/day			
32.14–281.85	43/139,813	1.00	1.00
281.86–463.36	47/140,131	1.07 (0.71–1.62)	0.82 (0.52–1.29)
463.37–2555.2	33/139,070	0.76 (0.48–1.19)	0.89 (0.52–1.51)
P for trend		0.16	0.44
Vitamin B-6, mg/day			
0.19–1.92	44/139,469	1.00	1.00
1.93–3.27	40/140,884	0.89 (0.58–1.36)	1.25 (0.76–2.05)
3.28–457.12	39/138,662	0.88 (0.57–1.35)	1.97 (1.08–3.62)
P for trend		0.62	0.03
Vitamin B-12, μg/day			
0.03–7.17	41/139,753	1.00	1.00
7.18–14.66	45/139,822	1.09 (0.72–1.67)	1.38 (0.88–2.16)
14.67–1,553.37	37/139,440	0.90 (0.58–1.40)	1.29 (0.78–2.14)
P for trend		0.60	0.35
Methionine, g/day			
0.18–1.49	47/140,432	1.00	1.00
1.50–2.04	39/137,982	0.85 (0.56–1.31)	0.98 (0.59–1.63)
2.05–10.57	37/140,600	0.80 (0.52–1.24)	1.03 (0.53–1.99)
P for trend		0.33	0.92
Alcohol, g/day			
<20	116/400,867	1.00	1.00
≥20	7/18,147	1.39 (0.65–2.97)	0.91 (0.39–2.10)

a: Adjusted for age only.
b: Adjusted for age, pack-years of cigarettes (none, 1–19, 20–39, or ≥40), oral contraceptive use (never, ever), and intakes of calcium, vitamin E, and energy (quintiles).

intake (RR = 0.44, 95% CI = 0.22–0.89) than among those with low folate and high alcohol intake. For all statistical tests for interactions, $P > 0.05$.

Discussion

We hypothesized that low intake of nutrients involved in DNA methylation (folate, methionine, and vitamins B-6 and B-12) would increase risk of colon and rectal cancers. Furthermore, we hypothesized that the association of folate with these cancers would vary by intake of alcohol, methionine, and vitamins B-6 and B-12. The results from this cohort study of older women provide limited support for these hypotheses.

We found intake of folate to be unrelated to colon and rectal cancers overall. These findings contribute to a conflicting literature regarding the association of folate to cancers of the colon and rectum. Although most studies have found some evidence of an inverse association between fo-

Table 3. RRs of Colon Cancer According to Level of Folate Intake, Stratified by Methionine, Vitamin B-6, Vitamin B-12, or Alcohol Intake in the IWHS, 1986–98[a]

	Total Colon		Proximal Colon		Distal Colon	
	<350.8 μg folate	≥350.8 μg folate	<350.8 μg folate	≥350.8 μg folate	<350.8 μg folate	≥350.8 μg folate
Vitamin B-6						
<2.41 mg/day	1.00	0.79 (0.55–1.12)	1.00	0.72 (0.43–1.19)	1.00	0.83 (0.51–1.37)
≥2.41 mg/day	0.95 (0.68–1.32)	0.90 (0.68–1.17)	1.04 (0.66–1.62)	0.65 (0.50–0.84)	0.82 (0.50–1.37)	1.13 (0.77–1.65)
Vitamin B-12						
<11.15 μg/day	1.00	0.91 (0.69–1.21)	1.00	0.82 (0.54–1.23)	1.00	0.98 (0.66–1.47)
≥11.15 μg/day	1.02 (0.81–1.29)	0.85 (0.65–1.12)	1.10 (0.79–1.53)	0.59 (0.39–0.89)	0.95 (0.68–1.33)	1.11 (0.77–1.61)
Methionine						
<1.76 g/day	1.00	0.97 (0.72–1.32)	1.00	0.83 (0.53–1.32)	1.00	1.07 (0.71–1.61)
≥1.76 g/day	1.06 (0.80–1.39)	0.85 (0.61–1.17)	1.32 (0.88–1.98)	0.74 (0.46–1.21)	0.84 (0.57–1.24)	0.92 (0.59–1.42)
Alcohol						
≥20 g/day	1.00	0.82 (0.37–1.82)	1.00	0.39 (0.12–1.26)	1.00	1.76 (0.55–5.66)
<20 g/day	0.90 (0.53–1.53)	0.79 (0.45–1.37)	0.65 (0.34–1.26)	0.44 (0.22–0.89)	1.36 (0.55–3.36)	1.43 (0.56–3.63)

a: Values are RRs, adjusted for age, pack-years of cigarettes (none, 0–19, 20–39, or ≥40), body mass index (quintiles), estrogen use (current, past, never), and intakes of calcium, vitamin E, and energy (quintiles), with 95% CIs in parentheses.

Table 4. RR of Rectal Cancer According to Level of Folate Intake, Stratified by Methionine, Vitamin B-6, Vitamin B-12, or Alcohol Intake in the IWHS, 1986–98[a]

	Folate Intake	
	<350.48 μg/day	≥350.48 μg/day
Vitamin B-6, mg/day		
<2.41	1.00	0.44 (0.16–1.24)
≥2.41	1.93 (1.07–3.46)	1.24 (0.71–2.16)
Vitamin B-12, μg/day		
<11.15	1.00	0.72 (0.37–1.40)
≥11.15	1.32 (0.83–2.11)	1.05 (0.59–1.85)
Methionine, g/day		
<1.76	1.00	0.80 (0.42–1.54)
≥1.76	1.12 (0.63–1.97)	0.89 (0.76–1.05)
Alcohol, g/day		
≥20	1.00	0.52 (0.10–2.85)
<20	0.98 (0.35–2.74)	0.61 (0.22–1.74)

a: Values are RRs, adjusted for age, pack-years of cigarettes (none, 0–19, 20–39, or ≥40), oral contraceptive use (never, ever), and intakes of calcium, vitamin E, and energy (quintiles), with 95% CIs in parentheses.

late and cancers and adenomas of the colon and rectum (14–19,21–26,28,30,38) while just two have not (27,29), it is important to note that, for a number of studies, the association was suggestive but not statistically significant (14,16,18,22,23,26,28,38). Also, many of the studies found the protective effect of folate to be limited to specific study population subgroups. For example, Su and Arab (30) found folate intake to be protective against colon cancer among male, but not female, participants in the National Health and Examination Survey Epidemiologic Follow-up Study. In contrast, Slattery et al. (18) found a nonsignificant inverse association between folate intake and colon cancer among women, but not men, in their case-control study. No discernable pattern of findings with respect to age, gender, or cancer site is evident, although it is likely that the association between dietary folate and cancers of the colon and rectum may be limited to certain population groups or cancer locations given the disparate findings across and within studies. In addition, recent studies suggest that genetic factors may influence the importance of folate in colon and rectal cancer development (26,38–41), thus providing another possible explanation for the inconsistent and weak associations reported in the literature.

In our study, vitamin B-12 was unrelated to cancers of the colon and rectum. Findings from previous research examining this association have produced similar results. No evidence of an association between vitamin B-12 and colon cancer was found in a population-based case-control study of men and women reported by Slattery et al. (18). Similarly, no association between vitamin B-12 and colorectal adenomas was found in a population-based case-control study in Spain (25). Benito et al. (24) found an inverse association between vitamin B-12 and cancers of the colon and rectum in a population-based case-control study conducted in Spain; however, the association was not statistically significant.

We found vitamin B-6 to be unrelated to colon cancer, whereas incidence of rectal cancer rose progressively with increasing intake of vitamin B-6, more attributable to vitamin B-6 from supplemental sources than vitamin B-6 from dietary sources. Previous research has found vitamin B-6 to be unrelated to (24,25) or inversely associated with (18) cancers and adenomas of the colon and rectum.

In our study, methionine was unrelated to colon and rectal cancers. Previous research examining the association of methionine with colorectal cancers is limited, providing minimal data for comparison. Results from two prospective studies (the Nurses' Health Study and the Health Professionals' Study) were suggestive of an inverse association between methionine and cancers and adenomas of the colon and rectum, although CIs included 1.0 (13,14). No evidence of an association between methionine and colon cancer was found in a case-control study of men and women (18).

In some cases, combinations of the nutrients involved in DNA methylation were associated with reduced risk of colon cancer. Most notably, RRs of proximal colon cancer were lower among those with 1) both high folate and high vitamin B-12 intake and 2) both high folate and high vitamin B-6 intake than among those with the lowest intake of these nutrients. In addition, risk estimates suggest lower incidence of cancer of the proximal colon among those with high folate and low alcohol intake than among those with low folate and high alcohol intake. For all statistical tests for interaction, $P > 0.05$, suggesting that reductions in risk observed for combinations of nutrients were no greater than would be expected given the independent effects of each nutrient. These findings contribute to a conflicting literature in which some studies have found the association of folate with colorectal cancers and adenomas to be similar across intake levels of alcohol (16,18), while others have found a low-folate and high-alcohol diet to be associated with higher risk of colorectal cancers and adenomas than a high-folate and low-alcohol diet (13,14,17,19,22,29,30).

Interestingly, several other studies have found the location of the cancer in the colon to influence findings with respect to the association of folate with colon cancer relative to alcohol, methionine, vitamin B-6, and vitamin B-12 intake. Consistent with our findings, Slattery et al. (38) found stronger associations between folate, vitamin B-6, and vitamin B-12 and cancer of the proximal colon (relative to the distal colon) among case-control study participants with a specific 5,10-methylenetetrahydrofolate reductase genotype. In contrast, however, Giovannucci et al. (14) found significantly higher risk of distal colon cancer among those with a high-alcohol-low-methionine-low-folate diet than among those with a low-alcohol-high-methionine-high-folate diet, while the risk ratio for proximal colon cancer for this comparison was lower (and not statistically significant).

Several explanations for our predominantly null findings are possible. First, if only very low intakes of folate, methionine, and vitamins B-6 and B-12 lead to disturbances in DNA methylation, it is possible that the "at-risk" group is insufficient within our study cohort. In >80% of women in the

cohort, intake of folate and vitamins B-6 and B-12 exceeded the Recommended Dietary Allowances (42,43) for these nutrients, suggesting that few women consumed very low levels of these nutrients. In addition, alcohol intake was low in our cohort, limiting our ability to examine possible modification of the association of folate and colorectal cancers by high alcohol consumption. Power was limited by the low number of incident rectal cancers, and power was limited for testing interactions. For vitamin B-12, it is possible that dietary intake does not correlate with blood levels of this nutrient, inasmuch as absorption is dependent on the availability of intrinsic factor, and deficiency of this factor is not uncommon in older populations. Measurement error is also of concern, inasmuch as dietary intake was assessed with a single FFQ administered at baseline. Although the FFQ used in the IWHS has been shown to provide reasonably valid intake estimates for folate, vitamin B-6, vitamin B-12, and alcohol (36), measurement error is inherent in retrospective dietary assessment of cancer (44). Use of biomarkers for micronutrient intake such as red blood cell folate would be preferable but not possible in this study, in which blood was not collected from study participants. Finally, it may be that intake of these nutrients earlier in life is more relevant to colorectal cancer than in the later years characterized in this study, where the average age of participants at baseline was 61.7 yr.

In conclusion, findings from this study contribute to an inconsistent body of literature on the association of nutrients involved in DNA methylation with cancers of the colon and rectum. Overall, our results provide limited support for the hypothesis that low intake of nutrients involved in DNA methylation (folate, methionine, and vitamins B-6 and B-12) increases risk of colon and rectal cancers. We found little evidence that the association of folate with risk of cancers of the colon and rectum varies by intake of alcohol, methionine, and vitamins B-6 and B-12. The only statistically significant or suggestive associations found in this study were a positive association between vitamin B-6 and rectal cancer and a lower risk of proximal colon cancer among those with 1) both high folate and high vitamin B-6 intake, 2) both high folate and high vitamin B-12 intake, and 3) both high folate and low alcohol intake. These latter three associations are consistent with our hypotheses; however, given the large number of statistical tests conducted, these findings also could be due to chance.

Acknowledgments and Notes

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