Short Communication

Dietary Intake of Folate and Riboflavin, *MTHFR C677T* Genotype, and Colorectal Adenoma Risk: A Dutch Case-Control Study

Maureen van den Donk,[1] Brian Buijsse,[1,2] Saskia W. van den Berg,[1,2] Marga C. Ocké,[2] Jan L. Harryvan,[1] Fokko M. Nagengast,[3] Frans J. Kok,[1] and Ellen Kampman[1]

[1]Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands; [2]Centre for Nutrition a nd Health, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; and [3]Department of Gastroenterology and Hepatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

Abstract

We investigated the associations between dietary intake of folate and vitamin B2, *MTHFR C677T* genotype, and colorectal adenomas in a Dutch case-control study. Data of cases with at least one histologically confirmed colorectal adenoma (n = 768) and controls with no history of any type of colorectal polyp (n = 709) were included. Dietary intake was assessed using a food-frequency questionnaire. Multivariable models included age and, if appropriate, dietary folate and calcium intake. The adjusted odds ratio (OR) and 95% confidence interval (CI) for the highest compared with the lowest sex-specific tertile of intake were 1.32 (95% CI, 1.01-1.73) for folate and 0.51 (95% CI, 0.36-0.73) for vitamin B2. Folate seemed to be a risk factor, especially when vitamin B2 intake was low; vitamin B2 was inversely associated with adenomas, especially with relatively high folate intake. No association was observed between *MTHFR C677T* genotype and colorectal adenomas. The inverse association between vitamin B2 intake and colorectal adenoma risk seemed to be more pronounced among those with the *MTHFR TT* genotype. We conclude that this study does not provide evidence for a decreased colorectal adenoma risk for subjects with high dietary intake of folate. It suggests, however, an inverse association between vitamin B2 and colorectal adenomas, which may be more relevant for those with the *MTHFR TT* genotype. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1562–6)

Introduction

Colorectal adenomas are highly prevalent in the Western world. Among asymptomatic, average-risk patients, prevalence of adenomas is ~25% in colonoscopy studies (1). As certain colorectal adenomas are considered precursors of colorectal cancer (2, 3), prevention of colorectal adenomas may decrease the occurrence of colorectal cancer.

Folate is hypothesized to have a beneficial effect on the development of colorectal adenomas and carcinomas. Folate is essential in DNA metabolism; deficiency affects DNA methylation and purine and pyrimidine synthesis (4). Vitamin B2 (riboflavin) plays a prominent role in folate metabolism. Flavin adenine dinucleotide, a metabolite of vitamin B2, serves as a cofactor for methylenetetrahydrofolate reductase (MTHFR; refs. 5-7). MTHFR is an important enzyme in folate metabolism; it catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (8). Flavin adenine dinucleotide was found to modify MTHFR activity in healthy subjects (9).

Most epidemiologic studies examining the association between folate intake or status and colorectal adenoma risk observed an inverse association (10-20), which was statistically significant in some studies (10-14), whereas two studies did not find an association (21, 22). We know of only two observational studies on vitamin B2 intake and colorectal adenoma risk: in one study no association was found (11), whereas the other study found a nonsignificant inverse association [odds ratio (OR) for highest versus lowest tertile of intake is 0.67; 95% confidence interval (95% CI) is 0.39-1.17; ref. 20].

A common C-to-T substitution in the *MTHFR* gene at nucleotide 677 converts an alanine to valine and is associated with decreased enzyme activity (23). Studies investigating the association of *MTHFR C677T* genotype and colorectal adenoma risk show nonsignificant relative risks ranging from 0.35 to 2.41 (13, 17, 19, 20, 22, 24-26). However, *MTHFR C677T* genotype may modify the association between intake of B-vitamins and colorectal adenomas; several studies indicate that the *MTHFR TT* genotype in combination with a low folate status may be a risk factor for colorectal adenomas (13, 18, 24, 26), although some studies do not show an interaction (17, 19). As far as we know, only one published study evaluated the interaction between vitamin B2 intake and *MTHFR C677T* genotype, in which there was no evidence of an interaction (20).

Most studies on folate and colorectal adenoma or cancer risk are conducted in the United States. Intake of folate and vitamin B2 in the United States is high compared with the Netherlands, where supplements are not regularly used and foods are not enriched with folate and only recently with vitamin B2. Therefore, we evaluated the associations between intake of folate and vitamin B2 and colorectal adenoma risk in a Dutch case-control study, taking into account potential confounding or effect-modifying variables, such as alcohol consumption, smoking, and intake of other B-vitamins. In addition, we examined whether these associations are modified by *MTHFR C677T* genotype.

Received 6/11/04; revised 2/21/05; accepted 3/22/05.

Grant support: The Netherlands Organization for Health Research and Development (ZonMw), grant 980-10-020, and the Netherlands Foundation for Digestive Diseases (MLDS), grant WS 99-72.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Ellen Kampman, Division of Human Nutrition (bode 62), Wageningen University and Research Centre, P.O. Box 8129, NL-6700 EV Wageningen, the Netherlands. Phone: 31-317-483867; Fax: 31-317-482782. E-mail: ellen.kampman@wur.nl

Materials and Methods

Study Population. The POLIEP study is a case-control study conducted in the Netherlands to investigate gene-environment interactions and risk of colorectal adenomas. Participants were recruited among those undergoing endoscopy in 10 outpatient clinics between June 1997 and October 2002. The study design has been previously described (27).

We defined cases as those with at least one histologically confirmed colorectal adenoma ever in their life. Controls had no history of any type of polyps, proven by complete visualization of the colon (i.e., full colonoscopy or sigmoidoscopy combined with X-ray). Eligibility criteria were Dutch speaking, of European origin, of ages 18 to 75 years at time of endoscopy, no hereditary colorectal cancer syndromes (i.e., familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer), no chronic inflammatory bowel disease, no history of colorectal cancer, and no (partial) bowel resection. Response rates varied from 35% to 91% in different outpatient clinics; overall response was 54%.

Of 1,526 eligible participants, we excluded 49 subjects with insufficient dietary data. Thus, the analyses included 1,477 participants: 768 cases and 709 controls. Of 24 participants, no DNA sample was available for *MTHFR* genotyping and the *MTHFR* gene could not be amplified in one DNA sample; therefore, analyses using data on *MTHFR* genotype included 1,452 participants: 751 cases and 701 controls.

Questionnaires. Participants filled out self-administered questionnaires on diet, medical history, and several lifestyle factors, according to their habits in the year previous to their colonoscopy or complaints. Dietary intake was assessed with a standardized and validated semiquantitative food-frequency questionnaire that was originally developed for the Dutch cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC; ref. 28). Subsequently, intake of energy and nutrients was calculated using the Dutch food composition table. Folate intake was calculated using recently updated information of folate content in Dutch foods from Konings et al. (29). Vegetables, bread, and meat contributed more than 50% of folate intake. The reproducibility of these products as assessed with the questionnaire was for men 0.76, 0.86, and 0.68, respectively, and for women 0.65, 0.78, and 0.80, whereas the relative validities compared with 24-hour recalls were 0.31, 0.76, and 0.47 for men and 0.38, 0.78, and 0.70 for women (28). The main sources of vitamin B2 were milk and milk products, accounting for about 50% of intake. The reproducibility for milk and milk products was 0.71 for men and 0.79 for women; the relative validity was 0.73 for men and 0.78 for women (28). The Dutch EPIC food-frequency questionnaire has not yet been validated for folate intake.

***MTHFR* Genotyping.** To determine the *MTHFR C677T* polymorphism, we used the PCR-RFLP method described in detail by Frosst et al. (23) in DNA isolated from whole blood. PCR was done with internal negative controls. Laboratory staff was blinded to case-control status. To study reproducibility, 20% of the samples were analyzed in duplicate and yielded the same result. In addition, we participated in an external quality control program. Results showed a 100% match with expected genotype.

Statistical Analysis. To investigate the association between nutrient intake and colorectal adenomas, we used logistic regression models. Intakes of nutrients were adjusted for total energy intake using the linear residual regression method of Willett and Stampfer (30). Sex-specific tertiles of intake were calculated based on the distribution among controls. We calculated ORs and 95% CIs to estimate the relative risk of developing colorectal adenomas. Reference groups were those with the lowest nutrient intake. To examine the association between *MTHFR C677T* genotype and colorectal adenomas, we used individuals with the *MTHFR CC* genotype as reference.

We examined whether the associations with vitamin intake were modified by sex, alcohol intake, or smoking habits, but no differences were observed between strata. We examined if potential confounding factors (i.e., age, body mass index, physical activity, educational level, smoking, use of nonsteroid anti-inflammatory drugs, indication for colonoscopy, family history of colorectal cancer, use of contraceptives, hormone replacement therapy, outpatient clinic, and intake of fat, fiber, alcohol, folate, vitamin B2, vitamin B6, vitamin B12, calcium, total meat, organ meat, fruits, and vegetables) were associated both with colorectal adenomas and vitamin intake, and changed the crude estimates by more than 10% when added to the logistic regression models. The final logistic regression models included the covariate age; the vitamin B2 models also included dietary intake of folate and calcium.

Interaction between *MTHFR C677T* genotype and intake of vitamins was studied by stratification to genotype, using those categorized in the lowest tertile of intake and with the *MTHFR CC* genotype as reference, and by testing for different slopes associated with nutrient intake across genotype.

To test for linear trend, we modeled the tertile of nutrient intake as a continuous variable in the logistic regression model, in which each tertile was assigned its median value.

All tests of statistical significance were two sided and the significance level was set at 5%. We used Statistical Analysis Software (SAS version 8, SAS Institute, Gary, NC) for all analyses.

Results

In Table 1, characteristics of the study population are shown. Compared with controls, cases were more likely to be male, older, have a slightly higher body mass index, and smoke more. Furthermore, cases usually had a colonoscopy for screening rather than because of complaints, used less contraceptives and hormone replacement therapy, and had a higher intake of total energy, vegetables, fat, alcohol, vitamin B6, and folate. Most of these differences remained after standardization for age and sex.

Table 2 shows the associations between dietary intake of folate and vitamin B2 and colorectal adenoma risk for the whole study population and stratified by *MTHFR C677T* genotype. A high dietary folate intake was positively associated with risk of colorectal adenomas, with a borderline significant test for trend ($P = 0.054$). A high intake of vitamin B2 was inversely associated with colorectal adenoma risk, with a statistically significant test for trend. No association was observed between intake of vitamins B6 and B12 and colorectal adenomas (data not shown). ORs did not change substantially when we excluded those using multivitamin supplements (data not shown).

Compared with individuals with the *MTHFR CC* genotype, *MTHFR CT* and *TT* genotypes were not related to colorectal adenoma risk. The age- and sex-adjusted ORs (95% CIs) were 1.06 (0.84-1.33) and 0.96 (0.66-1.39), respectively (data not shown). For intake of folate (Table 2), vitamin B6, and vitamin B12 (data not shown), no effect modification by *MTHFR C677T* genotype was observed. The inverse association for vitamin B2 intake was present in all *MTHFR* genotypes, but seemed more pronounced among those with the *MTHFR TT* genotype. The interaction term did not reach statistical significance (Table 2).

Folate seemed to be a risk factor with low or medium vitamin B2 intake (Table 3). Moreover, the inverse association between vitamin B2 and colorectal adenomas was particularly seen when folate intake was relatively high. Statistically, the interaction was not significant ($P = 0.64$).

Table 1. Characteristics of the study population

	Cases (n = 768)	Controls (n = 709)	P
Female (%)	46.5	61.6	<0.01
Age (y)*	59.1 ± 10.1	51.5 ± 13.6	<0.01
Body mass index (kg/m^2)*	26.1 ± 3.8	25.5 ± 4.1	<0.01
Smoking status (% ever)	67.0	55.3	<0.01
Physical activity (% high)	29.8	33.5	0.13
Educational level (% low)	35.7	33.0	0.30
Family history of colorectal cancer (% yes)	23.5	20.1	0.12
Regular nonsteroid anti-inflammatory drug use (≥12 times/y; % yes)	25.1	28.5	0.14
Indication for colonoscopy (%)			
Complaints	47.7	76.7	
Screening	44.7	11.3	
Other/unknown	7.6	12.0	<0.01
Contraceptive use (% ever)†	65.2	77.6	<0.01
Hormone replacement therapy (% ever)‡	21.1	29.6	0.03
MTHFRC677T genotype (%)			
CC	44.7	45.8	
CT	45.0	43.1	
TT	10.3	11.1	0.72
Dietary intake			
Energy (kJ/d)*	8,703 ± 2,484	8,434 ± 2,498	0.04
Total vegetables (g/d)*	129 ± 53	121 ± 46	<0.01
Total fruits (g/d)*	192 ± 138	185 ± 130	0.30
Fat (g/d)*	83 ± 30	79 ± 29	0.03
Alcohol (g/d)§	9.5 (1.0;24.1)	4.2 (0.3;15.5)	<0.01
Vitamin B2 (mg/d)*	1.62 ± 0.57	1.59 ± 0.56	0.27
Vitamin B6 (mg/d)*	1.65 ± 0.48	1.59 ± 0.45	0.02
Folate (μg/d)*	200 ± 60	190 ± 53	<0.01
Vitamin B12 (μg/d)*	4.87 ± 2.63	4.52 ± 2.00	<0.01
Calcium (mg/d)*	1,095 ± 433	1,083 ± 403	0.57
Fiber (mg/d)*	23.6 ± 6.7	23.1 ± 6.7	0.12
Supplementary multivitamin use (% yes)	17.5	17.9	0.82
Supplementary B-vitamin use (% yes)	6.9	6.5	0.75

*Mean ± SD.
† Among women only.
‡ Among postmenopausal women only.
§Median (25th percentile; 75th percentile).

Discussion

In this endoscopy-based case-control study, we observed a slightly positive association between dietary folate intake and colorectal adenoma risk, which was especially apparent for those with low vitamin B2 intakes. *MTHFR* genotype did not modify this association. An inverse association between dietary intake of vitamin B2 and colorectal adenoma risk was found, which may especially be important among those with relatively high folate intake or those with the *MTHFR TT* genotype.

As far as we know, one other publication exists on the interaction between vitamin B2 and folate in colorectal carcinogenesis, in which there was a pattern of decreased adenoma risk among those with high intakes of folate and vitamin B2 compared with those with low intakes of folate and vitamin B2. However, in that study the intake of vitamin B2 was substantially higher than in our study (20).

In a rat model, it was shown that MTHFR was affected by riboflavin deficiency (5). However, this does not explain why folate intake increases colorectal adenoma risk in those with a low vitamin B2 status. We speculate that folate will become protective in colorectal carcinogenesis only when vitamin B2 intake is high enough. One *in vitro* study showed that at low riboflavin level, nuclear buds decreased significantly with increasing folic acid level, whereas at high riboflavin level, nuclear buds decreased even more with increasing folic acid level. In that study, the interaction between folic acid and riboflavin was statistically significant (31). These observations might explain why we cannot reproduce most American results, suggesting a protective role of folate intake in colorectal carcinogenesis; cereals in the United States have been enriched with vitamin B2 since 1943 (32), and mean vitamin B2 intake is about 28% higher than in the Netherlands (33, 34).

The positive, but statistically nonsignificant, association between dietary folate intake and colorectal adenoma risk conflicts with results from other studies showing an inverse association (10-20) or no association at all (21, 22). Our study population had a low folate intake and the range of intake was narrow compared with that in other studies. Supplement use is not common in the Netherlands; in the Dutch Food Consumption Survey 1998, using a 2-day dietary record, 9.5% of participants reported having used multivitamin supplements, including B-vitamin supplements, on one or both days (34). Furthermore, as vitamin intake from supplements was not calculated in our study, we focused on dietary intake. In some studies, the inverse association between folate intake and colorectal adenoma risk weakened when the analyses were restricted to dietary folate (10, 24). A limitation of the present study and many other epidemiologic studies to date, is the use of a food-frequency questionnaire for the assessment of dietary folate intake. This EPIC questionnaire was not validated for folate intake. However, most food-frequency questionnaires that were validated for folate intake show poor correlation between erythrocyte folate and dietary folate intake (15, 35, 36). Validations for total folate intake show higher correlations (10).

Furthermore, the methods of assessment of folate contents from foods may differ between studies. In our study, we used data of folate contents in foods assessed using a high-performance liquid chromatography–based method, which overall leads to about 25% lower estimates of folate contents than data based on microbiological assays (29). However, in the Netherlands Cohort Study on Diet and Cancer, which used the same method of folate measurement, an inverse, although not statistically significant, association between

Table 2. Association between dietary intake of folate and vitamin B2 and colorectal adenomas, stratified by *MTHFR C677T* genotype

	MTHFR genotype		Dietary intake (tertiles)*			*P* trend	
			Low	Medium	High		
Folate†	All						
		N cases/controls	197/236	276/237	295/236		
		OR (95% CI)	1 (reference)	1.29 (0.98-1.69)	1.32 (1.01-1.73)	0.054	
	CC						
		N cases/controls	78/119	123/104	135/98		
		OR (95% CI)	1 (reference)	1.67 (1.12-2.51)	1.77 (1.18-2.65)	0.01	
	CT						
		N cases/controls	88/84	124/106	126/112		
		OR (95% CI)	1.55 (1.00-2.39)	1.59 (1.06-2.39)	1.52 (1.02-2.27)	0.88	
	TT						
		N cases/controls	25/30	25/23	27/25		
		OR (95% CI)	1.22 (0.65-2.30)	1.41 (0.73-2.74)	1.43 (0.75-2.73)	0.69	
P interaction, gene-nutrient							0.34
Vitamin B2‡	All						
		N cases/controls	250/237	288/235	230/237		
		OR (95% CI)	1 (reference)	0.84 (0.64-1.10)	0.51 (0.36-0.73)	0.0002	
	CC						
		N cases/controls	102/114	134/102	100/105		
		OR (95% CI)	1 (reference)	1.04 (0.69-1.55)	0.57 (0.36-0.90)	0.01	
	CT						
		N cases/controls	115/99	115/105	108/98		
		OR (95% CI)	1.21 (0.81-1.81)	0.84 (0.56-1.27)	0.68 (0.43-1.08)	0.09	
	TT						
		N cases/controls	26/22	34/24	17/32		
		OR (95% CI)	1.22 (0.63-2.38)	1.19 (0.64-2.22)	0.32 (0.16-0.67)	0.02	
P interaction, gene-nutrient							0.13

NOTE: Intake of folate and vitamin B2 was adjusted for total energy intake, according to Willett and Stampfer (30).
*Cut points for tertiles of daily dietary intake—women: vitamin B2, 1.27 mg/1.65 mg; folate, 160 μg/190 μg; men: vitamin B2, 1.51 mg/1.92 mg; folate, 191 μg/220 μg.
† Adjusted for age.
‡ Adjusted for age and dietary folate and calcium intake.

folate and colon cancer in men and women and between folate and rectal cancer in men was suggested. The intake of folate was about 210 μg/d, which is somewhat higher than in our study (37).

An alternative explanation for the positive association between folate intake and colorectal adenomas as found in our study might have been consumption of liver. Liver contains not only a high level of folate but also of carcinogens and thus may drive up the ORs for folate. Consumption of liver was not specifically assessed in our study. However, total organ meat was assessed and did not confound the results. Therefore, we assume that the influence of liver consumption, if present at all, will be small.

We found an inverse relationship between vitamin B2 intake and colorectal adenoma risk. An explanation may be that vitamin B2 deficiency reduces MTHFR activity (5). An inverse association was also found in one other study (20), whereas another study did not find an association (11).

We did not find an indication that *MTHFR C677T* genotype modifies the association between intake of folate and colorectal adenoma risk, which is in line with some studies (17, 19), but not with others (13, 18, 24, 26), not depending on study size. Our data suggest a nonsignificant interaction between vitamin B2 intake and *MTHFR C677T* genotype in colorectal adenoma occurrence; the inverse association between vitamin B2 intake and colorectal adenomas seemed more pronounced among *MTHFR TT* individuals. In a study examining the structure and properties of MTHFR from *Escherichia coli*, it was found that the *E. coli MTHFR Ala177Val* polymorphism, corresponding to the human *C677T* polymorphism, increases the tendency for MTHFR to lose its flavin adenine dinucleotide cofactor (6). This finding was also observed in recombinant human *MTHFR*, although more subtle (7), which may explain our finding of an interaction between vitamin B2 intake and *MTHFR* genotype. In human studies, it was observed that the homocysteine-lowering effect

Table 3. Interaction between dietary intake of folate and vitamin B2 in colorectal adenoma risk

Dietary vitamin B2 intake (tertiles)	Dietary folate intake (tertiles)			*P* trend
	Low	Medium	High	
Low				
N cases/controls	97/110	89/78	64/49	
OR (95% CI)	1 (reference)	1.26 (0.82-1.94)	1.34 (0.82-2.18)	0.22
Medium				
N cases/controls	67/78	111/90	110/67	
OR (95% CI)	0.73 (0.46-1.15)	1.04 (0.68-1.58)	1.42 (0.92-2.19)	0.01
High				
N cases/controls	33/48	76/69	121/120	
OR (95% CI)	0.56 (0.31-1.01)	0.74 (0.45-2.22)	0.69 (0.44-1.07)	0.56
P trend	0.31	0.01	0.02	
P interaction				0.64

NOTE: Intake of folate and vitamin B2 was adjusted for total energy intake, according to Willett and Stampfer (30), and for age and dietary calcium intake.

of vitamin B2 was essentially confined to subjects carrying the *MTHFR TT* genotype (9, 38), or even to subjects who carry the *MTHFR TT* genotype and have low folate status (39).

Case-control studies may be limited by selection and information bias. As screening for colorectal cancer is not common in the Netherlands, most endoscopies are conducted for bowel complaints, which may influence dietary patterns and introduce information bias. However, when we excluded people who had changed their dietary habits because of these complaints (166 cases and 232 controls), it did not affect the results. Information bias might also be caused by the fact that we included prevalent cases, but when we excluded prevalent cases (n = 363), results were essentially the same.

In summary, the results from this study do not provide evidence for a decreased colorectal adenoma risk for subjects with high levels of folate intake. Folate seems to be a risk factor for colorectal adenomas, especially when vitamin B2 intake is low. This may particularly be relevant for populations where products are not (yet) or only recently enriched with B-vitamins, such as the Netherlands.

Additionally, the study indicates an inverse association between vitamin B2 intake and colorectal adenoma risk, especially with higher folate intake. This study indicates that there may be an interplay between *MTHFR C677T* genotype and intake of vitamin B2, but not of folate, in association with colorectal adenoma risk. Although the study does not provide enough evidence to draw firm conclusions, it brings an interesting speculation about the interactive roles of folate and vitamin B2 in colorectal carcinogenesis, which should be further elucidated.

Acknowledgments

We are grateful to all the people who kindly participated in this study. The participants were recruited with help from the endoscopy staff of the following hospitals in the Netherlands: Slingeland Ziekenhuis (Doetinchem), Ziekenhuis Gelderse Vallei (Ede), Radboud University Nijmegen Medical Centre (Nijmegen), Antonius Ziekenhuis (Nieuwegein), Meander Medisch Centrum (Amersfoort), Ziekenhuis Rijnstate (Arnhem), Ziekenhuis Rivierenland (Tiel), Slotervaart Ziekenhuis (Amsterdam), Jeroen Bosch Ziekenhuis ('s Hertogenbosch), and Canisius Wilhelmina Ziekenhuis (Nijmegen). We thank Maria van Vugt and Elly Monster (Division of Human Nutrition, Wageningen University, the Netherlands) for collecting blood samples and assistance with the conduct of this study.

References

1. Neugut AI, Jacobson JS, Rella VA. Prevalence and incidence of colorectal adenomas and cancer in asymptomatic persons. Gastrointest Endosc Clin N Am 1997;7:387–99.
2. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
3. Peipins LA, Sandler RS. Epidemiology of colorectal adenomas. Epidemiol Rev 1994;16:273–97.
4. Ryan BM, Weir DG. Relevance of folate metabolism in the pathogenesis of colorectal cancer. J Lab Clin Med 2001;138:164–76.
5. Bates CJ, Fuller NJ. The effect of riboflavin deficiency on methylenetetrahydrofolate reductase (NADPH) (EC 1.5.1.20) and folate metabolism in the rat. Br J Nutr 1986;55:455–64.
6. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol 1999;6:359–65.
7. Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci U S A 2001;98:14853–8.
8. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr 2000;130:129–32.
9. Hustad S, Ueland PM, Vollset SE, Zhang Y, Bjorke-Monsen AL, Schneede J. Riboflavin as a determinant of plasma total homocysteine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. Clin Chem 2000;46:1065–71.
10. Giovannucci E, Stampfer MJ, Colditz GA, et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 1993;85:875–84.
11. Benito E, Cabeza E, Moreno V, Obrador A, Bosch FX. Diet and colorectal adenomas: a case-control study in Majorca. Int J Cancer 1993;55:213–9.
12. Boutron-Ruault MC, Senesse P, Faivre J, Couillault C, Belghiti C. Folate and alcohol intakes: related or independent roles in the adenoma-carcinoma sequence? Nutr Cancer 1996;26:337–46.
13. Ulvik A, Evensen ET, Lien EA, et al. Smoking, folate and methylenetetrahydrofolate reductase status as interactive determinants of adenomatous and hyperplastic polyps of colorectum. Am J Med Genet 2001;101:246–54.
14. Martinez ME, Henning SM, Alberts DS. Folate and colorectal neoplasia: relation between plasma and dietary markers of folate and adenoma recurrence. Am J Clin Nutr 2004;79:691–7.
15. Bird CL, Swendseid ME, Witte JS, et al. Red cell and plasma folate, folate consumption, and the risk of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 1995;4:709–14.
16. Tseng M, Murray SC, Kupper LL, Sandler RS. Micronutrients and the risk of colorectal adenomas. Am J Epidemiol 1996;144:1005–14.
17. Chen J, Giovannucci E, Hankinson SE, et al. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis 1998;19:2129–32.
18. Marugame T, Tsuji E, Kiyohara C, et al. Relation of plasma folate and *methylenetetrahydrofolate reductase* C677T polymorphism to colorectal adenomas. Int J Epidemiol 2003;32:64–6.
19. Giovannucci E, Chen J, Smith-Warner SA, et al. Methylenetetrahydrofolate reductase, alcohol dehydrogenase, diet, and risk of colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2003;12:970–9.
20. Boyapati SM, Bostick RM, McGlynn KA, et al. Folate intake, MTHFR C677T polymorphism, alcohol consumption, and risk for sporadic colorectal adenoma (United States). Cancer Causes Control 2004;15:493–501.
21. Baron JA, Sandler RS, Haile RW, Mandel JS, Mott LA, Greenberg ER. Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. J Natl Cancer Inst 1998;90:57–62.
22. Pufulete M, Al-Ghnaniem R, Leather AJ, et al. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. Gastroenterology 2003;124:1240–8.
23. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
24. Ulrich CM, Kampman E, Bigler J, et al. Colorectal adenomas and the C677T *MTHFR* polymorphism: evidence for gene-environment interaction? Cancer Epidemiol Biomarkers Prev 1999;8:659–68.
25. Marugame T, Tsuji E, Inoue H, et al. Methylenetetrahydrofolate reductase polymorphism and risk of colorectal adenomas. Cancer Lett 2000;151:181–6.
26. Levine AJ, Siegmund KD, Ervin CM, et al. The methylenetetrahydrofolate reductase 677C→T polymorphism and distal colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 2000;9:657–63.
27. Tiemersma EW, Wark PA, Ocké MC, et al. Alcohol consumption, alcohol dehydrogenase 3 polymorphism, and colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2003;12:419–25.
28. Ocké MC, Bueno-de-Mesquita HB, Goddijn HE, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26 Suppl 1: S37–48.
29. Konings EJ, Roomans HH, Dorant E, Goldbohm RA, Saris WH, van den Brandt PA. Folate intake of the Dutch population according to newly established liquid chromatography data for foods. Am J Clin Nutr 2001; 73:765–76.
30. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
31. Kimura M, Umegaki K, Higuchi M, Thomas P, Fenech M. Methylenetetrahydrofolate reductase C677T polymorphism, folic acid and riboflavin are important determinants of genome stability in cultured human lymphocytes. J Nutr 2004;134:48–56.
32. Backstrand JR. The history and future of food fortification in the United States: a public health perspective. Nutr Rev 2002;60:15–26.
33. Alaimo K, McDowell MA, Briefel RR, et al. Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988–91. Adv Data 1994;1–28.
34. Zo eet Nederland 1998. Resultaten van de Voedselconsumptiepeiling 1998. Voedingscentrum Den Haag 1998.
35. Pufulete M, Emery PW, Nelson M, Sanders TA. Validation of a short food frequency questionnaire to assess folate intake. Br J Nutr 2002;87:383–90.
36. Drogan D, Klipstein-Grobusch K, Wans S, Luley C, Boeing H, Dierkes J. Plasma folate as marker of folate status in epidemiological studies: the European Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Br J Nutr 2004;92:489–96.
37. Konings EJM, Goldbohm RA, Brants HAM, Saris WHM, van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. Cancer 2002;95:1421–33.
38. McNulty H, McKinley MC, Wilson B, et al. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. Am J Clin Nutr 2002;76:436–41.
39. Jacques PF, Kalmbach R, Bagley PJ, et al. The relationship between riboflavin and plasma total homocysteine in the Framingham Offspring cohort is influenced by folate status and the C677T transition in the methylenetetrahydrofolate reductase gene. J Nutr 2002;132:283–8.

Dietary Intake of Folate and Riboflavin, *MTHFR C677T* Genotype, and Colorectal Adenoma Risk: A Dutch Case-Control Study

Maureen van den Donk, Brian Buijsse, Saskia W. van den Berg, et al.

Cancer Epidemiol Biomarkers Prev 2005;14:1562-1566.