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Dietary Folate, Methionine, Riboflavin, and Vitamin B-6 and Risk of Sporadic Colorectal Cancer[1,2]

Stefan de Vogel,[3,4]* Vasundhara Dindore,[3] Manon van Engeland,[4] R. Alexandra Goldbohm,[5] Piet A. van den Brandt,[3] and Matty P. Weijenberg[3]

Departments of [3]Epidemiology and [4]Pathology, GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands and [5]TNO Quality of Life, Department of Prevention and Health, 2301CE, Leiden, The Netherlands

Abstract

Adequate intake of folate, methionine, riboflavin, and vitamin B-6 may prevent aberrant DNA methylation and thereby protect against colorectal cancer (CRC). However, previous epidemiological studies investigating associations between dietary intakes of these nutrients and CRC have been inconsistent. We investigated the associations between intakes of folate, methionine, riboflavin, and vitamin B-6 and CRC risk, accounting for the sublocalization of the tumor. Within the Netherlands Cohort Study on diet and cancer ($n = 120,852$), 2349 cases and 4168 subcohort members were available for data analyses from a follow-up period of 13.3 y after baseline. Gender-specific adjusted incidence rate ratios (RR) were calculated over quintiles of dietary intake in case-cohort analyses. Folate intake was not associated with CRC risk in either men or women. However, methionine was associated with decreased risk of proximal colon cancer among men (RR = 0.57 for highest vs. lowest quintile of intake; *P*-trend = 0.03) and rectal cancer among women (highest vs. lowest quintile; RR = 0.45; *P*-trend = 0.05). Riboflavin tended to be associated with decreased proximal colon cancer risk among women (RR = 0.61; *P*-trend = 0.07). Conversely, there was a strong positive association between vitamin B-6 and rectal cancer among women (RR = 3.57; *P*-trend = 0.01). Our findings suggest that relatively high methionine intake may protect against proximal colon cancer in men and rectal cancer in women but that folate may not have a protective effect. This is the 2nd prospective cohort study in which vitamin B-6 intake was associated with increased risk of rectal tumors in women, which might suggest that this vitamin enhances rectal cancer in women. J. Nutr. 138: 2372–2378, 2008.

Introduction

It has been proposed that folate may affect colorectal carcinogenesis because of its role in the synthesis of nucleic acid and DNA methylation (1). Folate deficiency may result in uracil misincorporation in DNA, which possibly leads to DNA instability and gene alterations. It may also cause aberrant DNA methylation such as CpG island promoter hypermethylation or global hypomethylation, which, in turn, may contribute to colorectal carcinogenesis. For example, hypermethylation of gene promoters is known to result in silencing of tumor suppressor genes and DNA repair genes, which in turn may enhance carcinogenesis (2). Therefore, it may be hypothesized that adequate folate levels possibly protect against colorectal cancer (CRC).[7] However, epidemiological studies on the relation between dietary folate intake and CRC risk have not consistently shown a protective effect of high folate intake (3,4). Also, it has been suggested that the effect of folate supplementation may depend on the stage of colorectal carcinogenesis, i.e. it would protect against carcinogenesis in normal colorectal tissue but might enhance already existing lesions (5,6). In this respect, the relation between dietary folate and the etiology of CRC continues to be the subject of debate.

Like folate, methionine is required for the synthesis of *S*-adenosylmethionine, which is the universal methyl group donor needed for methylation processes (7). Adequate intakes of folate or methionine through the diet ensure a sufficient supply of methyl groups and it may be hypothesized that this also prevents aberrant DNA methylation. Other B-vitamins such as riboflavin and vitamin B-6 are involved in the folate-mediated 1-carbon metabolism and may therefore modulate the bioavailability of methyl groups. Riboflavin, as flavin adenine dinucleotide, is the cofactor for methylenetetrahydrofolate reductase (MTHFR), the enzyme that influences homocysteine remethylation and DNA methylation. Low riboflavin status was previously

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[7] Abbreviations used: CRC, colorectal cancer; MTHFR, methylenetetrahydrofolate reductase; NLCS, Netherlands cohort study on diet and cancer; RR, incidence rate ratio.
* To whom correspondence should be addressed. E-mail: stefan.devogel@epid.unimaas.nl.

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observed to be associated with increased homocysteine concentration, which possibly results in lower availability of methyl groups (8). In addition, vitamin B-6 is involved in the conversion of tetrahydrofolate into 5,10-methylenetetrahydrofolate, one of the steps of the folate cycle (7). On the other hand, high alcohol intake potentially reduces the bioavailability of folate and such a disruption of the 1-carbon metabolism may affect DNA methylation in, for example, the colonic mucosa (9–11).

The associations between dietary intakes of riboflavin, vitamin B-6, and methionine have been investigated in a number of studies. However, whereas a potential protective effect of riboflavin intake on colorectal adenomas was observed in 1 study (12), others have not demonstrated an association with CRC at all (13,14). Similarly, relatively high methionine intake did not seem to protect against CRC (13,15–17). Several studies demonstrated an inverse association between vitamin B-6 intake and CRC (17–21), although in 1 study, the vitamin was positively associated with rectal tumors among women (16). There may be a number of reasons for these inconsistencies between studies, such as the danger of selection bias and possibly recall bias that could have played a larger role in case-control studies. In this respect, some of the studies had a prospective cohort design (15,16,18–21), whereas others were case-control studies (12–14,17). Moreover, some studies were conducted among women only (15,16,19–21) or in relation to colorectal adenomas (12) and there are considerable differences in the level of vitamin intake between studies.

The relation between folate and CRC is still controversial and previous studies on associations between folate, B-vitamins, and methionine with CRC have inconsistencies. This prompted us to investigate the combination of folate, riboflavin, vitamin B-6, and methionine in a prospective setting. The study was conducted among incident CRC patients and subcohort members of the Netherlands Cohort Study on diet and cancer (NLCS), which is a large population-based study with a prospective case-cohort design.

Subjects and Methods

Study population. The participants in this study were incident colon and rectal cancer patients and subcohort members from the NLCS, which has been described in detail elsewhere (22). Briefly, the study was initiated in September 1986 and included 58,279 men and 62,573 women aged 55–69 y at baseline, who originated from 204 Dutch municipalities with computerized population registers. At baseline, participants completed a self-administered FFQ that also provided information about age, sex, and other risk factors for cancer. The entire cohort is being monitored for cancer occurrence by annual record linkage to the Netherlands Cancer Registry (9 cancer registries in The Netherlands). Accumulation of person-time in the cohort has been estimated through biennial follow-ups of vital status in a subcohort of 5000 men and women who were randomly selected after baseline exposure measurement. Cases with prevalent cancer other than nonmelanoma skin cancer were excluded from this subcohort, which left 4774 men and women eligible for analysis. CRC was classified according to the International Classification of Diseases (ICD) as follows: colon, i.e. proximal colon (ICD-O-1 codes 153.0, 153.1, 153.4, 153.5, 153.6) and distal colon (153.2, 153.3, 153.7), rectosigmoid (154.0), rectum (154.1), or ICD-O-1 codes 153.8 and 153.9 if information of the disease site was not available. Follow-up information was available from a follow-up period up to 13.3 y after baseline, i.e. from September 1986 until January 2000. Within this period, 2679 CRC patients were identified.

FFQ. The self-administered questionnaire was a 150-item, semiquantitative FFQ, which concentrated on habitual consumption of food and beverages during the year preceding the start of the study and also contained questions about body weight and length, smoking status, physical activity, and family history of CRC. Daily mean nutrient intakes were calculated as the cumulated product of the frequencies and portion sizes of all food items and their tabulated nutrient contents from the Dutch Food Composition Table (NEVO table, 1986) (23). The questionnaire was validated through comparison with a 9-d diet record (24) and the reproducibility was determined (25). Questionnaire data were key-entered twice for all incident cases in the cohort and for all subcohort members in a blinded manner with respect to case/subcohort status. This was done to minimize observer bias in coding and interpretation of the data.

Folate data were derived from a validated liquid chromatography trienzyme method (26) used to analyze the 125 most important Dutch foods contributing to folate intake (27). Mean daily intakes of all other relevant nutrients were calculated using the computerized Dutch Food Composition Table (23). Dietary supplement data were also obtained via the FFQ. However, the use of B-vitamin supplements was low (7%) and folic acid was generally not included in these supplements in The Netherlands in the late 1980s. Therefore, folic acid supplement use most likely plays a very minor role in our study population and supplement use was not further accounted for in the analyses.

Statistical analyses. Analyses were performed for all CRC cases combined and for each sublocalization, i.e. proximal colon, distal colon, rectosigmoid, and rectum. Dietary factors and other baseline characteristics were evaluated for men and women separately for subcohort members and CRC cases by calculating means and SD for continuous variables and distributions of the categorical variables.

Cox proportional hazards regression models were used to estimate gender-specific multivariate-adjusted incidence rate ratios (RR) and corresponding 95% CI over quintiles of folate intake, methionine, riboflavin, and vitamin B-6 using the lowest quintiles as reference. Standard errors of the RR were estimated using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort (28). Tests for dose response trends over the quintiles of intake were estimated by fitting the ordinal exposure variables as continuous variables and evaluated using the Wald test. To account for potentially nonlinear effects due to skewed distributions of folate, methionine, riboflavin, and vitamin B-6, we also estimated the associations between the quintile variables and CRC by replacing the quintiles with the median intakes within each quintile and compared those RR with the main analyses. The proportional hazards assumption was tested using the scaled Schoenfeld residuals (29) and by fitting the main determinants as time-dependent variables. Analyses were additionally stratified for the first and 2nd half of the follow-up time. Because undetected preclinical disease may have affected exposure status, we excluded the first year of follow-up in additional subanalyses and compared these results with the analyses performed over the full 13.3 y of follow-up. In addition to gender-specific analyses, we also conducted overall analyses for men and women combined.

The dietary variables were adjusted for total energy intake by calculating nutrient residuals from the regression of nutrient intake on total energy intake, as described by Willett et al. (30). Such nutrient residuals are uncorrelated with total energy intake and the effect of the variation in nutrient intake can subsequently be estimated independently of a potential effect of energy intake. Tests for heterogeneity were performed to evaluate differences between sublocalizations of tumors using the competing risks procedure in Stata. However, the standard error for the difference of the log hazard ratio from this procedure assumes independence of both estimated hazard ratios, which would underestimate that SE and thus overestimate the *P*-values for their difference. Therefore, these *P*-values and the associated CI were estimated based on a bootstrapping method that was developed for the case-cohort design (31). For each bootstrap sample, X subcohort members were randomly drawn from the subcohort of X subjects and Y cases from the total of Y cases outside the subcohort, both with replacement, out of the dataset of X + Y observations. The log hazard ratios were obtained from this sample using Stata's competing risks procedure and recalculated for each bootstrap replication. The CI and *P*-value of the differences in hazard ratio of the subtypes were then calculated from the replicated statistics. Each bootstrap analysis was based on 1000 replications.

TABLE 1 Baseline dietary intake and other characteristics of subcohort members and cancer cases from the NLCS[1]

	Men						Women					
	Subcohort	All tumors	Proximal colon	Distal colon	Rectosigmoid	Rectum	Subcohort	All tumors	Proximal colon	Distal colon	Rectosigmoid	Rectum
Patient characteristics												
n^2	2090	1389	382	467	141	360	2078	960	386	296	78	176
Age, *y*	61.3 ± 4.2	62.0 ± 4.1	62.5 ± 4.0	62.0 ± 4.2	62.0 ± 3.9	61.6 ± 4.0	61.4 ± 4.3	62.6 ± 4.0	62.9 ± 4.0	62.1 ± 4.1	62.8 ± 3.6	62.4 ± 3.8
Family history of CRC, % yes	5.4	9.3	10.0	9.4	10.6	8.1	6.0	9.8	10.9	9.1	7.7	9.1
BMI, kg/m^2	24.9 ± 2.6	25.2 ± 2.6	25.1 ± 2.6	25.4 ± 2.8	25.6 ± 2.6	25.1 ± 2.5	25.0 ± 3.5	25.1 ± 3.5	25.3 ± 3.5	25.0 ± 3.4	25.1 ± 3.2	25.0 ± 3.6
Smoking status, %												
Never	12.8	11.0	11.5	12.0	9.9	9.4	57.6	59.8	61.4	62.1	51.3	58.5
Ex-smoker	52.6	59.9	57.3	64.5	58.2	57.5	20.8	21.2	20.7	21.0	28.2	19.3
Current smoker	35.6	29.1	31.1	23.5	31.9	33.1	21.6	19.0	17.9	16.9	20.5	22.2
Dietary factors												
Folate, *μg/d*	224.6 ± 66.6	222.1 ± 65.0	222.0 ± 68.1	217.4 ± 54.1	227.7 ± 80.5	225.9 ± 65.7	198.5 ± 61.1	199.1 ± 61.3	196.4 ± 58.2	199.4 ± 62.4	196.5 ± 51.7	204.8 ± 67.7
Methionine, *mg/d*	1713 ± 293	1701 ± 277	1696 ± 280	1697 ± 2.70	1714 ± 287	1710 ± 2.71	1489 ± 276	1482 ± 278	1474 ± 2.98	1483 ± 251	1450 ± 250	1512 ± 279
Riboflavin, *mg/d*	1.58 ± 0.37	1.55 ± 0.34	1.56 ± 0.35	1.54 ± 0.32	1.56 ± 0.34	1.55 ± 0.34	1.45 ± 0.34	1.43 ± 0.35	1.43 ± 0.35	1.46 ± 0.34	1.33 ± 0.30	1.48 ± 0.37
Vitamin B-6, *mg/d*	1.54 ± 0.27	1.55 ± 0.27	1.54 ± 0.27	1.53 ± 0.27	1.55 ± 0.25	1.57 ± 0.29	1.33 ± 0.24	1.33 ± 0.24	1.33 ± 0.25	1.32 ± 0.23	1.33 ± 0.21	1.36 ± 0.25
Alcohol, %												
0 g/d	14.6	12.4	12.0	14.5	9.9	11.1	32.3	33.8	34.7	33.8	29.5	34.1
<30 g/d	70.6	69.7	74.6	67.5	73.8	67.2	64.1	61.4	59.8	61.8	61.1	63.1
≥30 g/d	14.8	17.9	13.4	18.0	16.3	21.7	3.6	4.8	5.5	4.4	6.4	2.8
Energy, *kJ/d*	9080 ± 2134	8949 ± 2059	8923 ± 2006	8846 ± 2135	8841 ± 1946	9149 ± 2008	7062 ± 1659	6980 ± 1608	6994 ± 1698	6933 ± 1541	7071 ± 1944	6991 ± 1332
Meat, *g/d*	137 ± 52	136 ± 49.7	134 ± 49	136 ± 49	137 ± 53	137 ± 50	116 ± 46	115 ± 45	112 ± 46	115 ± 42	123 ± 40	117 ± 47
Fat, *g/d*	93.9 ± 14.2	94.0 ± 13.6	95.0 ± 12.4	94.8 ± 14.0	93.7 ± 14.3	92.2 ± 14.1	74.0 ± 10.3	74.1 ± 10.3	73.6 ± 10.8	74.5 ± 10.0	75.4 ± 8.6	74.0 ± 10.3
Fiber, *g/d*	28.7 ± 7.3	28.8 ± 7.0	28.6 ± 7.1	28.4 ± 6.6	28.9 ± 6.9	29.4 ± 7.1	25.3 ± 5.8	25.1 ± 5.9	25.2 ± 6.2	24.9 ± 5.5	24.8 ± 6.0	25.4 ± 5.8
Calcium, *mg/d*	948 ± 295	932 ± 281	947 ± 286	919 ± 267	932 ± 2.96	944 ± 287	901 ± 268	896 ± 274	898 ± 2.93	909 ± 2.54	804 ± 218	913 ± 2.81
Iron, *mg/d*	13.3 ± 2.4	13.3 ± 2.4	13.2 ± 2.26	13.3 ± 2.3	13.5 ± 2.5	13.5 ± 2.4	11.7 ± 2.0	11.6 ± 1.9	11.6 ± 2.0	11.5 ± 1.9	11.6 ± 1.8	11.8 ± 1.9
Vitamin C, *mg/d*	98.6 ± 41.6	99.7 ± 40.6	98.8 ± 40.5	96.7 ± 38.6	99.5 ± 40.3	103.1 ± 42.6	108.4 ± 42.7	109.3 ± 45.5	108.0 ± 46.1	109.9 ± 44.4	110.4 ± 36.5	109.5 ± 48.4
Fruits, *g/d*	155 ± 114	160 ± 112	158 ± 107	158 ± 107	161 ± 121	166 ± 120	197 ± 121	195 ± 83	194 ± 134	195 ± 117	201 ± 98	191 ± 123
Vegetables, *g/d*	191 ± 85	192 ± 86	191 ± 91	185 ± 76	192 ± 73	205 ± 95	196 ± 82	194 ± 83	193 ± 83	191 ± 77	206 ± 85	200 ± 88

[1] Values are mean ± SD or percentages if indicated otherwise.
[2] Numbers of subcohort members and patients are based on complete availability of the variables presented in this table. Information about the tumor localization was unavailable for 39 men and 22 women.

First, we estimated the individual associations of dietary folate, methionine, riboflavin, and vitamin B-6 with CRC in separate models. However, because the bioavailability of methyl groups donated by folate and methionine may be influenced by riboflavin and vitamin B-6, the effects of these nutrients may not be independent and should then be mutually adjusted for. There were no major differences between the associations of these individual models compared with the multivariate analysis including folate, methionine, riboflavin, and vitamin B-6 simultaneously, and these main determinants were therefore included simultaneously in all further analyses.

The covariates considered as potential confounders were suggested risk factors of CRC and those commonly included as confounders in observational studies on the associations of B-vitamins and CRC (12–21). Covariates were included when they substantially influenced the RR of any of the main determinants folate, methionine, riboflavin or vitamin B-6. This applied to the variables BMI, smoking status, energy, calcium and meat (RR of any of the main determinants changed by more than 5% when adding each of these variables separately) and alcohol, iron, fat, fiber (RR of main determinants changed even more than 10%). The variables age and having a family history of CRC may be strong determinants for CRC and were therefore included as well.

We also conducted analyses using models only including the 4 main determinants and the variable age and compared the results of these crude results to the multivariate-adjusted analyses. After excluding subjects with missing information on these covariates or those who did not complete the questionnaire, 4168 subcohort members and 2349 CRC cases remained for statistical analyses.

We determined possible interactions between dietary intakes of folate, methionine, riboflavin, vitamin B-6, and alcohol intake. This was done by first testing, in separate models, the gender-specific interaction terms between folate on the one hand, with methionine, riboflavin, vitamin B-6, and alcohol on the other, for all CRC tumors combined and for each of the 4 sublocalizations. The Cox proportional hazard analyses without the interaction terms were subsequently stratified by low or high intake of folate, methionine, riboflavin, and vitamin B-6 using the median intakes as cut-off values to define both strata within each variable. The strata used for alcohol intake were abstainers, subjects with intake <30 g/d, and subjects who consumed ≥30 g/d.

All statistical analyses were performed with the Stata statistical software package (version 9.1).

Results

During a period of 13.3 y of follow-up, 1389 men and 960 women with CRC were identified who also had complete information on the covariates used in the adjusted regression analyses. The mean intakes of the main determinants folate, methionine, riboflavin, and vitamin B-6 were generally similar between subcohort members and cases in most of the subgroups among both men and women (**Table 1**). Only the intakes of folate among men with a distal colon tumor and of riboflavin among women with a tumor in the rectosigmoid appeared slightly lower compared with subcohort members. The percentages of subjects with a family history of CRC were generally higher in cases than in subcohort members. The other variables did not differ between subcohort members and CRC patients.

We subsequently estimated multivariate-adjusted incident RR over quintiles of folate intake, methionine, riboflavin, and vitamin B-6 for men and women.

TABLE 2 Associations between dietary folate, methionine, riboflavin, and vitamin B-6 with CRC risk in men

		All tumors			Proximal colon		Distal colon		Rectosigmoid		Rectum	
Quintile[1]	PY[2]	*n*[3]	RR (95% CI)[4]	RR (95% CI)[5]	*n*	RR (95% CI)[5]	*n*	RR (95% CI)[5]	*n*	RR (95% CI)[5]	*n*	RR (95% CI)[5]
Folate, μg/d												
1 (160.8)	4840	292	1.00	1.00	77	1.00	111	1.00	29	1.00	65	1.00
2 (189.5)	4971	289	0.93 (0.74–1.17)	0.90 (0.71–1.13)	94	1.14 (0.80–1.61)	88	0.69 (0.50–0.97)	22	0.67 (0.37–1.22)	76	1.05 (0.72–1.55)
3 (212.1)	4848	271	0.88 (0.70–1.13)	0.85 (0.66–1.09)	61	0.77 (0.51–1.16)	94	0.72 (0.50–1.04)	37	1.18 (0.67–2.08)	74	1.03 (0.68–1.55)
4 (240.6)	4822	270	0.89 (0.69–1.14)	0.86 (0.66–1.11)	80	1.02 (0.68–1.51)	88	0.69 (0.47–1.01)	25	0.78 (0.40–1.50)	70	1.00 (0.64–1.56)
5 (297.2)	4829	267	0.93 (0.71–1.20)	0.87 (0.65–1.15)	70	0.97 (0.62–1.52)	86	0.71 (0.46–1.08)	28	0.83 (0.41–1.66)	75	1.01 (0.64–1.60)
P-trend[6]			0.43	0.29		0.65		0.16		0.72		0.92
Methionine, *mg/d*												
1 (1366)	4960	296	1.00	1.00	77	1.00	104	1.00	31	1.00	72	1.00
2 (1555)	4805	284	1.01 (0.80–1.28)	0.96 (0.74–1.26)	91	1.07 (0.70–1.64)	86	0.91 (0.62–1.33)	26	0.83 (0.43–1.58)	71	1.03 (0.66–1.60)
3 (1698)	4971	277	0.96 (0.75–1.22)	0.87 (0.64–1.17)	83	0.87 (0.54–1.41)	91	0.89 (0.58–1.37)	30	0.81 (0.37–1.74)	68	0.98 (0.60–1.61)
4 (1843)	4803	282	0.98 (0.77–1.27)	0.90 (0.63–1.26)	65	0.65 (0.37–1.14)	105	1.13 (0.69–1.87)	26	0.65 (0.28–1.50)	79	1.21 (0.69–2.11)
5 (2093)	4772	250	0.91 (0.68–1.22)	0.79 (0.50–1.25)	66	0.57 (0.28–1.18)	81	1.03 (0.52–2.04)	28	0.65 (0.22–1.93)	70	1.09 (0.53–2.24)
P-trend			0.47	0.36		0.03		0.58		0.34		0.53
Riboflavin, *mg/d*												
1 (1.17)	4944	315	1.00	1.00	82	1.00	93	1.00	35	1.00	89	1.00
2 (1.38)	4968	265	0.82 (0.66–1.03)	0.83 (0.65–1.05)	72	0.82 (0.56–1.20)	94	1.13 (0.80–1.60)	27	0.68 (0.38–1.20)	64	0.67 (0.45–0.99)
3 (1.53)	4815	284	0.91 (0.72–1.15)	0.92 (0.71–1.19)	78	0.91 (0.61–1.35)	108	1.44 (0.98–2.11)	22	0.56 (0.29–1.08)	71	0.73 (0.48–1.11)
4 (1.71)	4681	270	0.90 (0.70–1.16)	0.93 (0.69–1.24)	75	0.96 (0.60–1.52)	98	1.47 (0.95–2.29)	28	0.74 (0.38–1.43)	64	0.65 (0.40–1.03)
5 (2.03)	4903	255	0.83 (0.63–1.08)	0.86 (0.60–1.22)	75	0.91 (0.53–1.58)	74	1.22 (0.71–2.08)	29	0.72 (0.30–1.74)	72	0.66 (0.38–1.14)
P-trend			0.37	0.72		0.92		0.19		0.52		0.16
Vitamin B-6, *mg/d*												
1 (1.22)	4838	257	1.00	1.00	70	1.00	94	1.00	25	1.00	59	1.00
2 (1.40)	4766	272	1.12 (0.89–1.40)	1.12 (0.87–1.43)	77	1.17 (0.80–1.71)	93	1.01 (0.71–1.45)	26	1.09 (0.59–2.04)	69	1.22 (0.81–1.82)
3 (1.53)	4959	283	1.14 (0.89–1.45)	1.13 (0.86–1.49)	82	1.23 (0.81–1.87)	93	0.98 (0.66–1.45)	26	1.09 (0.55–2.18)	78	1.32 (0.84–2.07)
4 (1.67)	4884	302	1.27 (0.99–1.63)	1.30 (0.97–1.75)	76	1.29 (0.81–2.06)	103	1.17 (0.76–1.79)	37	1.62 (0.78–3.36)	76	1.31 (0.81–2.12)
5 (1.88)	4863	275	1.18 (0.90–1.56)	1.29 (0.90–1.84)	77	1.50 (0.86–2.62)	84	1.03 (0.60–1.76)	27	1.28 (0.51–3.24)	78	1.35 (0.76–2.41)
P-trend			0.14	0.12		0.14		0.87		0.32		0.31

[1] Quintile (median intake within quintile).
[2] Number of accumulated person years (PY) within quintiles of dietary intake.
[3] Number of cases.
[4] Unadjusted incidence RR from a regression model including the variables folate, methionine, riboflavin, vitamin B-6, and age.
[5] Adjusted RR from a regression model including the variables folate, methionine, riboflavin, vitamin B-6, age, family history of CRC, BMI, smoking status, and the intakes of alcohol, energy, meat, fat, fiber, calcium, and iron.
[6] *P*-value for linear trend over quintiles of intake.

After 13.3 y of follow-up, folate intake was not significantly associated with risk of CRC at any of the subsites in men (**Table 2**) or women (**Table 3**). Among men, methionine was associated with a decreased risk of proximal colon cancer (RR = 0.57; CI = 0.28–1.18; *P*-trend = 0.03), whereas among women, it was inversely associated with rectal cancer (RR = 0.45; CI = 0.17–1.20; *P*-trend = 0.05). Among women, riboflavin tended to be inversely associated with proximal colon cancer (RR for the highest vs. the lowest quintile of intake = 0.61; CI = 0.35–1.06; *P*-trend = 0.07). Conversely, there was a strong positive association between vitamin B-6 and rectal cancer risk in women (RR = 3.57; CI = 1.56–8.17; *P*-trend = 0.01). An increased RR for vitamin B-6 could also be observed for all CRC tumors combined among women, although this risk was not as high and was not significant (RR = 1.39; CI = 0.92–2.08; *P*-trend = 0.09). When performing the analyses for men and women together, the highest quintile of vitamin B-6 intake was also positively associated with overall CRC (RR = 1.34; CI = 1.03–1.74; *P*-trend = 0.02), with proximal colon cancer (RR = 1.37; CI = 0.93–2.01; *P*-trend = 0.05) and rectal cancer (RR = 1.86; CI = 1.17–2.95; *P*-trend = 0.02, data not shown).

Adjustment for potential confounders only slightly aggravated the estimated associations compared with age-adjusted analyses but did not result in different conclusions (Tables 2 and 3). There were no significant interactions between folate, methionine, riboflavin, or vitamin B-6 and alcohol intake for overall CRC or for the individual sublocalizations. In addition, we did not observe consistent differences when comparing the RR between strata of low or high intakes of folate, methionine, riboflavin, and vitamin B-6 or alcohol (data not shown).

Although the adjusted models did not meet the proportional hazards assumption according to the Schoenfeld residuals, fitting the main determinants as time-dependent variables showed that there was no interaction with time for these variables. Furthermore, when stratifying the analysis based on follow-up time, e.g. on the first and 2nd half of the follow-up period, we observed no consistent differences compared with analyses on the total follow-up period. This also applied to the results when excluding the first year of follow-up. Estimating the relative risks with the median intake within quintiles only slightly changed the *P*-values for dose-response trends and did not result in different conclusions. The tests for heterogeneity for the effects over the 4 subgroups of tumors were not significant.

Discussion

We investigated the associations between intakes of folate, methionine, riboflavin, and vitamin B-6 with CRC risk. Our

TABLE 3 Associations between dietary folate, methionine, riboflavin, and vitamin B-6 with CRC risk in women

		All tumors			Proximal colon		Distal colon		Rectosigmoid		Rectum	
Quintile[1]	PY[2]	*n*[3]	RR (95% CI)[4]	RR (95% CI)[5]	*n*	RR (95% CI)[5]	*n*	RR (95% CI)[5]	*n*	RR (95% CI)[5]	*n*	RR (95% CI)[5]
Folate, *μg/d*												
1 (139.0)	5097	198	1.00	1.00	89	1.00	56	1.00	16	1.00	32	1.00
2 (165.6)	5121	185	0.96 (0.75–1.25)	0.98 (0.75–1.28)	66	0.79 (0.54–1.15)	65	1.19 (0.79–1.80)	15	1.13 (0.48–2.66)	34	1.03 (0.60–1.77)
3 (187.5)	5313	180	0.90 (0.69–1.18)	0.95 (0.71–1.27)	71	0.83 (0.55–1.24)	59	1.11 (0.70–1.75)	14	1.06 (0.42–2.66)	31	0.90 (0.49–1.65)
4 (212.9)	5156	200	1.11 (0.84–1.46)	1.17 (0.87–1.58)	79	1.07 (0.71–1.63)	59	1.21 (0.76–1.94)	19	1.62 (0.67–3.94)	40	1.18 (0.65–2.14)
5 (267.3)	5119	197	1.12 (0.82–1.52)	1.25 (0.89–1.76)	81	1.24 (0.76–2.02)	57	1.34 (0.81–2.22)	14	1.38 (0.51–3.75)	39	1.06 (0.53–2.11)
P-trend[6]			0.31	0.10		0.17		0.37		0.28		0.73
Methionine, *mg/d*												
1 (1154)	5183	206	1.00	1.00	86	1.00	59	1.00	17	1.00	38	1.00
2 (1351)	5130	214	1.07 (0.84–1.38)	1.02 (0.76–1.36)	87	1.20 (0.80–1.82)	67	0.92 (0.59–1.45)	18	1.38 (0.61–3.15)	36	0.77 (0.43–1.38)
3 (1476)	5081	164	0.83 (0.63–1.10)	0.78 (0.56–1.10)	68	1.01 (0.62–1.65)	53	0.72 (0.42–1.22)	13	1.18 (0.43–3.24)	29	0.54 (0.27–1.05)
4 (1617)	5257	194	0.96 (0.73–1.28)	0.87 (0.59–1.29)	74	1.09 (0.61–1.92)	66	0.80 (0.44–1.45)	17	1.77 (0.56–5.56)	33	0.51 (0.24–1.08)
5 (1841)	5155	182	0.88 (0.65–1.21)	0.76 (0.46–1.26)	71	1.07 (0.51–2.22)	51	0.53 (0.24–1.19)	13	1.91 (0.52–6.92)	40	0.45 (0.17–1.20)
P-trend			0.30	0.18		0.86		0.16		0.31		0.05
Riboflavin, *mg/d*												
1 (1.04)	5124	211	1.00	1.00	99	1.00	51	1.00	25	1.00	33	1.00
2 (1.26)	5067	194	0.91 (0.71–1.17)	0.89 (0.68–1.17)	79	0.74 (0.51–1.06)	57	1.12 (0.71–1.77)	17	0.81 (0.40–1.65)	35	0.99 (0.57–1.72)
3 (1.42)	5130	188	0.92 (0.71–1.19)	0.92 (0.69–1.23)	62	0.62 (0.41–0.93)	73	1.53 (0.95–2.46)	12	0.65 (0.28–1.51)	34	0.99 (0.55–1.79)
4 (1.59)	5294	187	0.85 (0.65–1.11)	0.84 (0.60–1.16)	71	0.61 (0.38–0.96)	60	1.24 (0.72–2.12)	16	0.86 (0.35–2.07)	34	0.91 (0.47–1.74)
5 (1.89)	5190	180	0.85 (0.63–1.14)	0.79 (0.53–1.18)	75	0.61 (0.35–1.06)	55	1.22 (0.62–2.40)	8	0.55 (0.15–2.03)	40	0.92 (0.41–2.04)
P-trend			0.26	0.30		0.07		0.40		0.47		0.76
Vitamin B-6, *mg/d*												
1 (1.05)	5018	198	1.00	1.00	82	1.00	65	1.00	17	1.00	27	1.00
2 (1.21)	5278	189	0.98 (0.76–1.26)	1.04 (0.78–1.37)	75	1.02 (0.69–1.51)	61	0.90 (0.58–1.38)	10	0.67 (0.26–1.73)	38	1.73 (0.97–3.08)
3 (1.32)	5187	178	1.01 (0.75–1.34)	1.11 (0.80–1.52)	73	1.17 (0.75–1.82)	53	0.84 (0.50–1.39)	16	1.16 (0.43–3.11)	32	1.79 (0.91–3.54)
4 (1.44)	5324	194	1.08 (0.81–1.44)	1.23 (0.88–1.73)	85	1.35 (0.84–2.15)	60	0.97 (0.57–1.65)	18	1.31 (0.48–3.54)	28	1.73 (0.83–3.58)
5 (1.63)	4998	201	1.12 (0.81–1.55)	1.39 (0.92–2.08)	71	1.15 (0.65–2.04)	57	1.06 (0.56–2.03)	17	1.34 (0.38–4.64)	51	3.57 (1.56–8.17)
P-trend			0.42	0.09		0.33		0.86		0.36		0.01

[1] Quintile (median intake within quintile).
[2] Number of accumulated person years (PY) within quintiles of dietary intake.
[3] Number of cases.
[4] Unadjusted incidence RR from a regression model including the variables folate, methionine, riboflavin, vitamin B-6, and age.
[5] Adjusted RR from a regression model including the variables folate, methionine, riboflavin, vitamin B-6, age, family history of colorectal cancer, BMI, smoking status, and the intakes of alcohol, energy, meat, fat, fiber, calcium, and iron.
[6] *P*-value for linear trend over quintiles of intake.

results do not suggest an association between dietary folate and CRC. However, relatively high methionine intake was inversely associated with proximal colon cancer in men and rectal cancer in women. Also, a modest nonsignificant inverse association was observed between riboflavin intake and tumors of the proximal colon in women. Conversely, vitamin B-6 intake was positively associated with overall CRC, which was particularly strong for rectal cancer among women.

Previous observational studies did not consistently point to a clear inverse association between folate intake and CRC risk. Next to different sample sizes in those studies, this inconsistency may also have been caused by possible selection bias that is likely to occur in case-control studies but not in cohort studies, potential differences in the level of over- or underestimation of dietary intake using FFQ, and different adjustments for confounding factors between studies (3). In addition, the inconsistencies may be due to differences between study populations and inherent variation in levels of folate intake. The intake in our study was relatively low, with the median folate intake within quintiles ranging from 160 to 299 μg/d among men and 137 to 270 μg/d among women. However, among subjects with considerably higher levels of intake studied in prospective cohort settings, an inverse association was observed only twice (32,33), whereas 3 other studies did not suggest this (15,16,34). Moreover, a significant inverse association was observed in a cohort study among men with even lower intakes compared with men in our study (compared highest and lowest category >249 vs. <103 μg/d) (35), indicating that high ranges of intake do not necessarily lead to reduced CRC risk. Nevertheless, in several studies, the protective effect of folate was confined to either men or women or limited to a specific study population or subsite of CRC, which makes it difficult to generalize the results and hence to be conclusive about whether dietary folate has a chemopreventive effect on CRC.

Despite the current view of the importance of folate in cancer prevention, its role in colorectal carcinogenesis is still a subject of debate. Interestingly, it was recently observed that high plasma folate levels may be associated with increased CRC risk (36) and possibly with breast cancer (37). An alternative hypothesis in this regard is that although folate supplementation may protect against the occurrence of neoplasia in normal colorectal epithelium, it might promote already-existing early lesions into cancer, suggesting that the effect depends on the timing of intervention (5). It was also reported that folic acid supplementation was associated with increased risk of advanced lesions or recurrence of multiple adenomas after a follow-up of 3–5 y (38). Interestingly, the participants in that intervention study were people with a recent history of colorectal adenomas,

and, possibly, undetected neoplasms were present in these subjects, which may have had a growth advantage in the presence of high concentrations of folic acid. Furthermore, after the introduction of nationwide fortification of cereals with folic acid in the United States and Canada, CRC incidence increased in these countries, which may possibly be due to increased intakes folic acid (39). A potentially harmful effect of folate may also be confined to specific subgroups of cancers based on their molecular characteristics. In this respect, we previously observed positive associations between folate intake and colorectal carcinomas harboring mutations in the key genes *APC* or *BRAF* among men (40,41), indicating that folate may result in a growth advantage of such tumors.

We observed that a relatively high intake of methionine may be inversely associated with proximal colon cancer in men and rectal cancer in women. Previous studies examining the role of methionine intake did not demonstrate an individual inverse association with CRC risk (13,15–17), but 1 study showed that low methionine combined with low folate and high alcohol intake may lead to increased CRC risk (34). Although we did not observe an interaction between folate and methionine, our findings suggest that methionine, as a methyl donor, may be more effective in preventing CRC than folate in some of the colorectal sublocalizations. There was also a weak inverse association between riboflavin and proximal colon cancer in women only, whereas in men, riboflavin was unrelated to CRC. Riboflavin is a cofactor for MTHFR and a low intake of the vitamin may reduce the metabolic activity of this enzyme and thereby contribute to colorectal carcinogenesis (8). In addition, the 677C > T polymorphism in *MTHFR* may also affect enzymatic activity and the occurrence of this polymorphism was associated with reduced CRC risk among subjects with adequate folate status (42). It is worthwhile to further explore potential effect modification by such polymorphisms; however, as of today, this information is not yet available for our cohort members.

The reason why an effect of methionine or riboflavin would be site or gender specific remains unclear. Possibly, the development of tumors harboring promoter hypermethylation, which were observed more often in the proximal colon and among women (41,43,44), is more sensitive to these nutrients. We do not have information on global hypomethylation status; however, it is unknown whether the level of global DNA hypomethylation may differ between tumors in different sublocalizations of the colorectum or between men and women.

A remarkable finding in our study was the strong positive association between vitamin B-6 intake and rectal cancer in women, with the RR strongly increased in the highest quintile of intake. Also, when analyzing men and women and all colorectal tumors combined, we observed a modest positive association with the RR increasing evenly over quintiles of intake, suggesting a linear dose-response effect. Meat intake appeared to be the main contributor of vitamin B-6 intake (men, 23.8%; women, 24.4%) and could therefore have accounted for the positive association of vitamin B-6, because meat may also be a risk factor for CRC. However, all analyses were adjusted for meat intake. Interestingly, a positive association with rectal cancer in women has previously been observed in the Iowa women's health population-based cohort study (16), whereas the range of intake among women in that study was considerably higher compared with our study. Notwithstanding this difference, these observations suggest that vitamin B-6 may indeed be associated with increased CRC risk. Although these are observations based on subgroups of patients and thus caution should be taken in interpreting such findings, these are 2 prospective cohort studies showing a positive association between vitamin B-6 and rectal cancer. Moreover, we previously observed that a positive association between vitamin B-6 intake and CRC was most pronounced among individuals with tumors harboring human mut-L homolog 1 promoter hypermethylation (41). In view of this finding, we hypothesize that a relatively high vitamin B-6 intake may increase promoter hypermethylation and thereby enhance the development of tumors with a methylation-associated phenotype. However, because an inverse association between vitamin B-6 and CRC has been suggested in a number of previous prospective cohort studies (18–21), the role of vitamin B-6 clearly needs further attention. In future research, the role of vitamin B-6 in a hypermethylation-associated pathway should be studied and preferably be investigated in even larger cohort studies.

The current study was a population-based prospective cohort design with a long follow-up period. The large number of incident CRC patients is likely to have minimized the probability of reporting results based on chance alone. It is also unlikely that selection bias has occurred, because the follow-up of cases and subcohort members was almost complete. Recall bias was probably low, certainly if compared with retrospective case-control designs, although measurement error is unavoidable with self-administered FFQ and may still have biased results to some extent. After a validation study, however, it was concluded that the questionnaire is sufficiently able to rank subjects according to dietary intake (24), suggesting reasonably adequate validity of the single baseline measurement. Furthermore, potential preclinical disease is not likely to have affected exposure status, because the results did not substantially change after excluding the first year of follow-up.

This study could not account for potential changes in dietary habits over time, because there have been no follow-up measurements of dietary intake. We do nonetheless have information on the reproducibility of the baseline FFQ, which was determined from 5 annually repeated measurements in independent random samples from the cohort. The average decline of the correlation between follow-up measures was only minimal, indicating that the ability of the baseline FFQ to rank subjects according to dietary intake is maintained relatively well over a period of at least 5 y (25). Moreover, subjects aged 55–69 y at baseline and these elderly people tend to have a more stable dietary habits compared with younger individuals (22). Although follow-up was longer than 5 y, for these reasons, we consider that potential changes in dietary intake over time have presumably not influenced the estimated associations to a great extent.

In conclusion, the current large prospective cohort study suggests that dietary folate does not protect against CRC in this Dutch population and that methionine may reduce CRC risk of the proximal colon in men and rectum among women. Riboflavin also tended to reduce the risk of proximal colon tumors in women. Our study is the 2nd cohort study to show a positive association between vitamin B-6 and rectal cancer among women, which is interesting considering a possible similarity with the recent insight in a potentially harmful effect of high folate levels in colorectal carcinogenesis. However, we underscore the need for larger studies or meta-analyses and to further investigate the role of these nutrients in CRC development.

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