Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study

Intake of Dietary Folate Vitamers and Risk of Colorectal Carcinoma

Results from The Netherlands Cohort Study

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BACKGROUND. Several studies have reported inverse associations between folate intake and colorectal carcinoma risk. Few were prospective studies and none evaluated the association between the intake of individual folate vitamers and colorectal carcinoma risk.

METHODS. The aim of the current study was to investigate the relationship between dietary folate intake and the risk of colorectal carcinoma in a large prospective cohort study in The Netherlands comprising 120,852 men and women aged 55–69 years. After 7.3 years of follow-up, 760 colon and 411 rectal carcinoma cases were available for analysis. Data processing and analysis used the case–cohort approach. A new Dutch database was used to estimate intakes of total and individual folate vitamers.

RESULTS. Analyses adjusted for age, energy intake, family history of colorectal carcinoma, alcohol, vitamin C, iron, and dietary fiber intake yielded an inverse association between colon carcinoma risk and total dietary folate intake (rate ratio [RR]highest vs. lowest quintile, men: 0.73; 95% confidence interval [CI], 0.46–1.17, P trend = 0.03; women: 0.68; 95% CI, 0.39–1.20, P trend = 0.18). An inverse association between rectal carcinoma and total dietary folate intake was found only among men (RR highest vs. lowest quintile, men: 0.66; 95% CI, 0.35–1.21, P trend = 0.03). Analyses showed no clear difference in colorectal carcinoma risk associated with intake of different folate vitamers.

CONCLUSIONS. Dietary folate intake was related inversely to colon and male rectal carcinoma risk. ***Cancer* 2002;95:1421–33.** © *2002 American Cancer Society.*
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KEYWORDS: cohort study, colon carcinoma, rectal carcinoma, folates, diet.

The structure in Figure 1 is known as folic acid, but it is known more precisely as pteroylmonoglutamic acid. Folic acid with a fully oxidized pteridine ring does not occur naturally in significant amounts. It is the common most stable, synthetic form used for fortification. Natural folates comprise an extended family of monoglutamates and polyglutamates (usually five to seven glutamyl residues) of pteroic acid, which qualitatively exhibit the biologic activity of folic acid. The pteridine ring is reduced to give 5,6,7,8-tetrahydrofolate. These reduced forms can be substituted with a covalently bonded one-carbon adduct to the nitrogen positions 5 or 10 or bridged across these two positions. Besides tetrahydrofolate, the most substituted forms in the human diet are 5-formyl and 5-methyl. Other frequently occurring folates are 10-formyldihydrofolate and 10-formylfolic acid. Folic acid from fortified foods or supplements is absorbed mainly by a saturable pH-dependent process. Before enter-

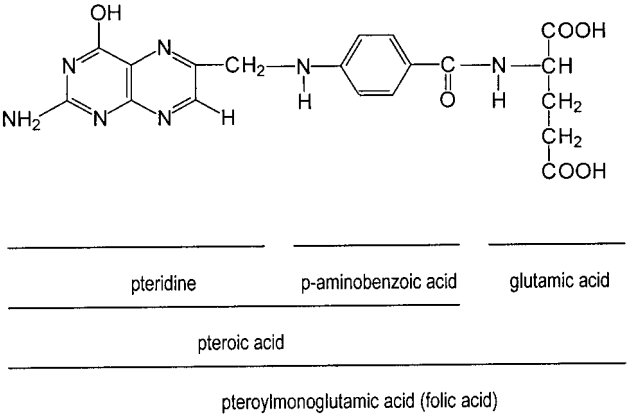

FIGURE 1. Structure of folic acid.

ing the blood circulation, folic acid undergoes reduction to tetrahydrofolate and either methylation or formylation in the mucosa cells.

Approximately 80% of all food folates exist as polyglutamates. They must be cleaved into folate monoglutamates by pteroylpolyglutamate hydrolase (conjugase), which is present in the intestinal brush border, before they can be absorbed in the intestine. Halsted[1] hypothesized that after deconjugation of dietary polyglutamates to monoglutamates by brush-border folate hydrolase and transport across the brush border into the enterocyte, folate polyglutamates are synthesized intracellularly by folate synthetase. This maintains the concentration gradient for uptake. Folate polyglutamate is the intracellular form that best serves the metabolic needs of the cell for folate-dependent reactions and is hydrolyzed intracellularly to folate monoglutamate for transport across the basolateral membrane to the portal circulation.[2,3]

Folates are significantly involved in a number of important processes. The folate-mediated transfer of one-carbon units from serine provides a major source of substrate in one-carbon metabolism. Folate coenzymes play a role in the synthesis of thymidylate and purines, the building blocks for RNA and DNA. Consequently, a deficiency of folate in tissues with rapidly replicating cells results in ineffective DNA synthesis, leading to reduced cell proliferation, impaired cellular physiology, and abnormal cytologic morphology. Although it is well established that folic acid deficiency will retard the growth of established cancers, it does not necessarily follow that nonneoplastic folate-deficient tissues are less likely to undergo neoplastic transformation.[4] The roles of folate deficiency and supplementation in colorectal carcinogenesis have been studied extensively since Freudenheim et al.[5] suggested this relationship. They found a lower risk of colorectal carcinoma in association with high dietary folate intake in a case–control study. Following this study, nine case–control studies[6–14] and three prospective cohort studies[15–17] investigated the relation between dietary folate intake and colorectal carcinoma. In general, a low folate status was associated with an increased risk of colorectal carcinoma, although the association was not statistically significant in all studies. Five studies investigated the relation between folate intake and risk of adenomatous polyps, the precursor lesions of colorectal carcinoma.[10,18–21] Colorectal carcinomas have different gender distributions, with essentially similar incidences between the genders, and a male predominance for rectal carcinoma.[22] However, there are inconsistencies in associations by subsite of the colon.[10]

A number of animal studies support the role of folate deficiency in colorectal carcinogenesis. However, not all animal studies support the protective role of folate supplementation in colorectal carcinogenesis.[4] In one study, dietary folate supplementation did not have a significant inhibitory effect on aberrant crypt foci, probably the earliest recognizable precursor lesion and a well established intermediate biomarker of colorectal carcinoma. In another study, folate supplementation significantly increased the size and multiplicity of colorectal tumors.[4] Some studies have shown that folate deficiency reduces the development of colorectal carcinoma in rats.[23] Cravo et al.[24] concluded from a rat study that folate-deficient rats developed microscopic and macroscopic colorectal neoplasms after initiation of dimethylhydrazine, suggesting that folate deficiency affects an early phase of colorectal carcinogenesis. However, no cancer was observed in two control groups that received a folate-depleted or control diet. In a subsequent study using the same model[25] a folate-deficient diet was associated with the development of macroscopic colorectal tumors. Dietary folate supplementation, up to four times the dietary requirement, retarded the progression from microscopic colorectal neoplastic foci to macroscopic tumors in a dose-dependent manner. This study suggested that folate also has a modulatory effect in a later stage of colorectal carcinogenesis. Levels of dietary folate greater than four times the dietary requirement did not convey further benefit. Therefore, supplemental folate may have two distinct actions. At lower levels of supplementation beyond the dietary requirement, it possesses an inhibitory effect on the genesis of microscopic foci of neoplasia. It also slows the progression of macroscopic neoplasms from microscopic neoplasms.[4] The relationship between blood levels of folate and colorectal carcinoma risk is less well defined than that observed between dietary folate intake and colorectal carci-

noma risk.[4] Results from small folate intervention trials are promising. Three large multicenter trials are underway to determine the chemopreventive role of folate supplementation in colorectal carcinogenesis.[4]

In all epidemiologic studies, dietary folate intake was based on the total folate content of foods, usually determined by microbiologic assay. Folates comprise an extended family of monoglutamates and polyglutamates of pteroic acid, all qualitatively exhibiting the biologic activity of folic acid. Many studies have determined that the relative bioavailability of polyglutamates versus corresponding monoglutamates ranges from 25% to 100%.[26] Several studies have determined the relative bioavailability of the most abundant folate monoglutamates, which may vary between 70% and 129% relative to folic acid.[26] One would expect a difference in risk associated with the intake of different folate vitamers, because of the wide range in bioavailability. No epidemiologic studies have investigated the association between individual folate vitamers and colorectal carcinoma.

In the current study, we investigated the association between the dietary intake of folates and the risk of colorectal carcinoma in The Netherlands Cohort Study on diet and cancer (NLCS). Separate analyses were performed for colorectal carcinoma, for both men and women, and for different subsites of the colon. Analyses were done for total dietary folate intake as well as for the intake of individual folate vitamers. The interaction with the intake of methionine and alcohol was taken into account.

MATERIALS AND METHODS

Cohort

The NLCS is a population-based prospective cohort study that was started in The Netherlands in 1986 and comprised 58,279 men and 62,573 women. The study population originated from 204 municipal population registries and had an age range of 55–69 years at baseline. At baseline, all participants completed a self-administered mailed questionnaire on habitual dietary intake, dietary supplement use, lifestyle characteristics, medical history, and other potential risk factors for cancer. The case–cohort approach was used for data processing and analysis. The case–cohort design randomly selects a subcohort from the entire cohort, which provides a comparison group at each disease occurrence time, i.e., those subjects in the subcohort still at risk for the disease under study at a given failure time function as controls for the occurring failure whether that failure occurs inside or outside the subcohort. This design allows the comparison group to be selected in advance of cohort follow-up. This is a distinct advantage because the subcohort can be used to monitor the achievement of intervention goals and the collection and processing of covariate information for all controls (the subcohort members) can be started immediately on inception. The subcohort consisted of 3500 subjects (1688 men and 1812 women) who were followed up biennially for vital status information. A detailed description of the design and characteristics of the cohort study has been reported previously.[27,28]

Follow-Up for Cancer

Follow-up for incident cancer was established by record linkage with all regional cancer registries in The Netherlands and the national pathology register (PALGA). The method of record linkage has been reported previously.[29] Follow-up was restricted to colon or rectal carcinoma incidence in the period from September 1986 to December 1992. During these 7.3 years of follow-up, 807 microscopically confirmed incident colon carcinoma and 453 rectal carcinoma cases were diagnosed. Subjects with incomplete or inconsistent dietary data were excluded from the analysis. Details of this procedure have been reported elsewhere.[30] After excluding subjects who reported a history of carcinoma other than skin carcinoma in the baseline questionnaire and subjects with colon and rectal carcinoma other than carcinoma, 760 colon carcinoma and 411 rectal carcinoma cases remained for analysis. Prevalent cancer cases other than skin carcinoma were excluded from the subcohort as well, leaving 3123 subcohort members for analysis. Completeness of follow-up of cancer incidence was more than 96%[30] and no subcohort members were lost to follow-up.

Dietary Intakes

A 150-item semiquantitative food frequency questionnaire (FFQ) was used to collect data on the habitual consumption of foods and beverages in the year preceding the start of the study. The questionnaire was validated against a 9-day diet record.[30] The validity was investigated in a subgroup of the cohort (59 men and 50 women). A dietary record, kept over three 3-day periods, 4–5 months apart, served as the reference method. Pearson correlation coefficients between nutrient intakes assessed by the record and the FFQ completed after ranged from 0.40 for vitamin B1 to 0.86 for alcohol intake, with correlations for most nutrients between 0.6 and 0.8. Intake of β-carotene was based on recent analytical data compiled by Goldbohm et al.[31] Folate data were derived from a validated liquid chromatography trienzyme method[32] used to analyze the 125 most important Dutch foods contributing to folate intake.[33] Vegetables, fruit, and potatoes provide greater than 33% of the daily dietary

folate intake (22%, 6%, and 7% respectively). Bread, milk products, meat, and meat products supply another 33% of the daily dietary folate intake (19%, 9%, and 11%, respectively).[33] The most abundant folate vitamers in foods were evaluated including tetrahydrofolate (H_4folate), 5-methyltetrahydrofolate (5-CH_3-H_4folate), 5-formyltetrahydrofolate (5-CHO-H_4folate), 10-formylfolic acid (10-HCO-folic acid), 10-formyldihydrofolate (10-HCO-H_2folate), and folic acid, as well as the grouped folates total monoglutamates (H_4folate, 5-CH_3-H_4folate 5-CHO-H_4folate, 10-HCO-folic acid, 10-HCO-H_2folate, and folic acid) and total polyglutamates (H_4folate, 5-CH_3-H_4folate 5-CHO-H_4folate, 10-HCO-folic acid, 10-HCO-H_2folate, and folic acid). Not all food products were analyzed for individual vitamers. Folate intake from foods analyzed for individual vitamers covered 90% of total folate intake. The mean daily intakes of all other relevant nutrients were calculated using the computerized Dutch food composition table.[34]

Population for Analysis

Ultimately, 760 colon carcinoma cases (400 men, 360 women), 411 rectal carcinoma cases (259 men, 152 women), and 3123 subcohort members (1525 men, 1598 women) were available for analysis. Of all malignant tumors in the colon, 378 (187 men, 191 women) originated in the proximal part (ICD-O topography[35] codes 153.0-1, 153.4-6), 348 (195 men, 153 women) originated in the distal part (ICD-O codes 153.2-3 and 153.7), 14 were unspecified (ICD-O code 153.9), and 10 had overlapping boundaries (ICD-O code 153.8). Malignant tumors of the rectosigmoid and/or rectum (ICD-O codes 154.0 and 154.1) were regarded as rectal carcinoma cases.

Statistical Analysis

Total folate intake was calculated and categorized into quintiles according to the distribution in the subcohort. Incidence rate ratios (RR) and corresponding 95% confidence intervals (CI) for colon or rectal carcinoma were estimated from exponentially distributed failure time regression models[28] (Stata statistical package, release 6.0[36]). Age-adjusted RRs for colorectal carcinoma and their 95% CIs were calculated. Tests for trend were based on two-sided likelihood ratio tests. Total energy, alcohol intake, smoking, physical activity, body mass index, meat consumption, and family history of colorectal carcinoma were evaluated as potential confounders. In our study, family history of colorectal carcinoma (yes/no) and alcohol intake were related to colorectal carcinoma.[37] Adjustment was made for these factors in a multivariate model. Because energy consumption (kcal/d) is frequently regarded as a potential confounder, it this was included in the multivariate model. In addition, models were used that adjusted for vitamin C, iron, and dietary fiber, as these nutrient intakes correlated highly with folate intake. To investigate whether a potential effect of total folate was caused by (a) specific folate vitamer(s), the association between folate vitamer intake as a continuous variable and colorectal carcinoma risk was calculated per increment of 50 μg/d. Two separate analyses were performed. In the analysis of monoglutamates, polyglutamates were included simultaneously and vice versa. In the analysis for each specific vitamer (5-methyltetrahydrofolate, 5-formyltetrahydrofolate, 10-formylfolic acid, tetrahydrofolate, 10-formyldihydrofolate, folic acid), all other vitamers were included simultaneously.

To date, The Netherlands does not fortify foods with folic acid. An intake of 200 g vegetables and two pieces of fruit daily is recommended to enhance intake of folate and other nutrients. This is approximately double the amount of fruit and vegetables consumed in 1992 according to the Dutch National Food Consumption Survey. The current population-based fruit and vegetable intake accounts for approximately 50 μg folate.[33] To calculate a possible risk reduction resulting from an additional folate intake of 50 μg/d, corresponding to the current recommendations of the Dutch Health Council, the association between folate intake as a continuous variable and colorectal carcinoma was calculated per increment of 50 μg/d. Additional analyses were performed after exclusion of cancer cases diagnosed in the first year of follow-up, because preclinical symptoms might have influenced the dietary habits. Separate analyses were conducted for subsite-specific colon carcinoma. To evaluate whether possible beneficial effects of folate might be limited to subjects with low methionine intake, associations between dietary folate intake and colorectal carcinoma were determined for low versus high methionine intake. The joint effects of low folate intake in combination with low methionine intake and high alcohol consumption were considered versus intermediate intake and high folate intake along with high methionine intake and low alcohol intake. Quintiles 1 and 2 were defined as low intake, intermediate intake was defined as Quintile 3, and high intake as Quintiles 4 and 5. Low and high alcohol intake were defined as 4 g/d or less and 15 g/d or more, respectively, with 5–14 g/d as the intermediate intake.

RESULTS

Table 1 presents the mean daily intake of total folate and individual folate vitamers, as well as several other relevant characteristics. The mean age of the subco-

TABLE 1
Nutrient Intake (Mean ± SD) and Other Characteristics among Cases and Subcohort Members: The Netherlands Cohort Study 1986–1993

	Men			Women		
	Subcohort	Colon carcinoma	Rectal carcinoma	Subcohort	Colon carcinoma	Rectal carcinoma
No.	1525	400	259	1598	360	152
Age (yrs)	61.4 ± 4.2	63.0 ± 4.2	62.3 ± 4.1	61.4 ± 4.3	62.7 ± 4.1	62.5 ± 3.8
Family history of colorectal carcinoma (%)	5.6	10.8	10.0	5.4	10.8	6.6
Total folate (μg/d)	224 ± 73	218 ± 72	219 ± 73	200 ± 65	191 ± 63	203 ± 69
Monoglutamates (μg/d)	70 ± 42	64 ± 37	69 ± 47	56 ± 33	55 ± 31	60 ± 43
Polyglutamates (μg/d)	133 ± 42	132 ± 44	130 ± 39	123 ± 39	117 ± 38	123 ± 38
Tetrahydrofolate (μg/d)	17 ± 27	15 ± 24	17 ± 32	14 ± 22	14 ± 20	16 ± 29
5-methyltetrahydrofolate (μg/d)	131 ± 45	130 ± 47	128 ± 42	123 ± 41	117 ± 41	125 ± 42
5-formyltetrahydrofolate (μg/d)	26 ± 10	24 ± 9	25 ± 10	22 ± 8	21 ± 8	22 ± 7
10-formylfolic acid (μg/d)	22 ± 9	21 ± 10	22 ± 9	16 ± 5	15 ± 6	15 ± 4
10-formyldihydrofolate (μg/d)	10 ± 5	10 ± 6	10 ± 5	9 ± 4	8 ± 4	9 ± 4
Folic acid (μg/d)	7 ± 7	6 ± 5	7 ± 6	6 ± 7	6 ± 5	6 ± 5
Methionine (g/d)	1.7 ± 0.4	1.7 ± 0.4	1.7 ± 0.4	1.5 ± 0.4	1.5 ± 0.3	1.5 ± 0.3
Nonalcohol drinkers (%)	15.3	14.8	11.3	32.1	36.7	30.8
Energy (kcal/d)	2160 ± 513	2097 ± 466	2136 ± 445	1690 ± 410	1654 ± 404	1682 ± 399
Iron (mg/d)	13.2 ± 3.2	13.2 ± 3.1	13.3 ± 3.2	11.7 ± 2.7	11.4 ± 2.5	11.6 ± 2.4
Vitamin C (mg/d)	98 ± 42	101 ± 42	102 ± 46	108 ± 44	105 ± 47	111 ± 42
Dietary fiber (g/d)	28.7 ± 8.8	28.3 ± 8.1	28.6 ± 8.5	25.4 ± 7.2	24.6 ± 7.2	24.2 ± 6.0

hort members was 61.4 years for both men and women. Cases were generally older (by approximately 1.2 years). Among subcohort members, approximately 5% had a family history of colorectal cancer. This percentage was approximately 10% among cases. Dietary folate intake among subcohort members and cases was comparable. Mean (± SD) dietary folate was 224 ± 73 μg/d for men in the subcohort versus 200 ± 65 μg/d for women. Comparable intakes among subcohort members and cases were also found for methionine, energy, iron, vitamin C, and dietary fiber. The percentages of alcohol abstainers among the subcohort members were 15.3% and 32.1% for men and women, respectively. These percentages were comparable for cases, with the exception of male rectal carcinoma cases, for which the percentage of alcohol abstainers was considerably lower (11.3%). Pearson correlation coefficients between folate intake and iron, vitamin C, and dietary fiber intake were 0.67, 0.53, and 0.60, respectively.

Table 2 shows the association between dietary folate intake and colon carcinoma. Generally, higher total dietary folate intake was related to a lower risk of colon carcinoma. However, among men, the RR increased again toward unity in the highest quintile of folate intake compared with the fourth quintile. Age-adjusted and multivariate analyses (including age, alcohol intake, energy intake, and family history of colorectal carcinoma) yielded comparable results. When iron intake, vitamin C intake, and dietary fiber intake were also included in the multivariate analyses, RRs were considerably more inverse, especially for men. A multivariate analysis including age, alcohol intake, energy intake, family history of colorectal carcinoma, iron intake, vitamin C intake, and dietary fiber intake revealed a statistical significant inverse association between dietary folate intake and colon carcinoma risk for men (RR high vs. low = 0.73; 95% CI, 0.46–1.17, P trend = 0.03). Age-adjusted analysis for women revealed a statistical significant inverse association between dietary folate intake and colon carcinoma. Adjustment with other covariates yielded similar results, although the P for trend increased. A multivariate analysis including age, alcohol intake, energy intake, family history of colorectal carcinoma, iron intake, vitamin C intake, and dietary fiber intake resulted in an RR for the highest versus lowest quintile of 0.68 (95% CI, 0.39–1.20, P trend = 0.18). In the continuous analysis, the RR decreased by approximately 10% in both men and women for each 50-μg increment of dietary folate intake per day. Exclusion of cases diagnosed in the first year of follow-up resulted in somewhat higher RRs.

Table 3 shows the association between dietary folate intake and rectal carcinoma. Dietary folate intake was not related to the risk of rectal carcinoma in age-adjusted and multivariate analyses including age, alcohol intake, energy intake, and family history of colorectal carcinoma. After additional adjustment for iron intake, vitamin C intake, and dietary fiber intake, the RRs showed an inverse association between dietary folate intake and rectal carcinoma risk among

TABLE 2
Incidence Rate Ratio (RR) of Colon Carcinoma According to Folate Intake Levels in Multivariate Analyses According to Years of follow-Up: The Netherlands Cohort Study, 1986–1993

Quintiles of folate intake Boundaries (μg/d)	Cases in cohort	Person yrs in subcohort	7.3 yrs of follow-up			First yr excluded
			RR (95% CI)[a]	RR (95% CI)[b]	RR (95% CI)[c]	RR (95% CI)[c]
Men, colon carcinoma						
1 (< 168)	98	2042	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 (168–198)	87	2122	0.88 (0.63–1.24)	0.92 (0.65–1.31)	0.84 (0.59–1.21)	1.01 (0.69–1.48)
3 (198–226)	66	2148	0.66 (0.46–0.95)	0.68 (0.46–0.99)	0.59 (0.40–0.88)	0.72 (0.47–1.10)
4 (226–266)	62	2104	0.66 (0.46–0.94)	0.70 (0.47–1.03)	0.58 (0.38–0.90)	0.71 (0.45–1.12)
5 (> 266)	87	2092	0.94 (0.67–1.32)	1.01 (0.68–1.50)	0.73 (0.46–1.17)	0.93 (0.57–1.52)
P value for linear trend			0.22	0.50	0.03	0.29
Increment 50 μg/d			0.96 (0.88–1.04)	0.97 (0.88–1.07)	0.88 (0.78–0.99)	0.90 (0.80–1.02)
Women, colon carcinoma						
1 (< 150)	91	2257	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 (150–176)	75	2276	0.86 (0.61–1.21)	0.85 (0.59–1.22)	0.84 (0.57–1.23)	0.91 (0.61–1.35)
3 (176–203)	70	2262	0.80 (0.56–1.13)	0.81 (0.55–1.19)	0.79 (0.52–1.21)	0.84 (0.54–1.30)
4 (203–243)	69	2262	0.80 (0.56–1.14)	0.84 (0.56–1.25)	0.81 (0.50–1.33)	0.87 (0.52–1.45)
5 (> 243)	55	2277	0.65 (0.45–0.95)	0.71 (0.46–1.08)	0.68 (0.39–1.20)	0.66 (0.36–1.21)
P value for linear trend			0.01	0.10	0.18	0.18
Increment 50 μg/d			0.91 (0.83–1.01)	0.95 (0.84–1.06)	0.96 (0.81–1.13)	0.95 (0.80–1.14)

CI: confidence interval.

[a] Adjusted for age (yrs).

[b] Adjusted for for age, alcohol intake, energy intake, and family history of colorectal carcinoma.

[c] Adjusted for age, alcohol intake, energy intake, family history of colorectal carcinoma, iron intake, vitamin C intake, and dietary fiber intake.

men (RR high vs. low = 0.66; 95% CI, 0.35–1.21, *P* trend = 0.03). Among women, there was no inverse association between dietary folate intake and rectal carcinoma. In the multivariate analysis, the RR for the highest versus lowest quintile was 1.26 (95% CI, 0.58–2.76, *P* trend = 0.55). RRs were slightly higher when cases diagnosed in the first year of follow-up were excluded.

Table 4 shows the results of multivariate analysis for individual folate vitamers. The RRs for colorectal carcinomas were lower for polyglutamates than for monoglutamates. The inverse association between folate polyglutamate intake and rectal carcinoma risk among men was significant (RR = 0.69; 95% CI, 0.52–0.91). Beneficial effects of folate intake might also vary between individual vitamers. Compared with other RRs, there was a strong inverse association between 5-formyltetrahydrofolate intake and colon carcinoma risk among men (RR = 0.25, 95% CI, 0.10–0.68).

Colon carcinoma cases were classified according to specific colon subsites. There were no differences in the association between folate intake and carcinoma at specific subsites. Inverse associations were found for total dietary folate intake and both distal and proximal colon carcinoma in men and women. However, none were statistically significant (Table 5). For men, the RRs for the highest versus the lowest quintile were 0.75 (95% CI, 0.39–1.44) and 0.74 (95% CI, 0.40–1.35) for proximal and distal colon carcinoma, respectively. Among women, the RR values for highest versus lowest quintile were 0.72 (95% CI, 0.33–1.54) and 0.69 (95% CI, 0.31–1.52) for proximal and distal colon carcinoma, respectively.

Results on the interaction between folate and methionine are shown in Table 6. The inverse association between folate and colorectal carcinoma in the low methionine intake was limited to men. (*P* values for statistical interaction were 0.30 and 0.04 for colon carcinoma for men and women, respectively, and 0.89 and 0.35 for rectal carcinoma for men and women, respectively.) Because alcohol acts as a methyl group antagonist, low intakes of folate and methionine among substantial alcohol users might be stronger risk factors for colorectal carcinoma. To examine this hypothesis, subgroups were formed of those with low intake levels of folate and methionine and with high intake levels of alcohol and vice versa. Results are shown in Table 7. For men, the RR estimates for low folate-low methionine-high alcohol intakes versus high folate-high methionine-low alcohol intakes were 1.26 (95% CI, 0.59–2.70) for colon carcinoma and 2.69 (95% CI, 0.99–7.29) for rectal carcinoma. For women, the RR values for high alcohol-low methionine-low folate intakes versus low alcohol-high methionine-high folate intakes were 1.01 (95% CI, 0.40–2.54) for colon carcinoma and 0.45 (95% CI, 0.14–1.50) for rec-

TABLE 3
Incidence Rate Ratio (RR) of Rectal Carcinoma According to Folate Intake Levels in Multivariate Analyses According to Years of Follow-Up: The Netherlands Cohort Study, 1986–1993

Quintiles of folate intake Boundaries (μg/d)	Cases in cohort	Person yrs in subcohort	7.3 yrs of follow-up			First yr excluded
			RR (95% CI)[a]	RR (95% CI)[b]	RR (95% CI)[c]	RR (95% CI)[c]
Men, rectal carcinoma						
1 (< 168)	54	2048	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 (168–198)	64	2124	1.17 (0.78–1.74)	1.19 (0.78–1.79)	1.07 (0.70–1.63)	1.06 (0.68–1.66)
3 (198–226)	46	2142	0.83 (0.54–1.27)	0.80 (0.51–1.26)	0.67 (0.42–1.09)	0.66 (0.40–1.09)
4 (226–266)	46	2108	0.86 (0.56–1.32)	0.86 (0.54–1.36)	0.68 (0.41–1.13)	0.72 (0.42–1.21)
5 (> 266)	49	2103	0.93 (0.61–1.41)	0.94 (0.56–1.58)	0.66 (0.35–1.21)	0.69 (0.36–1.31)
P value for linear trend			0.28	0.33	0.03	0.06
Increment 50 μg/d			0.96 (0.87–1.07)	0.96 (0.85–1.09)	0.87 (0.74–1.02)	0.89 (0.76–1.05)
Women, rectal carcinoma						
1 (< 150)	29	2261	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 (150–176)	30	2276	1.08 (0.63–1.84)	1.17 (0.66–2.09)	1.18 (0.65–2.15)	1.37 (0.73–2.60)
3 (176–203)	30	2270	1.06 (0.62–1.82)	1.18 (0.65–2.16)	1.22 (0.65–2.28)	1.35 (0.69–2.65)
4 (203–243)	32	2267	1.15 (0.68–1.96)	1.26 (0.68–2.36)	1.28 (0.63–2.59)	1.20 (0.56–2.58)
5 (> 243)	31	2280	1.14 (0.67–1.96)	1.28 (0.67–2.48)	1.26 (0.58–2.76)	1.25 (0.54–2.92)
P value for linear trend			0.56	0.40	0.55	0.81
Increment 50 μg/d			1.06 (0.93–1.20)	1.10 (0.94–1.28)	1.10 (0.92–1.32)	1.12 (0.92–1.36)

CI: confidence interval.
[a] Adjusted for age (yrs).
[b] Adjusted for for age, alcohol intake, energy intake, and family history of colorectal carcinoma.
[c] Adjusted for age, alcohol intake, energy intake, family history of colorectal carcinoma, iron intake, vitamin C intake, and dietary fiber intake.

TABLE 4
Incidence Rate Ratio (RR) of Colon and Rectal Carcinoma According to an Intake of 50 μg/d of Different Folate Vitamers: The Netherlands Cohort Study, 1986–1993[a]

	Men		Women	
	Colon carcinoma RR (95% CI)[b]	Rectal carcinoma RR (95% CI)[b]	Colon carcinoma RR (95% CI)[b]	Rectal carcinoma RR (95% CI)[b]
Monoglutamates	0.80 (0.65–0.98)	1.02 (0.84–1.24)	1.15 (0.96–1.37)	1.13 (0.87–1.45)
Polyglutamates	0.98 (0.79–1.21)	0.69 (0.52–0.91)	0.78 (0.59–1.01)	1.05 (0.76–1.46)
5-methyltetrahydrofolate	1.03 (0.84–1.27)	0.68 (0.51–0.91)	0.84 (0.62–1.13)	1.08 (0.76–1.53)
5-formyltetrahydrofolate	0.25 (0.10–0.68)	0.53 (0.16–1.74)	0.87 (0.28–2.78)	1.23 (0.26–5.89)
10-formylfolic acid	0.82 (0.40–1.65)	0.96 (0.43–2.13)	0.64 (0.09–4.50)	0.42 (0.04–3.88)
Tetrahydrofolate	0.83 (0.62–1.10)	1.14 (0.82–1.58)	1.30 (1.00–1.68)	1.10 (0.74–1.65)
10-formyldihydrofolate	1.31 (0.36–4.86)	5.07 (1.22–22.04)	1.03 (0.21–5.21)	1.61 (0.18–14.25)
Folic acid	0.35 (0.11–1.15)	0.70 (0.22–2.20)	0.79 (0.30–2.09)	1.22 (0.49–3.07)

CI: confidence interval.
[a] Two separate analyses were performed. In the analysis of monoglutamates, polyglutamates were included simultaneously and vice versa. In the analysis for each specific vitamer (5-methyltetrahydrofolate, 5-formyltetrahydrofolate, 10-formylfolic acid, tetrahydrofolate, 10-formyldihydrofolate, folic acid), all other vitamers were included simultaneously.
[b] Adjusted for age, alcohol intake, energy intake, family history of colorectal carcinoma, iron, vitamin C, and dietary fiber.

tal carcinoma. The following unstratified associations between alcohol and rectal carcinoma risk were found for men (RR high vs. low = 1.21; 95% CI, 0.88–1.66) and women (1.06; 95% CI, 0.62–1.82). There were 257 and 143 cases for men and women, respectively. These results suggest that men who consume higher amounts of alcohol combined with inadequate intakes of folate and methionine are at a higher risk of developing rectal carcinoma. Results are based on a small number of cases, however. Among men with low folate intake (< 168 μg/d) and high alcohol intake (≥ 15 g/d), the risk of rectal carcinoma was approximately 1.5 times the risk of rectal carcinoma for men with low folate intake combined with a low alcohol intake (≤ 4 g/d; data not shown).

TABLE 5
Incidence Rate Ratios (RR) of Colon Carcinoma According to Quintiles of Folate Intake and Subsite: The Netherlands Cohort Study, 1986–1993

Men[a]			Women[a]		
Quintiles of folate intake (μg/d)	Proximal colon carcinoma (186 cases) RR (95% CI)[b]	Distal colon carcinoma (194 cases) RR (95% CI)[b]	Quintiles of folate intake (μg/d)	Proximal colon carcinoma (185 cases) RR (95% CI)[b]	Distal colon carcinoma (152 cases) RR (95% CI)[b]
1 (< 168)	1.00 (reference)	1.00 (reference)	1 (< 150)	1.00 (reference)	1.00 (reference)
2 (168–198)	0.87 (0.53–1.42)	0.84 (0.53–1.33)	2 (150–176)	0.84 (0.51–1.40)	0.87 (0.51–1.48)
3 (198–226)	0.66 (0.38–1.15)	0.52 (0.31–0.88)	3 (176–203)	0.81 (0.47–1.42)	0.83 (0.46–1.51)
4 (226–266)	0.67 (0.38–1.21)	0.50 (0.28–0.89)	4 (203–243)	1.03 (0.55–1.95)	0.68 (0.33–1.41)
5 (> 266)	0.75 (0.39–1.44)	0.74 (0.40–1.35)	5 (> 243)	0.72 (0.33–1.54)	0.69 (0.31–1.52)
P value for trend	0.24	0.08		0.66	0.26

CI: confidence interval.

[a] Proximal colon carcinoma ICD codes: 153.0, 153.1, 153.4, 153.5, 153.6; distal colon carcinoma ICD codes: 153.2, 153.3, 153.7.

[b] Adjusted for age, alcohol intake, energy intake, family history of colorectal carcinoma, iron intake, vitamin C intake, and dietary fiber intake.

TABLE 6
Incidence Rate Ratios (RR) of Colon and Rectal Carcinoma According to Quintiles of Folate Intake and Level of Methionine Intake; The Netherlands Cohort Study, 1986–1993

Men			Women		
Quintiles of folate intake (μg/d)	Low methionine intake (≤ 1.58 g/d) RR (95% CI)[a]	High methionine intake (≥ 1.78 g/d) RR (95% CI)[a]	Quintiles of folate intake (μg/d)	Low methionine intake (≤ 1.38 g/d) RR (95% CI)[a]	High methionine intake (≥ 1.56 g/d) RR (95% CI)[a]
Colon carcinoma					
	176 cases	142 cases		146 cases	134 cases
1 (< 168)	1.00 (reference)	1.00 (reference)	1 (< 150)	1.00 (reference)	1.00 (reference)
2 (168–198)	0.85 (0.52–1.38)	1.09 (0.33–3.60)	2 (150–176)	0.80 (0.45–1.42)	0.71 (0.29–1.73)
3 (198–226)	0.46 (0.26–0.84)	1.17 (0.35–3.85)	3 (176–203)	1.06 (0.56–2.02)	0.41 (0.17–0.99)
4 (226–266)	0.41 (0.19–0.87)	0.95 (0.29–3.12)	4 (203–243)	1.72 (0.76–3.87)	0.48 (0.20–1.17)
5 (> 266)	0.40 (0.15–1.10)	1.46 (0.45–4.68)	5 (> 243)	0.50 (0.16–1.56)	0.45 (0.17–1.20)
P value for trend[b]	0.0004	0.34		0.76	0.09
Rectum carcinoma					
	109 cases	91 cases		51 cases	60 cases
1 (< 168)	1.00 (reference)	1.00 (reference)	1 (< 150)	1.00 (reference)	1.00 (reference)
2 (168–198)	1.10 (0.61–1.98)	2.06 (0.43–9.83)	2 (150–176)	1.21 (0.51–2.87)	0.48 (0.48–4.18)
3 (198–226)	0.71 (0.36–1.42)	1.12 (0.22–5.65)	3 (176–203)	1.30 (0.50–3.40)	0.53 (0.16–1.80)
4 (226–266)	0.43 (0.16–1.11)	1.05 (0.21–5.13)	4 (203–243)	1.97 (0.62–6.21)	0.57 (0.17–1.89)
5 (> 266)	0.50 (0.17–1.50)	1.23 (0.25–6.01)	5 (> 243)	0.86 (0.18–4.04)	0.74 (0.19–2.85)
P value for trend[b]	0.02	0.32		0.63	0.97

CI: confidence interval.

[a] Adjusted for age, alcohol intake, energy intake, family history of colorectal carcinoma, iron intake, vitamin C intake, and dietary fiber intake.

[b] Test for trend based on separate models stratified by methionine level.

DISCUSSION

Dietary folate intake is associated inversely with a risk of colon carcinoma in men and women and with rectal carcinoma in men. An inverse association of folate against rectal carcinoma was not found among women.

A number of mechanisms are involved in folate-associated carcinogenesis. First, folate plays a role in a number of metabolic pathways, such as the biosynthesis of purines and thymidines. Folate deficiency causes massive incorporation of uracil into human DNA as well as chromosome breaks, resulting in the initiation of neoplastic transformation.[38] These processes returned to normal when individuals received folic acid supplementation.[38] Second, a mutation in the methylenetetrahydrofolate reductase (MTHFR)

TABLE 7
Incidence Rate Ratios (RR) of Colon and Rectal Carcinoma According to a Combination of Folate Intake, Methionine Intake, and Alcohol Intake: The Netherlands Cohort Study, 1986–1993[a]

	Colon carcinoma		Rectal carcinoma	
Combined intake	Men (85 cases) RR (95% CI)[b]	Women (67 cases) RR (95% CI)[b]	Men (47 cases) RR (95% CI)[b]	Women (32 cases) RR (95% CI)[b]
High folate-high methionine-low alcohol	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Intermediate alcohol-methionine-folate	0.83 (0.26–2.67)	0.47 (0.10–2.18)	1.59 (0.37–6.90)	0.79 (0.16–3.82)
Low folate-low methionine-high alcohol	1.26 (0.59–2.70)	1.01 (0.40–2.54)	2.69 (0.99–7.29)	0.45 (0.14–1.50)

CI: confidence interval.

[a] Low alcohol intake is defined as ≤ 4 g/d and high alcohol intake is defined as ≥ 15 g/d. Low and high intakes of folate and methionine are Quintiles 1, 2 and 4, 5, respectively. Intermediate intakes of folate and methionine are Quintiles 3. Intermediate alcohol intake is defined as between 5 and 14 g/d. Low intake of folate are Quintiles 1 + 2 (< 198 μg/d for men, < 176 μg/d for women). High intake of folate are Quintiles 4 + 5 (≥ 226 μg/d for men, ≥ 203 μg/d for women). Intermediate intake of folate are Quintiles 3 (198–226 μg/d for men and 176–203 μg/d for women). Low intake of methionine are Quintiles 1 + 2 (< 1.58 g/d for men, < 1.38 g/d for women). High intake of methionine are Quintiles 4 + 5 (≥ 1.78 g/d for men, ≥ 1.56 g/d for women). Intermediate intake of methionine are Quintiles 3 (1.58–1.78 g/d for men and 1.38–1.56 g/d for women).

[b] Adjusted for age, energy intake, family history of colorectal carcinoma, iron intake, vitamin C intake, and dietary fiber intake.

gene causes reduced enzyme activity, leading to low plasma folate levels. Although the polymorphism is disadvantageous in homozygous carriers with low folate levels, its presence may reduce colorectal carcinoma in individuals with an adequate folate supply.[38–40] The findings, however, have not been consistent.[41] Third, folate is essential for methylation reactions in humans. Reduced methylation of DNA, caused by inadequate cellular levels of the methyl donor S-adenosylmethionine, may contribute to loss of normal controls on protooncogene expression. Because production of S-adenosylmethionine depends on both methionine and folate, diets deficient in these factors may cause an imbalance in DNA methylation. In humans, hypomethylation of DNA has been observed in individuals with colorectal carcinomas.[42] Site-specific hypermethylation also may be important in tumorigenesis.[43–45] However, genomic and gene-specific DNA hypomethylation is still considered to be an important epigenetic phenomenon in neoplastic transformation.[4] Sufficient methionine intake may also reduce the risk of colorectal carcinoma[15,19] and possibly attenuate the beneficial role of folate. The association between folate and colorectal carcinoma may be stronger in individuals with a low intake of methionine. A protective effect of folate and methionine against colorectal carcinoma may be impaired by the suppressive effect of high alcohol consumption on methyl-group metabolism.[46–50] Therefore, alcohol acts as a methyl-group antagonist. In combination with low folate and low methionine intake, heavy drinkers might be at greatest risk of colorectal carcinoma.

Many case–control studies have found inverse associations between folate intake and the risk of colorectal carcinoma.[6–14] These studies found on average a 35% reduction in the risk of developing colorectal carcinoma in subjects with the highest dietary folate intake compared with those with the lowest intake.[4] Five studies found a statistically significant reduction. Evidence from three prospective studies[15–17] supports an inverse association between folate intake and the risk of colon carcinoma. No prospective studies have been reported on rectal carcinoma. In the Health Professionals Follow-Up Study, dietary folate intake was not statistically significantly related to the risk of colon carcinoma with an RR (highest vs. lowest quintile) of 0.86,[15] nor did the Nurses' Health Study find a statistically significant association with colon carcinoma. The multivariate RR for highest versus lowest quintile in the Nurses' Health Study was 0.82 (95% CI, 0.56–1.20). The inverse association in the current study was stronger with RRs of about 0.70. This stronger association in our study occurred when we controlled for iron intake in the multivariate analysis, especially for men. In cohort studies reported previously, the use of supplemental folic acid for more than 15 years was associated with a lower risk of colon carcinoma. In the NLCS, 22% of the study population used vitamin B supplements.[51] However, due to legislative restrictions in The Netherlands, it was not allowed until 1994 to use folic acid in vitamin supplements. Therefore, the overall effect of folic acid supplements in this cohort is negligible. One of the strengths of our study is its prospective design. Preclinical symptoms are not likely to have influenced the dietary habits, because results were not much affected after exclusion of cases diagnosed in the first year of follow-up. Furthermore, selection bias was not likely because the follow-up of cases and subcohort members was almost complete.

Another strength of the current study was the use of a newly compiled folate database with food folate values. Based on Dutch products, the folate database was established with a validated trienzyme high-performance liquid chromatography method preceded by an affinity chromatography cleanup step.[33] Many comparisons were made and some significant associations might be the result of chance.

This is the first epidemiologic study to estimate associations between the intake of individual folate vitamers and cancer risk. Beneficial effects of folate intake might be stronger for folate monoglutamates than for folate polyglutamates because the relative bioavailability of polyglutamates versus their corresponding monoglutamates might be as low as 25%.[26] Analyses showed no clear difference in colorectal carcinoma risk associated with intake of polyglutamates versus monoglutamates. Among the associations between individual folate vitamers and colon or rectal carcinoma risk, a statistically significant inverse association between 5-formyltetrahydrofolate intake and colon carcinoma risk among men was found. The inverse association between 5-formyltetrahydrofolate intake and colon carcinoma risk among men seemed to be stronger than that between the other vitamers and colon carcinoma risk. This cannot be explained on the basis of current knowledge and might be the result of chance. Stern et al.[52] concluded that the intestinal tissue has the capacity to convert 5-formyltetrahydrofolate into 5-methyltetrahydrofolate. Conversion of 5-formyltetrahydrofolate into 5-methyltetrahydrofolate is unimpaired in folate-adequate persons homozygous for the C677T mutation in the MTHFR gene. As a result, there might be no difference in working mechanisms of these vitamers. The intakes of folic acid, 10-formyldihydrofolate tetrahydrofolate, and 10-formylfolic acid were low in this study. This resulted in much larger CIs (less statistical stability) of the observed RRs than those of the vitamers with a relatively high intake Also, when testing as many associations simultaneously, one significant observation is compatible with chance.

An inverse association between folate and colorectal carcinoma at low methionine intake seemed to be limited to men, although the interaction was not statistically significant. A greater benefit of folate at low methionine intake underlines the role of folate in methionine synthesis. An insufficient folate level combined with low methionine intake might result in inadequate DNA methylation, leading to a greater risk of colon carcinoma, as hypomethylation is regarded as one of the earliest events in colon caricinogenesis. Giovannucci et al.[16] found a statistically significant interaction between folate and methionine in the Nurses' Health Study ($P = 0.005$). This became weaker when folic acid supplement users were excluded from the analysis ($P = 0.02$), suggesting a stronger effect with higher folate intake at low methionine intake levels. The current study considered only dietary folate intakes, which might explain the weaker interaction effects. Folate deficiency has not been shown unequivocally to induce genomic or site-specific DNA hypomethylation but is still considered as a possible mechanism. Cravo et al.[53,54] observed that folate supplementation significantly reversed genomic DNA hypomethylation of the colonic mucosa. Kim et al.[55] reported that DNA methylation underwent improvement with folate supplementation. However, methylation also improved with a placebo, which might have been caused by participants who were informed about the possible cancer-protective effects of folate. The results of our study do not support a role of folate in DNA methylation. A stronger inverse association between folate and colorectal carcinoma at low methionine intake, which underlines the role of folate in methionine synthesis, was not confirmed by a statistically significant interaction.

We found a somewhat higher risk of colon carcinoma among men with a high alcohol intake combined with low folate and methionine intakes, although this was not significant. The effect was stronger for rectal carcinoma. In the Health Professionals Follow-Up Study, Giovannucci et al.[15] found an approximately twofold higher risk of colon carcinoma among men with a high alcohol intake combined with low folate and methionine intakes, compared to men with low alcohol intake combined with high folate and methionine intakes. Mean alcohol intake for the high intake level in our study was 32 g/d, slightly higher than the highest intake level in the Health Professionals Study, which was 29 g/d. The approximately 2.5 times higher folate intake level in the Health Professionals Follow-Up Study in the high intake group (755 μg/d vs. 290 μg/d in our study for the high folate intake level) might explain the differences in RR. Our study found a 2.5 times higher risk of rectal carcinoma among men with a high alcohol intake combined with low folate and methionine intakes. The inverse association between high alcohol intake combined with low folate and methionine intake and rectal carcinoma risk among women is hard to explain. This subgroup had the smallest number of cases, yet the absence of an inverse association between folate intake and rectal carcinoma risk among women would not plead for an effect of the folate antagonist alcohol. However, unstratified analysis revealed no association between alcohol consumption

and rectal carcinoma risk among women. Other studies have found elevated risks associated with alcohol predominantly in the rectum or distal colon.[42] The RRs for distal colon carcinoma in our study were not higher than those for total colon carcinoma (data not shown) among subjects with a high alcohol intake combined with low folate and methionine intakes.

There are subsite-specific differences in colon carcinoma risk associated with vegetable consumption.[37] However, this was not observed for folate intake, suggesting that another component in vegetables might be responsible for this effect. We included many dietary and nondietary determinants of colorectal carcinoma as potential confounders in the multivariate models. It is still possible, however, that another factor, not controlled for in our analysis, might be involved. A striking finding of the current study was a strong confounding effect of iron intake in the associations between folate intake and colorectal carcinoma among men, which was not found among women. This is consistent with findings by Nelson et al.[56,57] and Stevens et al.,[58] who reported positive associations between iron stores and dietary iron intake and between colon carcinoma and colonic adenoma, which might only apply to men. In our study, heme-iron from meat and meat products is the most important source that might be associated with an increased risk of colorectal carcinoma. It has been hypothesized that iron could catalyze the production of oxygen radicals, which can function as proximate carcinogens. Iron may be a limiting nutrient for the growth and development of cancer cells. Therefore, excess iron may stimulate the growth of cancer cells. Indicators of body iron stores are serum ferritin and total iron-binding capacity, which is related inversely to serum ferritin levels. Physiologically, women have lower iron stores, which might explain differences in cancer development. The presence of metals (Fe^{2+}) increases folate loss.[59] In addition, Free radical intermediates may be involved in the chemical oxidation of reduced pterins by Fe^{3+}.[60] This means that the biologic activity of folate may be reduced by the presence of iron.

Because many folate-containing foods also contain other possible risk-reducing vitamins that might affect our findings, associations between folate and colorectal carcinoma were investigated in subjects with low and high intakes of vitamin E, vitamin C, β-carotene, and vitamin A. No statistically significant interactions were found (data not shown), which underline the independent effect of dietary folate. Pearson correlation coefficients of folate with vitamin E, vitamin C, β-carotene, and vitamin A were, respectively, 0.28, 0.53, 0.50, and 0.68 for men and 0.32, 0.58, 0.52, and 0.66 for women. Given these relatively low correlations, the problem of multicollinearity probably did not exist.

In summary, the authors found evidence for an inverse association between dietary folate intake and colon carcinoma risk in men and women and rectal carcinoma risk in men only. Risk appeared to be independent of vitamins A,C,E, and β-carotene intakes as a proxi for fruit and vegetables. Analyses showed no clear difference in colorectal carcinoma risk associated with intake of different folate vitamers. The beneficial role of folate may not be limited to persons with a low methionine intake. The antagonism between alcohol and methyl group metabolism was most pronounced in men and mainly confined to rectal carcinomas.

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