Research Article

Cancer Epidemiology, Biomarkers & Prevention

Plasma Vitamins B2, B6, and B12, and Related Genetic Variants as Predictors of Colorectal Cancer Risk

Simone J.P.M. Eussen[1], Stein Emil Vollset[1,2], Steinar Hustad[1,3], Øivind Midttun[1,4], Klaus Meyer[1,4], Åse Fredriksen[1,4], Per Magne Ueland[1,4], Mazda Jenab[5], Nadia Slimani[5], Paolo Boffetta[5], Kim Overvad[6], Ole Thorlacius-Ussing[7], Anne Tjønneland[8], Anja Olsen[8], Françoise Clavel-Chapelon[9], Marie-Christine Boutron-Ruault[9], Sophie Morois[9], Cornelia Weikert[10], Tobias Pischon[10], Jakob Linseisen[11,12], Rudolf Kaaks[11], Antonia Trichopoulou[13,14], Demosthenes Zilis[13], Michael Katsoulis[13], Domenico Palli[15], Valeria Pala[16], Paolo Vineis[17,33], Rosario Tumino[18], Salvatore Panico[19], Petra H.M. Peeters[20,33], H. Bas Bueno-de-Mesquita[21], Fränzel J.B. van Duijnhoven[21], Guri Skeie[22], Xavier Muñoz[23], Carmen Martínez[24], Miren Dorronsoro[25], Eva Ardanaz[26], Carmen Navarro[27], Laudina Rodríguez[28], Bethany VanGuelpen[29], Richard Palmqvist[29], Jonas Manjer[30], Ulrika Ericson[31], Sheila Bingham[32†], Kay-Tee Khaw[32], Teresa Norat[33], and Elio Riboli[33]

Abstract

Background: B-vitamins are essential for one-carbon metabolism and have been linked to colorectal cancer. Although associations with folate have frequently been studied, studies on other plasma vitamins B2, B6, and B12 and colorectal cancer are scarce or inconclusive.

Methods: We carried out a nested case-control study within the European Prospective Investigation into Cancer and Nutrition, including 1,365 incident colorectal cancer cases and 2,319 controls matched for study center, age, and sex. We measured the sum of B2 species riboflavin and flavin mononucleotide, and the sum of B6 species pyridoxal 5′-phosphate, pyridoxal, and 4-pyridoxic acid as indicators for vitamin B2 and B6 status, as well as vitamin B12 in plasma samples collected at baseline. In addition, we determined eight polymorphisms related to one-carbon metabolism. Relative risks for colorectal cancer were estimated using conditional logistic regression, adjusted for smoking, education, physical activity, body mass index, alcohol consumption, and intakes of fiber and red and processed meat.

Results: The relative risks comparing highest to lowest quintile were 0.71 [95% confidence interval (95% CI), 0.56-0.91; P_{trend} = 0.02] for vitamin B2, 0.68 (95% CI, 0.53-0.87; P_{trend} <0.001) for vitamin B6, and 1.02 (95% CI, 0.80-1.29; P_{trend} = 0.19) for vitamin B12. The associations for vitamin B6 were stronger in males who consumed ≥30 g alcohol/day. The polymorphisms were not associated with colorectal cancer.

Conclusions: Higher plasma concentrations of vitamins B2 and B6 are associated with a lower colorectal cancer risk.

Impact: This European population-based study is the first to indicate that vitamin B2 is inversely associated with colorectal cancer, and is in agreement with previously suggested inverse associations of vitamin B6 with colorectal cancer. *Cancer Epidemiol Biomarkers Prev; 19(10); 2549–61. ©2010 AACR.*

Introduction

The one-carbon metabolism is related to carcinogenesis because of its involvement in the synthesis of purines and pyrimidines for subsequent DNA synthesis, and in the synthesis of methionine for DNA methylation. Aberrations in DNA synthesis and DNA methylation may contribute to colorectal carcinogenesis.

Authors' Affiliations: [1]LOCUS for homocysteine and related vitamins, Institute of Medicine, University of Bergen, and Haukeland University Hospital, [2]Medical Birth Registry, and Norwegian Institute of Public Health and Primary Health Care, [3]Hormone Laboratory, Haukeland University Hospital, and [4]Bevital A/S, Bergen, Norway; [5]International Agency for Research on Cancer (IARC-WHO), Lyon, France; Departments of [6]Clinical Epidemiology and [7]Surgical Gastroenterology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; [8]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; [9]INSERM (Institut National de la Santé et de la Recherche Médicale), and Institut Gustave Roussy, Villejuif, France; [10]Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany; [11]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany; [12]Institute of Epidemiology, Helmholtz Zentrum München, German Research Centre for Environmental Health (HMGU), Neuherberg, Germany; [13]Department of Hygiene and Epidemiology, Medical School University of Athens, Athens, Greece; [14]Hellenic Health Foundation, Greece; [15]Molecular and Nutritional Epidemiology Unit, Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Florence, Italy; [16]Department of Preventive and Predictive Medicine Nutritional Epidemiology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; [17]Department of Biomedical Science, University of Torino, Turin, Italy; [18]Cancer Registry Azienda Ospedaliera Civile-M.P. Arezzo, Ragusa, Italy; [19]Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy; [20]Julius Center for Health Sciences and Primary Care, University Medical Center,

B-vitamins and related genetic polymorphisms are essential for the one-carbon metabolism, and may therefore be associated with colorectal cancer (1). Among the B-vitamins, folate has been studied most extensively in relation to colorectal cancer. The majority of studies on folate intake indicate a 20% to 40% colorectal cancer risk reduction in individuals with the highest compared with the lowest intake, whereas associations between plasma concentrations of folate and colorectal cancer risk are inconsistent (1). Other B-vitamins, such as vitamins B2, B6, and B12, are also involved in the one-carbon metabolism.

Vitamins B2 and B6 are related because the interconversion of some vitamin B6 species require the vitamin B2 forms flavin mononucleotide (FMN) and flavin dinucleotide (FAD) as cofactors (2). Moreover, vitamin B2 serves as a cofactor for the methyl-metabolizing enzymes methylenetetrahydrofolate reductase (MTHFR), which regenerates 5-methyltetrahydrofolate from tetrahydrofolate, and methionine synthase reductase (MTRR), which activates methionine synthase (MS). Vitamin B6 is a cofactor for the enzyme cystathionine β-synthase (CBS), which is involved in the transsulfuration pathway where homocysteine is converted into cystathionine. Vitamin B12 acts as a cofactor for MTRR and MS, the latter catalyzing the remethylation of homocysteine to methionine. Transcobalamin II (TCN2) is essential for the uptake of vitamin B12 from the intestine (Fig. 1). Suboptimal concentrations of the B-vitamin cofactors as well as related genetic polymorphisms may affect the activity of these enzymes.

The majority of studies on associations of B2 (3–9) and vitamin B12 (4–14) with colorectal cancer have reported null findings. A recent meta-analysis of nine studies on vitamin B6 intake and four studies on plasma vitamin B6 concentrations revealed inverse associations with colorectal cancer risk (15). Previous research on associations between genetic variants and colorectal cancer risk has focused mainly on the *MTHFR 677C →T* and *1298C→A* polymorphisms, which were generally inversely associated with colorectal cancer (1). In contrast, genetic variants of *MTR* (14, 16, 17), *MTRR* (4, 18, 19), *CBS* (4, 20–23), and *TCN2* (18, 19) have been less studied in relation to colorectal cancer, and show inconsistent associations. In addition to potential interactions between B-vitamins and Single Nucleotide Polymorphisms (SNPs; refs. 24, 25), there is also some evidence for an interaction between plasma vitamin B6 and alcohol consumption (26).

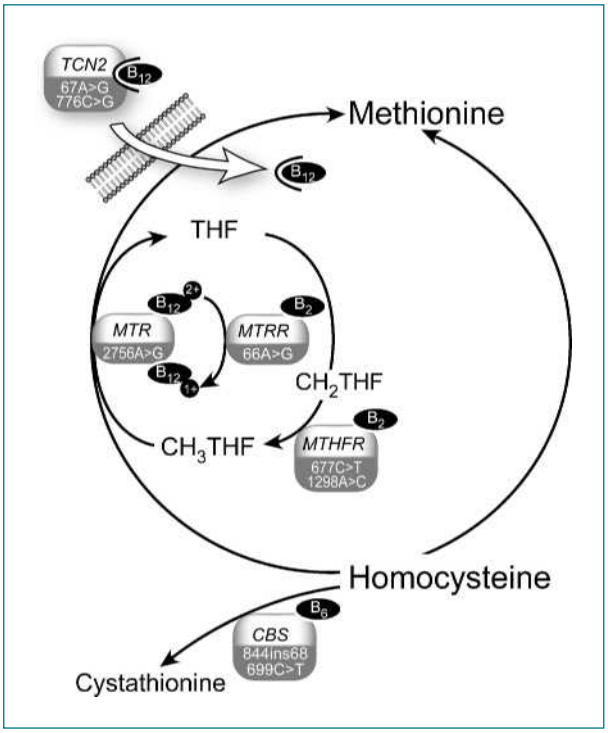

Figure 1. One-carbon metabolism and related enzymes and genetic polymorphisms. *CBS*, cystathionine β-synthase; CH_2THF, methylenetetrahydrofolate; CH_3THF, methyltetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase (provision of 5-methylfolate for homocysteine remethylation); MTR, methionine synthase (remethylation of homocysteine to methionine); MTRR, methionine synthase reductase (activation of methionine synthase); THF, tetrahydrofolate; TCN2, transcobalamin-II (vitamin B12 transport).

Studies on plasma vitamin B2 (9), vitamin B6 (15), and plasma vitamin B12 (9, 10) concentrations and colorectal cancer risk are sparse. In addition, associations between B-vitamins and colorectal cancer risk may be modified by SNPs related to one-carbon metabolism and alcohol consumption (24, 26). Therefore, we conducted a large nested case-control study (including 1,365 colorectal cancer cases and 2,319 matched controls) within the European Prospective Investigation into Cancer and Nutrition (EPIC), which is sufficiently large to address these questions.

Utrecht, the Netherlands; [21]National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; [22]Institute of Community Medicine, University of Tromsø, Tromsø, Norway; [23]Department of Epidemiology and Translational Research Laboratory, IDIBELL- Catalan Institute of Oncology, Barcelona, Spain; [24]Andalusian School of Public Health, Granada, Spain; [25]Department of Public Health of Guipuzkoa, San Sebastian, Spain; [26]Public Health Institute of Navarra, Pamplona, Spain, and CIBER Epidemiología y Salud Pública (CIBERESP), Spain; [27]Department of Epidemiology, Health Council of Murcia and CIBER Epidemiología y Salud Pública (CIBERESP), Spain; [28]Public Health Directorate, Health and Health Care Services Council, Asturias, Spain; [29]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden; [30]Department of Surgery, University Hospital, Malmö, Sweden; [31]Department of Clinical Sciences in Malmö/Nutrition Epidemiology, Lund University, Sweden; [32]MRC Dunn Human Nutrition Unit, Cambridge, UK&MRC Centre for Nutritional Epidemiology in Cancer Prevention and Survival, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; and [33]Department of Epidemiology and Public Health, Imperial College, London, United Kingdom.

[†]Deceased.

Corresponding Author: Simone J.P.M. Eussen, Section for Pharmacology, Department of Internal Medicine, University of Bergen, 5021 Laboratory Building, 9th floor, Bergen, Norway. Phone: 47-55975786; Fax: 47-55974605. E-mail: Simone.Eussen@farm.uib.no

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Materials and Methods

Study population and collection of blood samples

The design and methods of EPIC have previously been described (27). Briefly, the EPIC cohort included participants from 23 centers in 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom). Between 1992 and 1998, country-specific dietary questionnaires, standardized lifestyle and personal history questionnaires, and anthropometric data were collected from all participants, and a blood sample were taken from 80% of the cohort members. Follow-up is based on population cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom) or through health insurance records, pathology registries, and active contact with study subjects or next of kin (France, Germany, and Greece). The follow-up period for the present study was for cases included in reports received at the International Agency for Research on Cancer (IARC; Lyon, France) until June 2003 representing centers using cancer registry data, and until March 2004 for France, Germany, and Greece.

Fasting (46%) or nonfasting (54%) blood samples of ≥30 mL were drawn. B-vitamins and related metabolites did not significantly differ across fasting and nonfasting participants. Blood samples were stored at 5°C to 10°C while protected from light and transported to local laboratories for processing and aliquoting (27). Exceptions from this procedure were the EPIC-Oxford and EPIC-Norway centers, where whole blood samples were collected through a network of general practitioners (Oxford and Norway) and health-conscious people (Oxford) and transported to a central laboratory via mail. The whole blood samples were protected from light, but were exposed to ambient temperatures for up to 48 hours. As B-vitamins are partly degraded by such handling, all EPIC-Oxford (55 cases, 107 controls) and EPIC-Norway (5 cases, 9 controls) samples were excluded from the present analyses.

In all countries, except Denmark and Sweden, blood was separated into 0.5 mL fractions (serum, plasma, red cells and buffy coat for DNA extraction). Each fraction was placed into straws, which were heat sealed and stored in liquid nitrogen (−196°C). One half of all aliquots were stored at local study centers and the other half in the central EPIC biorepository at the IARC. In Denmark, blood fraction aliquots of 1.0 mL were stored locally at −150°C under nitrogen vapor. In Sweden, erythrocyte, plasma, and buffy coat samples were fractioned prior to freezing, and stored locally in −80°C freezers. This study was approved by the Ethical Review Board of the IARC and those of all EPIC centers. All EPIC participants have provided written consent for the use of their blood samples and all data.

Nested case-control study design and selection of study subjects

Colon cancer was defined as tumors in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, and descending and sigmoid colon (C18.0-C18.7 as per the 10th revision of the International Statistical Classification of Diseases, Injury and Causes of Death), as well as tumors that are overlapping or unspecified (C18.8 and C18.9). Colorectal cancer was defined as a combination of colon and rectal cancer. Cancers of the rectum were defined as tumors occurring at the rectosigmoid junction (C19) or rectum (C20). The present study included 1,365 incident colorectal cancer cases (colon, n = 846; rectal, n = 519). The distribution of colon/rectal cases by country was 204/174 in Denmark, 100/84 in Sweden, 30/3 in France, 14/13 in Greece, 98/61 in Germany, 106/43 in Italy, 102/49 in the Netherlands, 78/43 in Spain, and 114/49 in the United Kingdom.

Controls with available blood samples were randomly selected from cohort members still alive and free of cancer at the time of diagnosis of the cases. Controls were matched to cases by gender, age (±2.5 years), and study center (to account of center-specific differences in questionnaire design, blood collection procedures, etc.). An exception were Danish cases (n = 378) and controls (n = 373), who were posthoc matched using the "greedy" algorithm, a macro (gmatch) provided by the Mayo Clinic College of Medicine (28, 29) to be run in SAS. The greedy algorithm sorts cases and controls randomly and the macro matches the first case with the closest available control according to specified matching criteria, which is repeated until all cases are matched. The mean (range) difference in age between cases and controls in the overall study, except for the Danish population, and between Danish cases and controls within each caseset, was 0 (−2.4 to 1.8) and −1.03 (−5.0 to 4.9) years, respectively.

Laboratory measurements

Vitamin B2 measures included plasma concentrations of riboflavin and FMN, and pyridoxal' 5-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid (PA) were measured for vitamin B6 status. All B2 and B6 vitamers were determined by liquid chromatography-tandem mass spectrometry in the same laboratory (30). For PLP, PL, PA, and riboflavin, the within- and between-day coefficients of variation (CV) were <11%, whereas for FMN the CVs were 12% to 22%. Within- and between-day CVs for total-B2 were 7% to 11% and for total B6 they were 3% to 7%. Plasma vitamin B12 was determined by a *Lactobacillus leichmannii* microbiological assay (31), and plasma methylmalonic acid (MMA; inverse marker for vitamin B12 status) was measured with a method based on methylchloroformate derivatization and gas chromatography-mass spectrometry (32). Vitamin B12 and MMA concentrations were analyzed in the same laboratory, with within- and between-day CVs of <5% (32). Unspiked and spiked plasma samples with unknown endogenous concentrations were used for these experiments.

Eight polymorphisms of genes encoding enzymes involved in the one-carbon metabolism were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (33, 34). These included

cystathionine β-synthase (*CBS 699C→T;* rs234706, and the *CBS 844ins68 insertion)*, methylenetetrahydrofolate reductase (*MTHFR 677C→T*; rs1801133 and *MTHFR 1298A→C*; rs1801131), methionine synthase (*MTR 2756A→G*; rs1805087), methionine synthase reductase (*MTRR 66A→G*; rs1801394), and transcobalamin II (*TCN2 67A→G*; rs RsaI and *776C→G*; rs1801198).

Statistical methods

Because riboflavin and FMN are interconvertable (35), as are PLP and PL (36), and PA is formed from PL, we considered the sum of riboflavin and FMN as a measure for vitamin B2 status, and the sum of PLP, PL, and PA as a measure of vitamin B6 status. Both the summary variables as well as individual vitamin B2 and B6 species are presented. Differences in concentrations of vitamins B2, B6, and B12, and MMA between different groups were assessed by Mann-Whitney *U* tests or χ^2 tests where appropriate.

Relative risks [risk ratios (RR)] and 95% confidence intervals (95% CI) for colorectal cancer risk in relation to plasma B-vitamins were estimated by conditional logistic regression using the SAS LOGISTIC procedure, stratified by the case-control set. Relative risks for colorectal cancer were examined across quintiles with cutoff points based on the distribution of the B-vitamins in all 2,319 controls combined. Potential confounders included smoking status (never, former, current, missing), alcohol consumption (continuous), dietary fiber (continuous), intake of red and processed meat (continuous), physical activity (inactive, moderately inactive, moderately active, active), educational level (none, primary school completed, technical/professional school, secondary school, university degree, not specified), and body mass index (kg/m^2). Although none of these variables substantially altered the crude risk estimates, we present results of both the univariate and adjusted models. The adjusted models were additionally adjusted for mutual B-vitamins and folate. Likelihood ratio tests were used to assess linear trends across categories using values for quintile categories as the quantitative score of exposure. We also tested for effect modification by European region [north (Denmark, Sweden) versus central (France, United Kingdom, the Netherlands, Germany) versus south (Italy, Spain, Greece)], time from blood donation to cancer diagnosis (median follow-up time ≤3.6 years versus >3.6 years), age (≤60 years versus >60 years), sex, alcohol intake (0-30 g/day versus ≥30 g/day), and plasma folate concentrations (≤11.3 nmol/L versus >11.3 nmol/L; based on median concentrations in this cohort). Effect modification was tested by adding the product term of the B-vitamins (as categorical variables) and potential effect modifiers in the model. To investigate whether potential effect modification by alcohol intake was different for males and females, analyses were done by adding the product term of the B-vitamins, alcohol intake, and sex in the model (while retaining lower-order terms).

Associations between the polymorphisms and colorectal cancer risk were studied with conditional logistic regression. The risk estimates were calculated with the wild types (the most common genotypes in the natural population) as the reference categories. A trend test with equally spaced integer weights (0, 1, 2) for the genotypes was used to summarize the effect of each polymorphism. Effect modification of the SNP-colorectal cancer risk associations by B-vitamin concentrations and alcohol consumption were studied with conditional logistic regression, but by stratifying on country instead of the matched sets and with age and sex as covariates.

All statistical tests were done with SAS statistical software, version 9.1. Statistical tests were two-tailed and $P < 0.05$ was considered statistically significant.

Results

Characteristics of cases and controls

The distribution of sex, and mean age at recruitment was comparable among the 1,365 cases and 2,319 controls (Table 1). The median follow-up time between blood donation and the diagnosis of colorectal cancer was 3.6 years (range, 0.0-10.3). Apart from the concentration of the B6 vitamer PLP, which was higher in cases compared with their matched controls ($P = 0.02$), the sum variables of vitamins B2 and B6, vitamin B12 and MMA concentrations were similar between cases and controls. The distribution of vitamin B2 and B6 among controls was skewed, with a longer tail at higher concentrations. Concentrations of the vitamin B6 species correlated strongly with each other after adjustment for age, sex, and study center (Spearman correlation coefficients ranged from 0.64 to 0.74, all correlations $P < 0.01$; data not shown). In addition, riboflavin correlated with FMN (0.34; $P < 0.01$), and the correlation between vitamin B12 and MMA was −0.24 ($P < 0.01$).

Compared with women, among men concentrations of the vitamin B2 sum and vitamin B12 were lower, whereas the vitamin B6 sum concentration was higher (Table 2). Participants <60 years had lower vitamin B2 sum and MMA concentrations, and higher vitamin B12 concentrations, as compared with older individuals. Among current smokers, the vitamin B2 sum, vitamin B6 sum, and B12 concentrations were lower compared with former and never smokers. The sum variables of vitamin B2 and B6 were also lower among participants from southern European countries compared with those from central and northern European countries. Individuals consuming alcohol of ≥30 g/day had lower concentrations of the vitamin B2 sum and vitamin B12 compared with those drinking less, except for vitamin B6. Finally, vitamin B12 concentrations were slightly higher in those with the variant *TCN2* 677GG genotype, whereas concentrations of other vitamins did not differ according to genotype (*P* for all differences >0.05; data not shown).

Table 1. Characteristics of incident colorectal cancer cases and their matched controls

	Cases	Controls	$P_{difference}$
Number of individuals	1,365	2,319	
Sex, female, *n* (%)	697 (51)	1213 (52)	0.54
Mean age in years (min-max)			
At recruitment	58.9 (30.1-76.9)	58.7 (30.0-76.6)	0.40
At blood donation	59.1 (36.8-77.0)	58.9 (36.6-76.6)	0.44
At diagnosis	62.8 (37.7-81.2)	N.a.	
Lag time	3.6 (0.01-10.3)	N.a.	
Smoking status, *n* (%)			0.02
Never	559 (41)	1,024 (44)	
Former	447 (33)	774 (33)	
Current	344 (25)	508 (22)	
Alcohol drinking, *n* (%)			<0.01
Abstainers	93 (7)	166 (7)	
1-30 g/day	983 (72)	1784 (77)	
≥30 g/day	281 (21)	372 (16)	
Median (5th-95th percentile)			
Vitamin B2 sum, nmol/L	20.6 (9.4-65.7)	20.6 (10.1-74.7)	0.36
Riboflavin, nmol/L	14.8 (6.1-60.6)	14.8 (5.9-53.7)	0.37
FMN, nmol/L	5.4 (2.1-16.3)	5.3 (1.9-15.3)	0.20
Vitamin B6 sum, nmol/L	63 (31-197)	65 (32-222)	0.20
PLP, nmol/L	33.2 (14.2-111)	32.1 (13.2-93.8)	<0.01
PL, nmol/L	13.4 (6.7-44.0)	13.1 (6.2-38.3)	0.16
PA, nmol/L	16.5 (7.8-72.5)	17.3 (7.8-74.3)	0.17
Cobalamin, pmol/L	288 (162-498)	288 (161-501)	0.97
MMA, μmol/L	0.17 (0.12-0.32)	0.17 (0.12-0.30)	0.32

NOTE: Differences in plasma concentrations of the vitamins B2, B6, and B12, and MMA were assessed by Wilcoxon signed rank test, whereas categorical variable differences were assessed by McNemar's tests.
Abbreviation: N.a., not applicable.

Associations between B-vitamins and colorectal cancer

Matched analyses revealed that vitamins B2 and B6 were inversely associated with colorectal cancer, with RRs per quintile of 0.94 (95% CI, 0.89-0.99; P_{trend} = 0.02) for the sum of vitamin B2, and 0.94 (95% CI, 0.89-0.99; P_{trend} = 0.01) for the sum of vitamin B6 (Table 3). These associations were similar after further adjustment. Regarding the individual vitamers, risk reductions were strongest for FMN (5th versus 1st quintile, 35%; P_{trend} < 0.01) and for PLP (41%; P_{trend} < 0.01). When stratifying by anatomic site, these associations were observed for colon cancer risk, but not for rectal cancer risk with an exception for PLP. Vitamin B12 was not associated with colorectal cancer risk (Table 3), nor was plasma folate, as presented elsewhere (37). All the models as presented in Table 3 were also additionally adjusted for mutual B-vitamins and folate. However, these analyses did not materially change associations (data not shown).

We also assessed potential effect modification of the vitamin-colorectal cancer associations by sex, age, European region, time between blood donation and cancer diagnoses, and plasma folate concentrations. The association between vitamin B2 and colorectal cancer was modified by folate status ($P_{interaction}$ = 0.03), whereby an inverse association of the sum of vitamin B2 and colorectal cancer was observed among individuals with folate concentrations >11.3 nmol/L (51% risk reduction for the 5th versus 1st quintile; P_{trend}<0.01), and a 7% risk increase among subjects with folate concentrations <11.3 nmol/L (P_{trend} = 0.71). Although none of the other interaction terms were statistically significant, we also observed significant trends for the associations between vitamin B2 sum and vitamin B6 sum with colorectal cancer risk among females, individuals <60 years, and those living in central European countries. Furthermore, the association of vitamin B6 with colorectal cancer risk was stronger in individuals diagnosed within the first 3.6 years after enrollment compared with associations in individuals diagnosed later. Relative risks of 5th versus 1st quintile observed within the first 3.6 years were 0.61 (95% CI, 0.43-0.86; P_{trend} < 0.01) for the sum of vitamin B6, 0.55 (95% CI, 0.39-0.77; P_{trend} <0.01) for PLP, and 0.59 (95% CI, 0.41-0.83; P_{trend} <0.01) for PL. Relative risks observed after the first 3.6 years were 0.80 (95% CI, 0.56-1.14; P_{trend} = 0.43)

Table 2. Median concentrations (5th-95th percentile) of indices for vitamin B2, B6, and B12 by demographic and lifestyle characteristics in control cohort members (n = 2,319)

		n	Vitamin B2 indices		
			Vitamin B2 sum (nmol/L)	Riboflavin (nmol/L)	FMN (nmol/L)
Sex	Male	1,105	19.2 (9.5-71.3)	13.4 (5.6-58.9)	5.3 (2.2-17.9)
	Female	1,213	21.8 (10.9-77.0)	16.2 (6.9-64.5)	5.4 (2.0-15.7)
	$P_{difference}$*		<0.001	<0.001	0.60
Age	<60 years	1,274	20.0 (9.8-73.3)	13.8 (5.9-58.9)	5.7 (2.2-16.4)
	≥60 years	1,044	21.4 (10.4-74.7)	15.9 (6.8-64.2)	4.9 (2.0-15.8)
	$P_{difference}$*		<0.005	<.001	<0.001
Region†	North	731	23.4 (12.3-83.1)	16.6 (6.8-64.2)	6.5 (3.1-16.3)
	Central	999	21.5 (10.8-80.9)	16.0 (7.4-66.2)	4.8 (1.9-16.5)
	South	588	16.3 (8.1-64.0)	11.2 (4.8-51.5)	4.9 (2.0-14.9)
	$P_{difference}$‡		<0.001	<0.001	<.001
Smoking	Never	1,024	21.8 (11.2-83.4)	16.3 (7.0-68.7)	5.5 (2.1-16.5)
	Former	774	21.0 (9.6-87.7)	15.0 (6.3-71.8)	5.3 (2.1-16.8)
	Current	507	18.4 (9.5-57.8)	12.3 (5.1-47.0)	5.4 (2.1-14.2)
	P_{trend}§		<0.005	<0.005	0.22
Alcohol (g/day)	Abstainers	166	19.3 (8.9-87.6)	14.3 (5.7-65.3)	5.0 (2.2-15.0)
	1-30	1,784	21.0 (10.4-73.3)	15.3 (6.5-62.6)	5.3 (2.0-15.8)
	≥30	372	19.4 (9.4-76.2)	12.7 (5.4-53.7)	6.0 (2.3-21.4)
	$P_{difference}$*		0.03	<0.001	<0.005

(*Continued on the following page*)

for the sum of vitamin B6, 0.67 (95% CI, 0.48-0.94; P_{trend} = 0.05) for PLP, and 0.88 (95% CI, 0.63-1.25; P_{trend} = 0.86) for PL. However, lag time did not significantly modify the overall association between the sum of vitamin B6, PLP, and PL with colorectal cancer risk, as indicated by $P_{interaction}$ of 0.48, 0.57, and 0.29, respectively.

In addition, we explored the associations between the vitamins and colorectal cancer risk by subgroups of alcohol intake, and observed stronger inverse associations of the vitamin B2 sum and the vitamin B6 sum with colorectal cancer risk in individuals drinking alcohol of ≥30 g/day (Table 4). Further stratification of these alcohol analyses by sex revealed even stronger inverse associations for the vitamin B6 sum in males consuming alcohol ≥30 g/day compared with males who drink less alcohol (P_{trend} < 0.01; $P_{interaction}$ = 0.01). Associations between individual vitamin B2 and B6 species with colorectal cancer risk in subgroups of alcohol intake did not differ materially from those associations observed for sum scores. Furthermore, stratified analyses using a cutoff point of 15 g/day of alcohol did not materially alter associations between any of the B-vitamins and colorectal cancer risk (data not shown).

The polymorphisms and their associations with colorectal cancer risk

All the SNPs were in Hardy-Weinberg equilibrium (χ^2 test P values >0.05 for all SNPs). Table 5 shows that the genotype distributions of the *CBS 699C→T*, *CBS 844ins68*, *TCN2 67A→G*, and *TCN2 776C→G* polymorphisms did not differ among colorectal, colon, and rectal cases and their matched controls, nor were these polymorphisms associated with cancer risk. Stratification by European region yielded similar associations between the studied genotypes and colorectal cancer risk (data not shown).

Most of the associations between the SNPs and colorectal cancer were not modified by quintiles of B-vitamin status ($P_{interaction}$ > 0.19 for all relevant interactions; data not shown). A previous report (37) included *MTHFR 677C→T*, *MTHFR 1298A→C*, *MTR 2756A→G*, and *MTRR 66A→G* polymorphisms, which were not independently associated with colorectal cancer risk. However, among these SNPs, we observed effect modification of the association between *MTRR 66A→G* and colorectal cancer risk by vitamin B2 status as measured in the current study ($P_{interaction}$ = 0.04). The variant *MTRR 66GG* genotype conferred a statistically significantly lower colorectal cancer risk in individuals with concentrations <18.1 nmol/L of the sum of B2, with RR of 0.57 (95% CI, 0.35-0.93; P_{trend} = 0.02), whereas a statistically nonsignificantly higher colorectal cancer risk was observed in individuals with concentrations >33.4 nmol/L, with RR for *GG* versus *AA* of 1.24 (95% CI, 0.75-2.04, P_{trend} = 0.51). Finally, the SNP-colorectal cancer associations were neither modified by B-vitamin concentrations, nor by alcohol consumption ($P_{interactions}$ > 0.05; data not shown).

Table 2. Median concentrations (5th-95th percentile) of indices for vitamin B2, B6, and B12 by demographic and lifestyle characteristics in control cohort members (n = 2,319) (Cont'd)

Vitamin B6 indices				Vitamin B12 indices	
Vitamin B6 sum (nmol/L)	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	Cobalamin (pmol/L)	MMA (μmol/L)
69.5 (33.5-190.5)	35.6 (15.1-97.4)	13.8 (6.8-36.1)	18.4 (8.5-56.5)	274 (154-467)	0.17 (0.12-0.32)
60.5 (31.1-288.5)	31.2 (13.4-130.0)	13.1 (6.6-58.1)	15.2 (7.3-106)	303 (170-517)	0.17 (0.11-0.31)
<0.001	<0.001	0.10	<0.001	<0.001	0.49
62.9 (32.1-217.8)	33.9 (14.7-115.0)	12.8 (6.5-41.9)	15.6 (7.5-67.0)	294 (167-488)	0.16 (0.11-0.27)
66.2 (32.1-228.2)	32.8 (13.8-105.0)	13.9 (6.9-45.9)	17.7 (8.3-81.8)	279 (153-506)	0.18 (0.12-0.35)
0.21	0.02	<0.005	<0.001	<0.005	<0.001
75.0 (34.0-328.6)	36.9 (14.3-141.5)	16.2 (6.5-76.0)	21.1 (8.9-125)	290 (166-478)	0.17 (0.12-0.31)
65.4 (32.4-211.3)	33.0 (14.8-110.0)	13.6 (7.3-37.1)	16.3 (8.1-64.8)	276 (161-481)	0.18 (0.12-0.32)
55.2 (28.6-115.4)	29.9 (13.3-73.2)	11.0 (6.2-22.9)	13.5 (6.5-29.6)	304 (160-550)	0.16 (0.11-0.31)
<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
65.4 (33.3-225.9)	34.2 (15.5-109.0)	13.7 (7.2-47.0)	16.5 (8.1-75.9)	296 (166-503)	0.17 (0.12-0.30)
69.1 (34.0-216.1)	35.1 (15.5-120.0)	14.2 (7.1-40.1)	18.2 (8.5-71.0)	283 (161-498)	0.17 (0.12-0.32)
57 (26.5-220.6)	28.9 (11.8-105.0)	11.9 (5.4-41.6)	14.7 (6.6-64.9)	277 (147-482)	0.16 (0.11-0.33)
0.91	0.05	0.76	0.78	<0.005	0.91
59.4 (25.7-228.2)	29.5 (12.2-120.0)	13.2 (5.2-54.8)	16.7 (6.8-83.5)	321 (183-527)	0.16 (0.11-0.32)
63.4 (32.0-227.9)	32.2 (14.3-109.0)	13.2 (6.6-44.7)	16.3 (7.8-74.3)	287 (161-496)0	0.17 (0.12-0.32)
75.5 (35.2-207.7)	41.7 (16.4-116.0)	14.8 (7.7-38.4)	19.2 (8.1-57.3)	280 (162-481)	0.16 (0.11-0.28)
<0.001	<0.001	<0.001	0.001	<0.005	<0.005

*$P_{difference}$ (two-sided) calculated by Mann Whitney U test; abstainers were excluded from statistical analyses on alcohol consumption.
†North: Sweden and Denmark; Central: United Kingdom.
‡$P_{difference}$ (two-sided) calculated by Kruskall Wallis test.
§P_{trend} (two-sided) calculated by regression models.

Discussion

In this large European cohort, we investigated the associations of plasma vitamins B2, B6, and B12, and genetic variants of the one-carbon metabolism with colorectal cancer risk. Overall, plasma vitamin B2 and B6 status was inversely associated with colorectal cancer. In addition, we observed that the inverse association between vitamin B6 and colorectal cancer was more pronounced among males who consumed >30 g/day of alcohol. None of the SNPs were associated with colorectal cancer risk, and generally the vitamins did not modify these associations.

The present study is the largest prospective study on plasma B-vitamins and colorectal cancer risk published so far, allowing for well-powered subgroup analyses. Extensive information on lifestyle factors enabled us to control for potential confounders and assessment of possible effect modifications. Another important strength of this study is the collection of blood samples prior to cancer diagnosis. Moreover, the study centers collected and stored blood samples according to a standardized protocol (27), and all biochemical analyses were done in one laboratory, thereby optimizing sample treatment and avoiding between-laboratory method variability. The overall observed associations between the vitamins and age, sex, and smoking status (23, 33, 38) are in line with previous findings, supporting the integrity of biochemical data. Furthermore, the range of plasma concentrations observed for vitamin B12 (10) and vitamin B6 (9) were comparable with those in other European studies, whereas vitamin B6 concentrations were slightly lower compared with those observed in American studies (39–41), which may be explained by widespread supplement use in the United States. Data on supplement use, and specifically use of B-vitamins, in the EPIC cohort are sparse. However, in a subsample of the EPIC population, single 24-hour recalls revealed a clear north-south gradient in supplement use, with higher consumption in northern than in southern European countries, and higher consumption for women than for men (42). Moreover, none of the European countries applied mandatory fortification of any of the B-vitamins. As national fortification policies vary considerably throughout the European Union, the European Commission aims to harmonize voluntary food fortification across European countries in the near future (43).

Two of four studies on the associations between vitamin B6 and colorectal cancer (9, 39–41) reported median follow-up time of 6 years (9) and 10 years (41), compared with the present study with a median follow-up time of

Table 3. Relative risks (95% CI) for colorectal, colon, and rectal cancer by quintiles of indices for vitamins B2, B6, and B12 status

Site	Matched analyses			Matched + covariate adjusted analyses							
	Cases/Controls	RR/quintile	P_{trend}*	Cases/Controls	RR/quintile	P_{trend}†	Q1	Q2	Q3	Q4	Q5
Colorectal cancer											
Vitamin B2 sum	1,336/2,254	0.94 (0.89-0.99)	0.02	1,296/2,175	0.94 (0.88-0.99)	0.02	1	0.73 (0.58-0.92)	0.83 (0.65-1.05)	0.73 (0.57-0.93)	0.71 (0.56-0.91)
Riboflavin	1,358/2,315	0.97 (0.92-1.02)	0.21	1,318/2,236	0.97 (0.92-1.02)	0.27	1	0.88 (0.70-1.10)	0.84 (0.66-1.07)	0.84 (0.66-1.07)	0.87 (0.68-1.11)
FMN	1,337/2,254	0.89 (0.85-0.95)	<0.001	1,297/2,175	0.89 (0.84-0.94)	<0.001	1	0.77 (0.61-0.97)	0.81 (0.64-1.02)	0.54 (0.42-0.69)	0.65 (0.50-0.83)
Vitamin B6 sum	1,338/2,276	0.94 (0.89-0.99)	0.01	1,298/2,197	0.93 (0.88-0.98)	0.02	1	0.91 (0.72-1.13)	0.83 (0.66-1.05)	0.92 (0.73-1.17)	0.68 (0.53-0.87)
PLP	1,359/2,316	0.90 (0.85-0.95)	<0.001	1,319/2,237	0.89 (0.84-0.94)	<0.001	1	0.80 (0.64-1.00)	0.73 (0.58-0.91)	0.73 (0.57-0.92)	0.59 (0.47-0.76)
PL	1,355/2,302	0.95 (0.90-1.00)	0.06	1,315/2,223	0.90 (0.85-0.95)	0.03	1	0.79 (0.63-0.99)	0.73 (0.57-0.93)	0.84 (0.66-1.07)	0.70 (0.54-0.90)
PA	1,346/2,288	1.00 (0.95-1.06)	0.90	1,306/2,209	0.94 (0.89-0.99)	0.83	1	1.04 (0.82-1.31)	1.24 (0.98-1.56)	1.07 (0.84-1.37)	1.01 (0.78-1.30)
Cobalamin	1,362/2,303	1.02 (0.96-1.07)	0.58	1,322/2,225	1.03 (0.97-1.08)	0.19	1	0.86 (0.69-1.08)	1.02 (0.81-1.28)	1.08 (0.85-1.35)	1.02 (0.80-1.29)
MMA	1,365/2,313	0.99 (0.94-1.04)	0.72	1,325/2,234	1.02 (0.96-1.08)	0.97	1	1.09 (0.87-1.36)	1.16 (0.92-1.46)	1.18 (0.94-1.49)	0.94 (0.74-1.21)
Colon											
Vitamin B2 sum	630/1,436	0.91 (0.85-0.97)	<0.005	805/1,392	0.91 (0.85-0.97)	0.01	1	0.74 (0.56-1.00)	0.72 (0.54-0.97)	0.66 (0.49-0.89)	0.66 (0.48-0.90)
Riboflavin	842/1,467	0.95 (0.89-1.02)	0.15	817/1,423	0.96 (0.89-1.02)	0.19	1	0.88 (0.66-1.16)	0.81 (0.60-1.09)	0.74 (0.55-1.00)	0.86 (0.63-1.15)
FMN	831/1,436	0.86 (0.80-0.92)	<0.001	806/1,392	0.87 (0.81-0.93)	<0.001	1	0.76 (0.58-1.01)	0.76 (0.57-1.01)	0.53 (0.39-0.73)	0.59 (0.43-0.81)
Vitamin B6 sum	829/1,446	0.93 (0.87-1.00)	0.05	804/1,402	0.93 (0.86-1.00)	0.01	1	0.93 (0.70-1.23)	0.75 (0.56-1.01)	0.89 (0.67-1.20)	0.69 (0.50-0.96)
PLP	843/1,468	0.90 (0.85-0.97)	<0.005	818/1,424	0.90 (0.84-0.97)	<0.005	1	0.79 (0.60-1.04)	0.62 (0.47-0.83)	0.74 (0.55-0.99)	0.63 (0.46-0.86)
PL	839/1,460	0.95 (0.88-1.02)	0.13	814/1,416	0.94 (0.87-1.01)	0.08	1	0.73 (0.54-0.97)	0.67 (0.49-0.91)	0.80 (0.59-1.08)	0.68 (0.49-0.94)
PA	834/1,454	1.02 (0.95-1.09)	0.58	809/1,410	1.02 (0.95-1.09)	0.63	1	1.11 (0.83-1.50)	1.28 (0.96-1.72)	1.13 (0.83-1.54)	1.07 (0.78-1.48)
Cobalamin	844/1,461	1.00 (0.94-1.07)	0.97	819/1,,418	1.01 (0.94-1.08)	0.05	1	0.76 (0.57-1.01)	1.03 (0.77-1.36)	0.90 (0.67-1.22)	0.95 (0.70-1.28)
MMA	846/1,467	0.97 (0.90-1.04)	0.34	821/1,423	0.99 (0.92-1.06)	0.67	1	1.32 (0.99-1.76)	1.08 (0.81-1.46)	1.24 (0.93-1.67)	0.95 (0.69-1.30)
Rectal											
Vitamin B2 sum	506/818	1.00 (0.92-1.09)	0.96	491/783	0.99 (0.90-1.09)	0.81	1	0.73 (0.48-1.09)	1.14 (0.75-1.73)	0.94 (0.61-1.43)	0.84 (0.55-1.28)
Riboflavin	516/848	1.00 (0.91-1.09)	0.95	501/813	1.00 (0.91-1.10)	0.93	1	0.88 (0.59-1.32)	0.95 (0.63-1.45)	1.06 (0.69-1.62)	0.92 (0.60-1.40)
FMN	506/818	0.95 (0.87-1.04)	0.27	491/783	0.91 (0.83-1.01)	0.06	1	0.80 (0.53-1.20)	0.91 (0.60-1.37)	0.55 (0.36-0.85)	0.74 (0.48-1.15)
Vitamin B6 sum	509/830	0.95 (0.87-1.03)	0.19	494/795	0.94 (0.86-1.03)	0.20	1	0.85 (0.57-1.25)	1.06 (0.71-1.57)	1.01 (0.67-1.51)	0.69 (0.46-1.04)
PLP	516/848	0.90 (0.83-0.98)	0.01	501/813	0.89 (0.81-0.97)	0.01	1	0.84 (0.57-1.23)	1.01 (0.68-1.49)	0.74 (0.49-1.11)	0.58 (0.39-0.87)
PL	516/842	0.96 (0.88-1.04)	0.31	501/807	0.95 (0.87-1.04)	0.28	1	0.93 (0.63-1.37)	0.86 (0.58-1.27)	0.96 (0.64-1.45)	0.77 (0.51-1.17)
PA	512/834	0.98 (0.90-1.08)	0.72	497/799	1.00 (0.91-1.10)	0.98	1	0.93 (0.63-1.38)	1.20 (0.82-1.76)	1.02 (0.68-1.53)	0.95 (0.63-1.45)
Cobalamin	518/842	1.04 (0.96-1.13)	0.31	503/807	1.06 (0.97-1.15)	0.23	1	1.15 (0.78-1.70)	1.00 (0.68-1.46)	1.42 (0.97-2.07)	1.17 (0.80-1.73)
MMA	519/846	1.03 (0.95-1.12)	0.50	504/811	1.03 (0.94-1.12)	0.54	1	0.82 (0.56-1.20)	1.33 (0.91-1.93)	1.15 (0.79-1.69)	0.96 (0.64-1.43)

NOTE: Counts do not necessarily add to the total sum due to missing data.
The lower quintile is the reference category. All analyses are matched for age, sex, study center, and date of blood collection. The matched + covariate adjusted analyses are further adjusted for smoking status, education level, physical activity, fiber intake, intake of red and processed meat, alcohol consumption, and body mass index (BMI).
The cutoff values for the quintiles of the vitamin B2 sum were 14.2, 18.1, 23.5, and 33.4 μmol/L; for riboflavin they were 9.4, 12.8, 17.2, and 25.4 μmol/L; for FMN they were 3.3, 4.6, 6.3, and 8.8 μmol/L; for the vitamin B6 sum they were 45.4, 57.7, 72.6, and 105.3 μmol/L; for PLP they were 21.7, 29.1, 38.4, and 56.6 μmol/L; for PL they were 9.4, 11.8, 15.0, and 20.6 μmol/L; for PA they were 11.1, 14.6, 19.2, and 29.0 μmol/L; for vitamin B12 they were 220, 266, 312, and 380 pmol/L; and for MMA they were 0.14, 0.16, 0.18, and 0.22 μmol/L.
*P_{trend} (two-sided) in risk calculated with conditional logistic regression models without any covariates in the model using values for quintile categories as the quantitative score of exposure.
†P_{trend} (two-sided) in risk calculated with conditional logistic regression models with the covariates in the model using values for quintile categories as the quantitative score of exposure.

Table 4. Adjusted relative risks (95% CI) for colorectal cancer by quintiles of indices for vitamins B2, B6, and B12 status by alcohol consumption and sex

	Alcohol	Cases/Controls	Q1	Q2	Q3	Q4	Q5	P_{trend}*	$P_{interaction}$†	$P_{interaction}$‡
Vitamin B2 sum										
Overall	Abstainer1-15s	83/144	1	0.88 (0.30-2.54)	1.51 (0.48-4.72)	1.01 (0.31-3.23)	1.06 (0.31-3.72)	0.82		
	1-30 g/d	939/1671	1	0.83 (0.63-1.09)	0.92 (0.70-1.20)	0.85 (0.65-1.12)	0.83 (0.63-1.09)	0.27		
	≥30 g/d	274/360	1	0.55 (0.33-0.92)	0.65 (0.39-1.10)	0.60 (0.34-1.04)	0.56 (0.32-0.98)	0.07	0.21	
Males	1-30 g/d	386/700	1	0.80 (0.53-1.22)	1.23 (0.82-1.85)	1.16 (0.76-1.76)	0.87 (0.56-1.35)	0.82		
	≥30 g/d	220/285	1	0.50 (0.28-0.89)	0.66 (0.37-1.19)	0.76 (0.41-1.40)	0.50 (0.26-0.96)	0.15	0.14	
Females	1-30 g/d	553/971	1	0.84 (0.58-1.20)	0.75 (0.52-1.08)	0.69 (0.48-0.99)	0.80 (0.57-1.14)	0.17		0.27
	≥30 g/d	54/75	1	0.58 (0.14-2.40)	0.53 (0.14-1.94)	0.20 (0.04-0.99)	0.52 (0.15-1.80)	0.22	0.55	0.91
Vitamin B6 sum										
Overall	Abstainer1-15s	86/149	1	0.53 (0.20 1.41)	0.73 (0.27-1.96)	1.04 (0.39-2.80)	0.39 (0.11-1.39)	0.54		
	1-30 g/d	940/1691	1	0.92 (0.71-1.18)	0.78 (0.60-1.02)	0.91 (0.70- 1.19)	0.76 (0.58-1.01)	0.09		
	≥30 g/d	272/357	1	0.94 (0.49-1.80)	0.91 (0.47-1.76)	0.70 (0.37-1.35)	0.55 (0.28-1.07)	0.03	0.12	
Males	1-30 g/d	392/718	1	1.18 (0.77-1.80)	0.80 (0.52-1.23)	1.17 (0.77-1.77)	0.80 (0.51-1.26)	0.44		
	≥30 g/d	218/282	1	0.56 (0.25-1.25)	0.50 (0.22-1.13)	0.41 (0.19-0.91)	0.34 (0.15-0.76)	0.01	0.01	
Females	1-30 g/d	548/973	1	0.79 (0.58-1.08)	0.80 (0.57-1.12)	0.77 (0.54-1.10)	0.76 (0.53-1.09)	0.14		0.50
	≥30 g/d	54/75	1	2.21 (0.52-9.36)	1.79 (0.40-8.07)	3.19 (0.67-15.20)	0.92 (0.19-4.49)	0.86	0.34	0.03
Cobalamin										
Overall	Abstainer1-15s	88/154	1	0.36 (0.12-1.11)	0.77 (0.27-2.26)	0.75 (0.28-2.01)	0.56 (0.21-1.53)	0.66		
	1-30 g/d	959/1708	1	0.94 (0.73-1.22)	1.10 (0.86-1.42)	1.06 (0.82-1.38)	1.05 (0.80-1.37)	0.49		
	≥30 g/d	275/363	1	0.76 (0.46-1.26)	0.68 (0.41-1.15)	1.00 (0.60-1.68)	0.93 (0.54-1.60)	0.91	0.76	
Males	1-30 g/d	401/722	1	0.97 (0.67-1.41)	1.27 (0.88-1.83)	1.11 (0.75-1.66)	1.07 (0.69-1.65)	0.48		
	≥30 g/d	221/289	1	0.93 (0.53-1.64)	0.73 (0.41-1.31)	1.07 (0.59-1.96)	0.99 (0.53-1.84)	0.93	0.66	
Females	1-30 g/d	558/986	1	0.89 (0.62-1.29)	0.96 (0.68-1.37)	1.03 (0.73-1.45)	1.00 (0.71-1.41)	0.72		0.76
	≥30 g/d	54/74	1	0.45 (0.11-1.76)	0.58 (0.14-2.31)	0.73 (0.22-2.45)	0.71 (0.19-2.59)	0.80	0.90	0.78
MMA										
Overall	Abstainer1-15s	88/155	1	1.16 (0.45-2.96)	1.97 (0.68-5.73)	0.92 (0.33-2.59)	1.20 (0.43-3.36)	0.93		
	1-30 g/d	961/1714	1	1.07 (0.82-1.40)	1.10 (0.85-1.43)	1.17 (0.90-1.53)	0.91 (0.69-1.21)	0.80		
	≥30 g/d	276/365	1	1.45 (0.88-2.39)	1.28 (0.75-2.17)	1.28 (0.76-2.17)	1.28 (0.73-2.27)	0.53	0.63	
Males	1-30 g/d	400/723	1	1.19 (0.79-1.81)	1.33 (0.88-2.03)	1.22 (0.80-1.85)	1.01 (0.66-1.54)	0.98		
	≥30 g/d	222/290	1	1.62 (0.92-2.84)	1.56 (0.86-2.84)	1.49 (0.83-2.69)	1.51 (0.81-2.83)	0.24	0.40	
Females	1-30 g/d	561/991	1	1.01 (0.71-1.43)	0.98 (0.70-1.37)	1.19 (0.84-1.68)	0.86 (0.59-1.24)	0.80		0.98
	≥30 g/d	54/75	1	1.30 (0.37-4.61)	0.58 (0.13-2.52)	0.87 (0.23-3.28)	0.72 (0.14-3.67)	0.52	0.32	0.31

NOTE: Counts do not necessarily add to the total sum due to missing data.
The lower quintile is reference category. All analyses are matched for age, sex, study center, and date of blood collection and further adjusted for smoking status, education level, physical activity, fiber intake, intake of red and processed meat, alcohol consumption, and BMI.
The cutoff values for the quintiles of vitamin B2 sum were 14.2, 18.1, 23.5, and 33.4 μmol/L; for the vitamin B6 sum they were 45.4, 57.7, 72.6, and 105.3 μmol/L; for vitamin B12 they were 220, 266, 312, and 380 pmol/L; and for MMA they were 0.14, 0.16, 0.18, and 0.22 μmol/L.
*P_{trend} (two-sided) in risk calculated with conditional logistic regression models with the covariates in the model using values for quintile categories as the quantitative score of exposure.
†$P_{interaction}$ (two-sided) of the vitamin-colorectal cancer association by alcohol consumption (abstainers excluded) for the overall model, and separately for males and females.
‡$P_{interaction}$ (two-sided) of the vitamin-colorectal cancer association by sex, separately for individuals with low alcohol consumption (abstainers excluded) and high alcohol consumption.

Table 5. Distribution of genotypes by cancer site, and their associations with colorectal cancer risk

SNP		Colorectal cancer-All		Colon		Rectal	
		Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)
CBS 699	*CC*	583/1,056	1	356/702	1	226/361	1
	CT	603/1,040	1.04 (0.90-1.21)	374/656	1.12 (0.94-1.35)	230/389	0.93 (0.74-1.18)
	TT	141/253	1.00 (0.79-1.26)	83/148	1.11 (0.82-1.50)	58/103	0.87 (0.60-1.25)
	P_{trend}		0.78		0.25		0.39
CBS ins	*0 ins*	1,233/2,150	1	761/1,367	1	472/783	1
	1 ins	212/358	1.02 (0.85-1.23)	137/231	1.04 (0.83-1.31)	75/127	1.00 (0.73-1.36)
	2 ins	9/18	0.89 (0.40-2.00)	4/12	0.65 (0.21-2.04)	5/6	1.29 (0.39-4.34)
	P_{trend}		0.89		0.96		0.87
TCN2 67	*AA*	1,026/1,789	1	622/1,147	1	406/649	1
	AG	263/490	0.95 (0.80-1.12)	162/312	0.98 (0.79-1.21)	100/181	0.87 (0.66-1.15)
	GG	19/18	0.87 (0.50-1.53)	13/22	1.03 (0.51-2.08)	5/16	0.54 (0.20-1.51)
	P_{trend}		0.42		0.88		0.15
TCN2 776	*CC*	415/749	1	253/482	1	162/273	1
	CG	648/1,170	0.99 (0.85-1.16)	401/762	0.99 (0.81-1.20)	248/412	1.01 (0.78-1.30)
	GG	266/435	1.10 (0.90-1.33)	160/266	1.13 (0.88-1.46)	105/169	1.04 (0.76-1.43)
	P_{trend}		0.45		0.42		0.81

NOTE: Wild type is the reference category.

3.6 years. A potential drawback of cohort studies with relatively short follow-up is reverse causality, i.e., the phenomenon that preclinical disease influences exposure status. This is more likely to affect individuals diagnosed early than those diagnosed later. In this respect, Lee et al. (41) previously observed a stronger inverse association between vitamin B6 and colorectal cancer in an earlier compared with a later follow-up period. Although in our study the associations between vitamin B6 sum and all its individual species and risk of colorectal cancer seemed more pronounced in those diagnosed within the first years of follow-up compared with those diagnosed later, it should be emphasized that there was no statistical evidence for effect modification by the duration of follow-up. Nevertheless, to present analyses according to different lengths of follow-up time should be recommended in future studies.

The most important dietary sources of vitamin B2 are milk and dairy products (44), whereas vitamin B6 may be obtained from various food groups, including fruit, vegetables, and meat (45). After ingestion, free FMN and FAD are converted to riboflavin, whereas all vitamin B6 species are converted into PLP and PL. FMN acts as a cofactor and PA is the catabolite product of these reactions (36, 45). As the sum variables for vitamin B2 and vitamin B6 might account for any interconversion between the two B2 species (35, 46–48) and the three B6 species (36, 45, 49), they are used as supplementary variables to determine vitamin B2 and vitamin B6 status, respectively.

Few epidemiologic studies have investigated plasma concentrations of B-vitamins in relation to colorectal cancer risk (9, 10, 39, 41). The present study observed a risk reduction of 29% for individuals in the highest quintile of the sum of vitamin B2 concentration compared with those in the lowest quintile. Although riboflavin has been suggested as the best plasma marker of vitamin B2 status (50), no relation with colorectal cancer was found for riboflavin, whereas it was found for FMN. FMN serves as a cofactor in the synthesis of PLP (2, 51), which is the active form of vitamin B6. Interestingly, in line with previous studies on plasma PLP (39, 41), we observed an inverse association between the sum of vitamin B6 and colorectal cancer. We did not observe an association between plasma vitamin B12 and its marker MMA and colorectal cancer, whereas Dahlin et al. (10) observed that plasma vitamin B12 was inversely associated with rectal cancer. In the Aspirin/Folate Polyp Prevention Study, which investigated the effects of folic acid supplementation on incidence of new colorectal adenomas in persons with a history of adenomas, high plasma concentrations of PLP and riboflavin at baseline seemed to protect against colorectal adenomas (52). Methodologic differences in cross-sectional, prospective, and intervention studies, as well as differences between data on intake (3–8, 11–13, 26) and plasma concentrations (9, 10, 14, 39, 41), may have resulted in inconsistencies between studies. However, taking all epidemiologic studies into account, current evidence suggests a role for the vitamins B2 and B6 in colorectal carcinogenesis.

Notably, folate and vitamin B12 are carriers of one-carbon units, whereas vitamins B2 (53) and B6 (54) are involved in many pathways other than one-carbon metabolism. Vitamin B2 serves as cofactor in fat, amino acid,

carbohydrate, and vitamin metabolisms (53), whereas vitamin B6 has been shown to reduce oxidative stress and affects cell proliferation and angiogenesis (55). As colorectal cancer risk was not related to concentrations of folate (37) and vitamin B12 in this cohort, the inverse association between risk and vitamin B2 and B6 may reflect mechanisms not involving one-carbon metabolism. Both vitamin B2 and B6 are cofactors within the kynurenine metabolism which is related to inflammation (56). An inverse association of PLP with the inflammatory marker C-reactive protein, which has been related to several cancer types, has also been reported (57).

Alcohol consumption may reduce bioavailability of folate (58) and vitamin B6 (59). So far, only two studies have investigated the interaction among vitamin B6 status, alcohol consumption, and colorectal cancer (39, 60), and both studies suggest that a sufficient vitamin B6 status may prevent the development of colorectal cancer particularly in persons with high alcohol consumption, results which are in agreement with data for males of the present study.

Regarding the role of genetic variants in the one-carbon metabolism and colorectal cancer, we did not observe that the polymorphisms investigated were associated with colorectal cancer. Although some associations have been reported, previous studies did not consistently show associations of similar one-carbon SNPs and colorectal cancer (4, 18–21, 23). Furthermore, generally variable B-vitamin status did not modify the associations between SNPs and colorectal cancer risk. Despite the large study population, the sample size might still have been too small to detect associations with colorectal cancer risk, interactions between genes and European region, and interactions between genes and vitamins, and may also have resulted in chance findings. Nevertheless, we observed that the association between *MTRR 66A→G* and colorectal cancer was modified by vitamin B2 status. Furthermore, the polymorphisms presented in this study may not cover all genetic variability of the studied genes, but they represent a collection of polymorphisms in genes encoding central enzymes of the one-carbon metabolism. All these variants have been shown to influence one-carbon metabolism (61) and some have been related to cancer (18, 19, 23). Future studies could adopt a more pathway-oriented approach to the data analysis in a mathematical model, integrating all the B-vitamins and polymorphisms involved in the one-carbon metabolism (62).

In summary, this large prospective European multicenter study revealed that plasma concentrations of vitamins B2 and B6 were inversely associated with colorectal cancer risk. However, unlike previous studies, none of the studied polymorphisms related to one-carbon metabolism were associated with colorectal cancer in this large European cohort.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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