

Research Article

Vitamins B2 and B6 and Genetic Polymorphisms Related to One-Carbon Metabolism as Risk Factors for Gastric Adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition

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Abstract

B vitamins and polymorphisms in genes coding for enzymes involved in one-carbon metabolism may affect DNA synthesis and methylation and thereby be implicated in carcinogenesis. Previous data on vitamins B2 and B6 and genetic polymorphisms other than those involving *MTHFR* as risk factors for gastric cancer (GC) are sparse and inconsistent. In this case-control study nested within the European Prospective Investigation into Cancer and Nutrition cohort, cases ($n = 235$) and controls ($n = 601$) were matched for study center, age, sex, and time of blood sampling. B2 and B6 species were measured in plasma, and the sum of riboflavin and flavin mononucleotide was used as the main exposure variable for vitamin B2 status, whereas the sum of pyridoxal 5'-phosphate, pyridoxal, and 4-pyridoxic acid was used to define vitamin B6 status. In addition, we determined eight polymorphisms related to one-carbon metabolism. Relative risks for GC risk were calculated with conditional logistic regression, adjusted for *Helicobacter pylori* infection status and smoking status. Adjusted relative risks per quartile (95% confidence interval, P_{trend}) were 0.85 (0.72-1.01, 0.06) for vitamin B2 and 0.78 (0.65-0.93, <0.01) for vitamin B6. Both relations were stronger in individuals with severe chronic atrophic gastritis. The polymorphisms were not associated with GC risk and did not modify the observed vitamin-cancer associations. In summary, results from this large European cohort study showed an inverse association between vitamin B2 and GC risk, which is borderline significant, and a significant inverse association between vitamin B6 and GC risk. *Cancer Epidemiol Biomarkers Prev*; 19(1); 28–38. ©2010 AACR.

Introduction

Although the incidence of gastric cancer (GC) has declined in industrialized countries over the last century, it is still the second most important cause of cancer mortality

in many developing countries (1). *Helicobacter pylori* (Hp) infection of the gastric mucosa has been associated with noncardia GC, whereas severe chronic atrophic gastritis (SCAG) has been associated with cardia GC (2). The relatively high prevalence of Hp infection in populations with

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low GC risk (3) suggests that also other factors, such as diet and genetic predisposition, may play an etiologic role.

One-carbon metabolism (Fig. 1) is of special interest with respect to carcinogenesis. The activity of enzymes involved can be affected by low concentrations of B vitamin cofactors and by genetic polymorphisms. Derangement of one-carbon metabolism (4) might be implicated in carcinogenesis via impaired synthesis and methylation of DNA (5). Folate (6-9) and cobalamin (9-13) are among the B vitamins that have been studied most frequently in relation to GC risk and show inconsistent associations. Riboflavin (vitamin B2) and pyridoxine (vitamin B6) have been studied less frequently. Only a few case-control studies have investigated associations of vitamin B2 (10, 13-21) and B6 (10, 13, 15-18) intake with GC risk. The results suggest either no association (10, 13-17), an increased (18, 19) or decreased (20, 21) risk with higher vitamin B2 intake, or a decreased risk with higher vitamin B6 intake (10, 16-18). The vitamins B2 and B6 are interrelated because the interconversion of some vitamin B6 species requires the vitamin B2 species flavin mononucleotide (FMN) and flavin dinucleotide as cofactors (22, 23). In one-carbon metabolism, vitamin B2 serves as a cofactor for the enzymes methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR), whereas vitamin B6 serves as a cofactor for the enzyme cystathionine β -synthase (CBS) and cystathionine γ -lyase. The majority of previous research on effects of genetic variation and GC risk has focused on *MTHFR* 677C \rightarrow T and 1298C \rightarrow A polymorphisms (9, 24), whereas few results have been published on the CBS 844ins68 (25), *MTRR* 66A \rightarrow G (26, 27), methionine synthase (*MTR*) 2756A \rightarrow G (25, 27), methylenetetrahydrofolate dehydrogenase (*MTHFD1*) 1958G \rightarrow A (28), and reduced folate carrier [solute carrier family 19 (*SLC19A1*)] 80G \rightarrow A (29) polymorphisms. An association with GC risk has previously been observed for variant genotypes of the *MTHFR* 677C \rightarrow T (24), *MTHFR* 1298C \rightarrow A (9, 24), and the *MTHFD1* 1958G \rightarrow A (28) polymorphisms.

Most previous studies on vitamins B2 (10, 13-21) and B6 (10, 13, 15-18) in GC have been relatively small, and none measured plasma concentrations of vitamin B2 and B6 species. Moreover, in all previous studies, intake of vitamin B2 and B6 was reported after diagnosis of the disease, which may have affected the results. To obtain a

clearer understanding of the role of prediagnostic plasma concentrations of vitamin B2 and B6, and their possible interactions with eight one-carbon polymorphisms in GC etiology, this case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC; refs. 30, 31) was conducted.

Materials and Methods

Study Population and Collection of Blood Samples

The design and methods of the EPIC study have previously been described in detail (30, 31). Briefly, the EPIC cohort consists of 23 centers in 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and United Kingdom). Between 1992 and 1998, country-specific dietary questionnaires, standardized lifestyle and personal history questionnaires, anthropometric data, and blood samples were collected from the majority of the participants.

The present study includes GC cases, which were diagnosed after blood collection, and matched control cohort members free of cancer from all EPIC countries. In each of the recruitment centers, fasting and nonfasting blood samples of at least 30 mL were drawn from all participants and stored at 5°C to 10°C while protected from light and transported to local laboratories for processing and aliquoting, as previously described (30, 31), except for the EPIC-Oxford center. The EPIC-Oxford center collected blood samples from a network of general practitioners and health conscious individuals, and blood samples were transported to a central laboratory in Oxford via mail. While protected from light, the whole blood samples from Oxford were exposed to ambient temperatures for up to 48 h. As B vitamins are partly degraded by such handling, all EPIC-Oxford (three cases, nine controls) samples were excluded from the present analyses. GC cases were not diagnosed among the Norwegian cohort members.

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 the local study center and the other half in the central EPIC biorepository at the IARC (Lyon, France). In Denmark, blood fraction aliquots of

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1.0 mL were stored locally in Nunc tubes at -150°C under nitrogen vapor. In Sweden, samples were stored in -70°C freezers.

Follow-up for Cancer Incidence

In EPIC, follow-up is based on population cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom), health insurance records, pathology registries, and active contact of 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 IARC until the end of October 2002, representing complete follow-ups until either December 2000 or December 2001 for all centers using cancer registry data and until 2002 for France, Germany, and Greece. Cancers of the stomach included cancers coded as C16 (10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death). The diagnosis, tumor site classification, and morphology (according to ICD02 and Lauren classifications) of each identified cancer were confirmed and validated by an independent panel of pathologists with a representative from each EPIC country and a coordinator (32). The pathologist panel reviewed original histologic slides and/or recuts from the paraffin blocks and original histopathology reports that were provided by each EPIC center.

Nested Case-Control Study Design and Selection of Study Subjects

Incident GC cases were cohort members who developed cancer after recruitment into EPIC. The present study includes a total of 221 gastric adenocarcinomas and 14 adenocarcinomas of the gastroesophageal junction (GEJ). In this study, these 235 cases are grouped together. For each identified cancer case, control subjects with available blood samples were randomly selected from all cohort members who were alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the case patient. Controls ($n = 601$) were matched by gender, age group (± 2.5 y), study center, and date of blood sample collection (± 45 d). GC cases were divided into three groups according to anatomic subsite: (a) tumors originating from the gastric cardia ($n = 62$), combining tumors that reached the GEJ, either crossing the GEJ or located below the GEJ (all 14 GEJ cancers) or not; (b) noncardial tumors ($n = 102$) grouping cases from other sites in the stomach; and (c) tumors from unknown or mixed sites ($n = 71$). When divided by histologic subtype, of the 235 cancer cases, 82 were classified as diffuse and 81 as intestinal according to the Lauren classification. The remaining cases ($n = 72$) were of unknown or mixed histologic types. All gastric lymphomas, gastric stump cancers, other gastric nonadenocarcinoma, esophageal nonadenocarcinomas, and otherwise unspecified malignant neoplasms of the stomach were excluded from this analysis. This study was approved by the Ethical Review Board of the IARC and those of all individual EPIC centers.

Laboratory Measurements

Vitamin B2 measures included citrate plasma concentrations of riboflavin and FMN, and vitamin B6 measures included pyridoxal 5'-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid (PA). All vitamin species were determined by liquid chromatography–tandem mass spectrometry assay in the same laboratory in Bergen, Norway (33). Within- and between-day coefficients of variation (CV) of vitamin B2 and B6 species were 3% to 18% and 6% to 22%, respectively. The largest CVs are for FMN (within CV = 18%; between CV = 22%), which is present in low concentrations and has a relatively low signal (33).

Eight polymorphisms (reference, amino acid change) of genes coding for enzymes involved in one-carbon metabolism were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (34) in Bergen, Norway. These included *CBS* ($699\text{C}\rightarrow\text{T}$; rs 234706, *Tyr*²³³*Tyr*) and, in addition, the *CBS 844ins68 insertion*, *MTHFD1* ($1958\text{G}\rightarrow\text{A}$; rs 2236225, *Arg*⁶⁵³*Gln*), *MTR* ($2756\text{A}\rightarrow\text{G}$; rs 1805087, *Asp*⁹¹⁹*Gly*), *MTRR* ($66\text{A}\rightarrow\text{G}$; rs 1801394, *Ile*²²*Met*), *SLC19A1* ($80\text{G}\rightarrow\text{A}$; rs 1051266, *Arg*²⁷*His*), and transcobalamin II (*TCN2 67A* \rightarrow *G*; rs RsaI, *Ile*²³*Val* and *TCN2 776C* \rightarrow *G*; rs 1801198, *Pro*²⁵⁹*Arg*). These single-nucleotide polymorphisms (SNP) are “nonsynonymous” and are often called “potentially functional” (35). Quantification of anti-*Hp* (*Hp*) antibodies in stored plasma samples was done by an ELISA technique using incubation with lysates of the CCUG *Hp* strain (36). Pepsinogen A (PGA) was assayed in plasma by a commercial microplate-based quantitative ELISA kit (Biohit). SCAG was serologically defined as a PGA circulating concentration lower than 22 $\mu\text{g/L}$ (36).

Statistical Methods

Because riboflavin and FMN are interconvertible (37–40), as are PLP and PL (41, 42), and PA is formed from PL, we calculated the sum of riboflavin and FMN as an index for vitamin B2 status and the sum of PLP, PL, and PA as an index of vitamin B6 status. In the present study, these sums of B2 and B6 vitamins are therefore considered as the main exposure variables for vitamin B2 and B6 status, respectively. In addition, results are also presented for the individual B2 and B6 species.

The Mann-Whitney *U* test was used to assess potential differences in plasma concentrations of vitamin B2 and B6 species according to sex, age (<60 y versus >60 y), European region (north versus central), *Hp* infection status, and SCAG status, whereas the Kruskal-Wallis test was used to assess differences in concentration among categories of smoking categories (nonsmokers, ex-smokers, current smokers, and missing) in the 601 controls. Differences in plasma concentrations between cases and controls were investigated by the Mann-Whitney *U* test.

Relative risks (RR) and 95% confidence intervals (95% CI) for GC in relation to indices of vitamin B2 and B6 status were calculated by conditional logistic regression using the SAS LOGISTIC procedure (SAS statistical

software, version 9.1; SAS Institute) stratified by the case-control set. In our study, the RR indicated the incidence rate ratio, which is reflected by the odds ratio calculated from conditional logistic regression (43). Risk estimates were adjusted for *Hp* infection status and smoking categories. The RRs on GC were examined by quartiles with cutoff points based on the distribution of B2 and B6 indices in all 601 controls combined. Likelihood ratio tests were used to assess linear trends in RRs across the categories using values for quartile categories as the quantitative score of exposure. Models were also carried out separately for each anatomic subsite (cardia versus noncardia), histologic subtype (diffuse versus intestinal), European region [north-central Europe (Sweden, Denmark, Germany, the Netherlands, United Kingdom, and France) versus southern Europe (Italy, Greece and Spain)], time from blood donation to cancer diagnosis (<1 y versus ≥ 1 y), age at recruitment (<60 y versus ≥ 60 y), sex, and *Hp* status (infection or no infection). Heterogeneity was tested by adding the product term of the vitamin indices and potential effect modifiers in the model. In addition, subgroup analyses by SCAG (yes or no) were done for cases with and without SCAG and their matched controls.

The associations between the polymorphisms and GC risk were studied with conditional logistic regression but by stratifying on country instead of the matched sets and with age and sex as covariates. The risk estimates were calculated with the wild-type as the reference category. An ordinal level variable with equally spaced integer weights (0, 1, 2) for the genotypes was used to test for trend to summarize the effect of each polymorphism. Effect modification of the SNP-GC associations by vitamin concentrations was studied with conditional logistic regression.

Results

Characteristics of the Study Population

Selected characteristics of the 235 GC cases and 601 matched controls are summarized in Table 1. Forty-one percent of the cases were female, mean age at diagnosis was 62 years, and the mean time from blood donation to cancer diagnosis was 3.2 years. GC cases had a higher prevalence of *Hp* infection, SCAG, and current smoking than their matched controls.

Concentrations of Vitamins B2 and B6

The distributions of vitamin B2 and B6, and the individual vitamins, in the control group were skewed, with a longer tail at higher concentrations (Table 2). Plasma concentrations of all vitamin B6 species correlated strongly with each other after adjustment for age, sex, and study center (correlation coefficients ranged from 0.51 to 0.74; P for all correlations < 0.01), and the correlation between plasma concentrations of riboflavin and FMN was 0.32 (P < 0.01; data not shown).

Table 2 shows that median vitamin B2 concentrations were higher in females, whereas vitamin B6 concentrations were higher in males. Furthermore, concentrations

of vitamin B2 and B6 were lower in southern European countries compared with northern European countries ($P_{\text{difference}} < 0.01$) and in smokers compared with former and never smokers ($P_{\text{trend}} < 0.01$). Concentrations did not differ with respect to age (<60 y versus ≥ 60 y), *Hp* infection (yes versus no), and SCAG (yes versus no) in controls (Table 2). A trend toward higher concentrations of all B6 species was observed for the homozygote GG variants of the *MTRR* ($P_{\text{trend}} < 0.05$) and *TCN2* ($P_{\text{trend}} < 0.05$) polymorphisms, whereas concentrations of the B2 and B6 species did not differ across other polymorphic genetic variants (data not shown).

Median concentrations (nmol/L) of the vitamin B2 sum, riboflavin, FMN, the vitamin B6 sum, PLP, PL, and PA in the GC cases were 15.6, 12.1, 2.9, 51.9, 22.1, 13.4, and 15.6 nmol/L, respectively. Furthermore, concentrations did not differ significantly among cases and controls with *Hp* infection ($P_{\text{difference}} > 0.05$) and SCAG ($P_{\text{difference}} > 0.05$; data not shown).

Associations between Plasma Indices of Vitamin B2 and B6 Status with GC

GC risk decreased borderline significantly with increasing vitamin B2 sum (RR per increase in quartile of 0.85; $P_{\text{trend}} = 0.06$) and with strongest associations for FMN ($P_{\text{trend}} = 0.01$). The vitamin B6 sum was significantly inversely associated with GC risk (RR per increase in quartile of 0.78; $P_{\text{trend}} < 0.01$), and among the separate B6 species, only high PA concentrations were significantly associated with lower GC risk ($P_{\text{trend}} = 0.01$; Table 3). Exclusion of cohort members with extremely high concentrations of the B6 species, attributable to vitamin B6 supplements as indicated by the presence of pyridoxine in plasma ($n = 81$; ref. 44), did not alter any of the observed associations (data not shown).

We also analyzed the associations between vitamin B2 and B6 indices with GC risk separately in cases with ($n = 42$) and without ($n = 191$) SCAG (Table 4). Generally, associations of the vitamins with GC risk were more pronounced in those with SCAG compared with those without SCAG. Associations were stronger, with RR/quartile (P_{trend}) of 0.38 (<0.01) for FMN and 0.51 (0.01) for PA. Moreover, the difference in associations in those with and without SCAG was significant for FMN ($P_{\text{interaction}} = 0.03$; Table 4). Because gastritis was not a matching criterion, 2% of controls matched to cases without SCAG had SCAG, and 32% of controls matched to cases with SCAG had SCAG, which may have biased these associations. Therefore, we additionally analyzed these associations stratified for gastritis comparing cases and controls with or without SCAG. These analyses revealed even stronger inverse associations between vitamin B2 and B6 and GC in individuals with SCAG (data not shown).

We further studied whether the associations between GC and vitamin B2 and B6 were modified by *Hp* infection status (yes or no), time between blood sampling and cancer diagnosis (<1st year or ≥ 1 st year), sex, age

($P_{\text{interaction}} = 0.05$). None of the associations between the SNPs and GC risk was statistically significantly modified by folate and vitamin B12 status ($P_{\text{interaction}} > 0.19$ for all relevant interactions; data not shown).

Discussion

The present nested case-control study investigated the association of GC risk with indices of vitamin B2 and B6

status, and nine polymorphisms in genes encoding for enzymes involved in one-carbon metabolism. Overall analyses indicated a tendency for an inverse association between vitamin B2 and GC risk and a significant inverse association between vitamin B6 and GC risk. Furthermore, the associations were more pronounced in individuals with SCAG. None of the studied polymorphisms was related to GC risk, nor did they modify the observed associations for vitamin B2 and B6.

Table 2. Vitamins B2 and B6 [mean/median (5-95 percentile)] in relation to demographic characteristics and risk factors of GC in control cohort members ($n = 601$)

		<i>n</i>	Vitamin B2 sum	Riboflavin	FMN	Vitamin B6 sum	PLP	PL	PA
	Overall	601	23.9/16.6 (7.6-59.1)	18.8/12.8 (5.0-46.0)	5.1/3.2 (1.2-12.6)	89/56 (28-174)	32.4/23.5 (8.5-78.0)	23.6/14.5 (7.2-36.9)	33.0/16.4 (8.7-66.5)
Sex	Male	356	24.1/15.3 (7.7-55.6)	18.7/12.2 (5.0-43.3)	5.4/2.9 (1.2-13.2)	98/59 (30-178)	34.2/26.2 (9.0-79.8)	26.2/14.7 (7.4-36.9)	37.4/17.1 (8.4-71.4)
	Female	245	23.6/18.5 (7.6-61.6)	18.9/14.6 (5.3-50.8)	4.7/3.5 (1.2-12.2)	76/55 (26-164)	29.7/20.6 (7.3-71.3)	20.0/13.8 (7.2-37.0)	26.7/16.0 (8.9-60.9)
Age	$P_{\text{difference}}^*$		<0.01	<0.01	0.08	<0.01	<0.01	0.16	0.13
	<60 y	289	23.9/15.6 (7.5-62.9)	18.8/12.0 (5.1-50.8)	5.1/3.2 (1.2-13.2)	97/54 (27-148)	33.8/23.9 (8.9-73.1)	27.6/14.2 (7.1-33.2)	35.9/15.1 (7.8-51.9)
	≥60 y	312	23.8/17.6 (7.7-51.5)	18.8/13.5 (5.0-44.7)	5.1/3.0 (1.1-11.4)	81/58 (28-185)	31.1/23.4 (7.9-78.0)	20.0/14.6 (7.4-38.2)	30.3/18.7 (9.3-76.8)
European region [†]	$P_{\text{difference}}^*$		0.12	0.06	0.41	0.05	0.92	0.23	<0.01
	North	338	27.5/19.0 (9.3-60.6)	22.1/14.9 (6.8-49.0)	5.4/3.2 (1.3-14.4)	113/64 (32-236)	37.8/27.2 (10.0-90.5)	30.0/15.7 (8.0-51.1)	44.9/20.8 (9.6-97.8)
	South	263	19.3/14.4 (6.7-47.7)	14.6/10.7 (4.2-38.9)	4.6/3.2 (1.2-12.1)	59/48 (25-101)	25.5/20.0 (7.0-55.6)	15.5/12.9 (6.7-24.5)	17.8/14.1 (7.8-28.3)
Smoking status	$P_{\text{difference}}^*$		<0.01	<0.01	0.87	<0.01	<0.01	<0.01	<0.01
	Never	256	25.5/18.2 (7.6-58.0)	20.7/13.8 (5.4-48.2)	4.7/3.4 (1.4-13.2)	103/59 (28-189)	33.4/24.7 (8.5-79.8)	29.0/15.1 (7.2-35.4)	40.5/17.2 (9.7-59.4)
	Former	198	27.8/18.0 (7.8-69.5)	21.1/13.8 (5.8-53.2)	6.7/3.3 (1.2-17.0)	89/59 (33 -174)	35.4/26.7 (9.0-82.4)	21.5/14.8 (7.8-37.3)	31.8/17.6 (9.9-76.8)
	Current	136	15.7/13.1 (6.3-39.0)	12.2/9.9 (4.3-30.2)	3.5/2.7 (0.9-8.0)	65/47 (24-148)	26.3/19.1 (7.3-62.5)	17.5/12.8 (6.6-38.2)	21.4/13.7 (6.5-52.4)
<i>Hp</i> infection	Unknown	11	15.7/14.2 (5.4-26.9)	12.1/11.7 (4.4-20.5)	3.6/2.4 (0.7-8.1)	69/51 (21-232)	29.9/20.1 (5.5-89.7)	15.3/11.6 (6.5-54.7)	23.5/16.1 (8.9-87.7)
	$P_{\text{trend}}^{\ddagger}$		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	No	196	25.0/16.5 (7.4-69.5)	19.1/12.8 (5.1-53.5)	5.8/2.8 (1.3-16.3)	107/57 (31-168)	32.8/25.1 (9.4-78.0)	30.7/14.3 (7.4-35.3)	43.7/18.1 (9.6-71.4)
SCAG	Yes	404	23.3/16.6 (7.7-51.5)	18.6/12.8 (5.0-43.8)	4.7/3.3 (1.1-12.1)	80/56 (27-185)	32.1/22.3 (7.6-78.0)	20.2/14.6 (7.2-38.2)	27.8/16.1 (8.2-66.5)
	$P_{\text{difference}}^*$		0.71	0.57	0.18	0.36	0.30	0.70	0.13
	No	548	24.5/16.9 (7.6-60.5)	19.3/12.9 (5.1-48.2)	5.2/3.2 (1.2-13.0)	91/56 (28-172)	32.7/23.9 (8.9-72.1)	24.3/14.5 (7.4-36.5)	34.2/16.5 (8.4-64.8)
SCAG	Yes	45	17.2/15.0 (7.9-31.8)	13.3/11.0 (4.4-28.4)	3.7/3.0 (0.9-8.2)	68/48 (26-232)	29.2/18.8 (7.0-110.0)	16.6/13.2 (7.0-50.7)	22.3/15.0 (9.1-76.3)
	$P_{\text{difference}}^*$		0.12	0.11	0.20	0.08	0.08	0.24	0.35

* P for difference based on median concentrations.

[†]North: Scandinavia, United Kingdom, and Central Europe; south: Italy, Spain, and Greece.

[‡]Category unknown not included in the test for trend.

Study Design

An important strength of our study is the availability of blood samples before cancer diagnosis. One could argue that a mean lag time of 3 years between blood donation and cancer diagnosis may be too short to obtain unbiased associations between the vitamins and GC. Vitamin concentrations measured shortly before diagnosis should be considered with caution because low vitamin status may be a consequence of malignancy rather than causing it. In this regard, we previously observed that cobalamin was inversely associated with GC risk, particularly among individuals with severe atrophic chronic gastritis (9). This agrees with a reduced bioavailability of cobalamin due to infection with *H. pylori* and atrophic gastritis (45), which may precede GC (46). Notably, in the present study, the inverse association between cancer risk and concentrations of vitamin B2 and B6 was strongest in cases with SCAG. This may suggest that impaired vitamin B2 and B6 status contributes to the development of GC or that atrophic gastritis may adversely affect the status of these vitamins. In line with the latter possibility, reduced PLP concentrations are consistently associated with inflammation (47-49). In the present study, vitamin B2 and B6 concentrations did not differ significantly from people with and without SCAG, and correction for low pepsinogen concentrations did not confound the association between the vitamins and GC. It should be noted that low PGA concentrations are a marker for severe gastritis (50). Results of the present study do therefore only apply to severe gastritis and not for milder forms of gastritis. Previous studies on the association of the vitamins B2 (10, 13-21) and B6 (10, 13, 15-18) with GC investigated the effects of dietary intake rather than plasma concentrations. Blood sampling is less likely to result in bias due to measurement errors that are inherent to food frequency questionnaires. Within this multicenter study, all study centers collected and stored blood samples according to

a standardized protocol (31), and all biochemical analyses were carried out in one laboratory, thereby optimizing sample treatment during analysis. Moreover, the present study included plasma concentrations of several B2 and B6 species. Although individual vitamin B2 and B6 species reflect vitamin B2 (51) and B6 (47) status, there is no clear consensus as to which vitamin species is the best marker. We also used the sum of the individual species as indices of vitamin B2 and B6 status because the B2 species (37-40) and the B6 species (41, 42) are interconvertible, and the plasma concentrations are positively correlated, as shown here and by others (44).

Vitamin B2 and B6 Intake and GC

The most important dietary sources of vitamin B2 are milk and dairy products (52). Contrary to our results showing an inverse association of plasma vitamin B2 with GC risk, other studies observed that vitamin B2 (18, 19) and milk intake (15, 53) are associated with increased risk. Although information on dairy intake was not taken into account in the present study, it is possible that milk intake is increased to reduce gastric pain during disease progression. Vitamin B6 may be obtained from various food groups, including fruit, vegetables, and meat (41), and once ingested, all vitamin B6 species are converted into PLP and PL (41, 42). No strong overall evidence was observed for a protective role of fruit and vegetable intake in GC in our study population (54) or in other cohort studies (55, 56). In contrast, meat intake has been found to be associated with increased risk of the noncardia subsite of GC in the EPIC cohort (57).

Mechanism

Studies in riboflavin-deficient rats show an increased GC risk, increased induction of DNA repair enzymes (58), and increased carcinogen binding to DNA (59) compared with rats on a riboflavin-replete diet. Moreover,

Table 3. RRs (95% CI) for GC risk by quartiles of plasma vitamins B2 and B6 indices

	Case/control	Q1*	Q2	Q3	Q4	RR/quartile	P _{trend}
Vitamin B2 sum	231/590	1	0.78 (0.48-1.27)	0.71 (0.43-1.17)	0.61 (0.36-1.03)	0.85 (0.72-1.01)	0.06
Riboflavin	231/591	1	1.02 (0.64-1.64)	0.73 (0.44-1.23)	0.71 (0.42-1.19)	0.87 (0.74-1.03)	0.09
FMN	231/590	1	1.18 (0.76-1.85)	0.64 (0.38-1.08)	0.55 (0.32-0.96)	0.79 (0.67-0.95)	0.01
Vitamin B6 sum	233/596	1	0.70 (0.44-1.11)	0.59 (0.35-0.98)	0.46 (0.26-0.80)	0.78 (0.65-0.93)	0.006
PLP	235/600	1	0.82 (0.51-1.30)	0.61 (0.36-1.05)	0.64 (0.36-1.13)	0.85 (0.71-1.02)	0.08
PL	234/599	1	1.02 (0.65-1.60)	0.56 (0.33-0.95)	0.74 (0.44-1.26)	0.86 (0.72-1.02)	0.07
PA	234/599	1	0.87 (0.56-1.35)	0.81 (0.50-1.30)	0.48 (0.28-0.83)	0.81 (0.68-0.96)	0.01

NOTE: Calculated by conditional logistic regression, stratified by the case-control set and adjusted for Hp infection status and smoking. Controls were matched to cases by age, sex, study center, and date of blood sample collection. The cutoff values for the quartiles were as follows: vitamin B2 sum, 11.88, 16.63, and 25.15 $\mu\text{mol/L}$; riboflavin, 8.52, 12.80, and 20.00 $\mu\text{mol/L}$; FMN, 2.13, 3.15, and 5.21 $\mu\text{mol/L}$; vitamin B6 sum, 40.8, 55.7, and 80.6 $\mu\text{mol/L}$; PLP, 14.90, 23.50, and 36.60 $\mu\text{mol/L}$; PL, 10.60, 14.50, and 18.97 $\mu\text{mol/L}$; and PA, 12.60, 16.40, and 24.90 $\mu\text{mol/L}$.

*Reference category.

Table 4. RRs (95% CI) for GC risk by quartiles of plasma concentrations of vitamin B2 and B6 indices in cases with and without SCAG

	SCAG*	Case/control	Q1 [†]	Q2	Q3	Q4	RR/quartile	P _{trend}	P _{interaction}
Vitamin B2 sum	Yes	42/106	1	1.10 (0.32-3.85)	0.46 (0.12-1.78)	0.31 (0.07-1.39)	0.64 (0.40-1.04)	0.07	
	No	187/474	1	0.76 (0.44-1.31)	0.76 (0.44-1.33)	0.77 (0.43-1.37)	0.92 (0.77-1.11)	0.40	0.23
Riboflavin	Yes	42/106	1	1.49 (0.47-4.79)	0.61 (0.15-2.50)	0.33 (0.07-1.45)	0.65 (0.41-1.02)	0.06	
	No	187/475	1	0.91 (0.54-1.55)	0.74 (0.42-1.30)	0.89 (0.50-1.58)	0.94 (0.78-1.13)	0.53	0.21
FMN	Yes	42/106	1	0.66 (0.19-2.33)	0.11 (0.02-0.61)	0.06 (0.01-0.44)	0.38 (0.20-0.73)	<0.01	
	No	187/474	1	1.25 (0.77-2.02)	0.79 (0.45-1.39)	0.73 (0.41-1.32)	0.87 (0.73-1.05)	0.16	0.03
Vitamin B6 sum	Yes	41/105	1	0.44 (0.14-1.42)	0.57 (0.15-2.09)	0.20 (0.04-0.94)	0.64 (0.40-1.03)	0.07	
	No	190/481	1	0.74 (0.44-1.25)	0.58 (0.32-1.05)	0.52 (0.28-0.96)	0.80 (0.66-0.98)	0.03	0.78
PLP	Yes	42/106	1	0.84 (0.28-2.56)	0.55 (0.15-2.03)	0.50 (0.13-1.96)	0.78 (0.50-1.22)	0.27	
	No	191/484	1	0.82 (0.49-1.38)	0.63 (0.34-1.15)	0.65 (0.34-1.24)	0.86 (0.69-1.05)	0.14	0.95
PL	Yes	42/105	1	0.80 (0.25-2.55)	0.97 (0.29-3.22)	0.50 (0.11-2.19)	0.85 (0.54-1.33)	0.47	
	No	190/484	1	1.09 (0.65-1.80)	0.47 (0.26-0.87)	0.81 (0.44-1.46)	0.86 (0.71-1.04)	0.12	0.59
PA	Yes	42/105	1	0.46 (0.16-1.27)	0.35 (0.10-1.20)	0.10 (0.02-0.62)	0.51 (0.30-0.86)	0.01	
	No	190/484	1	1.04 (0.63-1.71)	1.02 (0.60-1.74)	0.63 (0.35-1.15)	0.88 (0.73-1.06)	0.19	0.15

NOTE: RR (95% CI) calculated by conditional logistic regression, stratified by the case-control set and adjusted for Hp infection status and smoking. Of the 45 matched controls with SCAG, 32% (34 of 106) were matched to cases with SCAG and 2% (11 of 484) to cases without SCAG. The cutoff values for the quartiles were as follows: vitamin B2 sum, 11.88, 16.63, and 25.15 $\mu\text{mol/L}$; riboflavin, 8.52, 12.80, and 20.00 $\mu\text{mol/L}$; FMN, 2.13, 3.15, and 5.21 $\mu\text{mol/L}$; vitamin B6 sum, 40.8, 55.7, and 80.6 $\mu\text{mol/L}$; PLP, 14.90, 23.50, and 36.60 $\mu\text{mol/L}$; PL, 10.60, 14.50, and 18.97 $\mu\text{mol/L}$; and PA, 12.60, 16.40, and 24.90 $\mu\text{mol/L}$.

*SCAG defined as PGA concentrations <22 $\mu\text{g/L}$.

[†]Reference category.

some carcinogens are metabolized by flavin-dependent enzymes, and vitamin B2 status may modify effects of the carcinogen (58). PLP catalyzes ~100 essential enzymatic reactions in human metabolism (60). Although it is unclear whether low vitamin concentrations are a cause or a consequence of the disease, low PLP concentrations have been associated with high concentrations of inflammatory markers (47-49), which may explain our finding of stronger associations among those cases with SCAG. Furthermore, low PLP concentrations cause a decreased enzyme activity of serine hydroxymethyltransferase, which results in a lack of methylene groups for 5,10-methylenetetrahydrofolate production (61). This may lead to increased chromosome strand breaks (62), impaired DNA repair (63, 64), and DNA hypomethylation (65, 66), which has been observed in different types of tumors.

The majority of previous studies on genetic variations related to one-carbon metabolism in GC focused on the *MTHFR* gene and were mainly conducted in Asian populations (24). The overall result of a meta-analysis shows that in East Asian populations, but not in Caucasians, the risk of GC increases with the number of T alleles of the *MTHFR* 677C→T polymorphism (24). Published results for this EPIC population showed no association between the *MTHFR* 677C→T polymorphism and GC (9), and the null findings in the present report are in line with previous studies investigating

polymorphisms of the *MTRR* 66A→G (26, 27), *MTR* 2756A→G (27), and *SLC19A1* 180G→A (29) genes, but not with one study showing increased GC risk with variant genotypes of the *MTHFD1* 1958G→A (28) polymorphism. Notably, we observed opposite trends for the *CBS* 844ins68, the *TCN2* 776, and the *SLC19A1* 180 polymorphisms in northern and southern European countries. Furthermore, none of the polymorphisms modified the associations of the vitamins B2 and B6 with GC risk. However, sample sizes might have been too small to detect genetic associations with GC risk, interactions between genes and European region, and interactions between genes and vitamins and might have resulted in chance findings.

In summary, this large prospective European multicenter study revealed that higher concentrations of vitamin B2 suggest to decrease GC risk, whereas vitamin B6 were associated with a decreased GC risk, with more pronounced associations in a subgroup with SCAG. Furthermore, none of the polymorphisms related to one-carbon metabolism was associated with GC risk, nor did they modify the associations of vitamin B2 and B6 with this type of cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Table 5. Distribution of genotypes by European region and their associations with GC risk

Gene and SNP	Genotype	GC (all)		GC (northern Europe)		GC (southern Europe)	
		Case/control	OR (95% CI)	Case/control	OR (95% CI)	Case/control	OR (95% CI)
CBS 699	CC	103/282	1	62/147	1	41/135	1
	CT	118/251	1.26 (0.91-1.74)	72/141	1.18 (0.77-1.81)	46/110	1.37 (0.84-2.24)
	TT	16/69	0.58 (0.31-1.08)	11/37	0.62 (0.28-1.37)	5/32	0.52 (0.19-1.43)
		MAF = 0.32	$P_{\text{trend}} = 0.61$	MAF = 0.33	$P_{\text{trend}} = 0.65$	MAF = 0.31	$P_{\text{trend}} = 0.81$
CBS*	0 insertions	210/536	1	128/312	1	82/224	1
	1 insertion	34/91	0.96 (0.62-1.49)	24/40	1.44 (0.82-2.53)	10/51	0.54 (0.26-1.12)
	2 insertions	3/4	2.00 (0.43-9.35)	2/1	4.40 (0.36-53.5)	1/3	1.10 (0.11-10.8)
		MAF = 0.08	$P_{\text{trend}} = 0.81$	MAF = 0.07	$P_{\text{trend}} = 0.10$	MAF = 0.09	$P_{\text{trend}} = 0.15$
MTR 2756	AA	171/415	1	104/219	1	68/196	1
	AG	64/188	0.80 (0.57-1.12)	42/108	0.77 (0.49-1.21)	22/80	0.82 (0.48-1.40)
	GG	8/13	1.58 (0.62-3.99)	4/11	0.76 (0.23-2.47)	4/2	13.1 (1.43-120)
		MAF = 0.17	$P_{\text{trend}} = 0.56$	MAF = 0.18	$P_{\text{trend}} = 0.26$	MAF = 0.15	$P_{\text{trend}} = 0.62$
MTRR 66	AA	58/156	1	33/72	1	24/84	1
	AG	100/286	0.96 (0.65-1.41)	55/149	0.82 (0.48-1.40)	45/137	1.13 (0.65-1.98)
	GG	81/165	1.25 (0.82-1.91)	59/109	1.20 (0.70-2.07)	22/56	1.26 (0.64-2.48)
		MAF = 0.52	$P_{\text{trend}} = 0.28$	MAF = 0.57	$P_{\text{trend}} = 0.39$	MAF = 0.46	$P_{\text{trend}} = 0.51$

NOTE: RR (95% CI) calculated by conditional logistic regression, stratified by country and adjusted for age and sex.

Abbreviation: MAF, minor allele frequency (cases and controls combined).

*Significant interaction term ($P = 0.03$) for CBS 844ins68 with European region.

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References

- Verdecchia A, Mariotto A, Gatta G, Bustamante-Teixeira MT, Ajiki W. Comparison of stomach cancer incidence and survival in four continents. *Eur J Cancer* 2003;39:1603-9.
- Webb PM, Law M, Varghese C, et al. Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001;49:347-53.
- Schlemper RJ, van der Werf SD, Vandenbroucke JP, Biemond I, Lamers CB. Seroepidemiology of gastritis in Japanese and Dutch

- working populations: evidence for the development of atrophic gastritis that is not related to *Helicobacter pylori*. *Gut* 1995;37:199–204.
4. Hustad S, Midttun Ø, Schneede J, et al. The methylenetetrahydrofolate reductase 677C→T polymorphism as a modulator of a B vitamin network with major effects on homocysteine metabolism. *Am J Hum Genet* 2007;80:846–55.
 5. Bailey LB, Moyers S, Gregory JF. Present knowledge in nutrition. pp. 214–29. Washington (DC): International Life Sciences Institute; 2001.
 6. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology* 2006;131:1271–83.
 7. Larsson SC, Giovannucci E, Wolk A. Folate intake and stomach cancer incidence in a prospective cohort of Swedish women. *Cancer Epidemiol Biomarkers Prev* 2006;15:1409–12.
 8. Botterweck AA, van den Brandt PA, Goldbohm RA. Vitamins, carotenoids, dietary fiber, and the risk of gastric carcinoma: results from a prospective study after 6.3 years of follow-up. *Cancer* 2000;88:737–48.
 9. Vollset SE, Igland J, Jenab M, et al. The association of gastric cancer risk with plasma folate, cobalamin, and methylenetetrahydrofolate reductase polymorphisms in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol Biomarkers Prev* 2007;16:2416–24.
 10. Mayne ST, Risch HA, Dubrow R, et al. Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10:1055–62.
 11. Hsing AW, Hansson LE, McLaughlin JK, et al. Pernicious anemia and subsequent cancer. A population-based cohort study. *Cancer* 1993;71:745–50.
 12. Karlson BM, Ekblom A, Wacholder S, McLaughlin JK, Hsing AW. Cancer of the upper gastrointestinal tract among patients with pernicious anemia: a case-cohort study. *Scand J Gastroenterol* 2000;35:847–51.
 13. Munoz N, Plummer M, Vivas J, et al. A case-control study of gastric cancer in Venezuela. *Int J Cancer* 2001;93:417–23.
 14. Graham S, Haughey B, Marshall J, et al. Diet in the epidemiology of gastric cancer. *Nutr Cancer* 1990;13:19–34.
 15. Cornee J, Pobel D, Riboli E, Guyader M, Hemon B. A case-control study of gastric cancer and nutritional factors in Marseille, France. *Eur J Epidemiol* 1995;11:55–65.
 16. Harrison LE, Zhang ZF, Karpeh MS, Sun M, Kurtz RC. The role of dietary factors in the intestinal and diffuse histologic subtypes of gastric adenocarcinoma: a case-control study in the U.S. *Cancer* 1997;80:1021–8.
 17. Zhang ZF, Kurtz RC, Yu GP, et al. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. *Nutr Cancer* 1997;27:298–309.
 18. Kaaks R, Tuyns AJ, Haelterman M, Riboli E. Nutrient intake patterns and gastric cancer risk: a case-control study in Belgium. *Int J Cancer* 1998;78:415–20.
 19. Risch HA, Jain M, Choi NW, et al. Dietary factors and the incidence of cancer of the stomach. *Am J Epidemiol* 1985;122:947–59.
 20. Ji BT, Chow WH, Yang G, et al. Dietary habits and stomach cancer in Shanghai, China. *Int J Cancer* 1998;76:659–64.
 21. Kim HJ, Kim MK, Chang WK, et al. Effect of nutrient intake and *Helicobacter pylori* infection on gastric cancer in Korea: a case-control study. *Nutr Cancer* 2005;52:138–46.
 22. Lowik MR, van den Berg H, Kistemaker C, Brants HA, Brussaard JH. Interrelationships between riboflavin and vitamin B6 among elderly people (Dutch Nutrition Surveillance System). *Int J Vitam Nutr Res* 1994;64:198–203.
 23. McCormick DB. Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol Rev* 1989;69:1170–98.
 24. Zintzaras E. Association of methylenetetrahydrofolate reductase (MTHFR) polymorphisms with genetic susceptibility to gastric cancer: a meta-analysis. *J Hum Genet* 2006;51:618–24.
 25. Ott N, Geddert H, Sarbia M. Polymorphisms in methionine synthase (A2756G) and cystathionine β-synthase (844ins68) and susceptibility to carcinomas of the upper gastrointestinal tract. *J Cancer Res Clin Oncol* 2008;134:405–10.
 26. Stolzenberg-Solomon RZ, Qiao YL, Abnet CC, et al. Esophageal and gastric cardia cancer risk and folate- and vitamin B(12)-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomarkers Prev* 2003;12:1222–6.
 27. Zhang FF, Terry MB, Hou L, et al. Genetic polymorphisms in folate metabolism and the risk of stomach cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:115–21.
 28. Wang L, Ke Q, Chen W, et al. Polymorphisms of MTHFD, plasma homocysteine levels, and risk of gastric cancer in a high-risk Chinese population. *Clin Cancer Res* 2007;13:2526–32.
 29. Gotze T, Rocken C, Rohl FW, et al. Gene polymorphisms of folate metabolizing enzymes and the risk of gastric cancer. *Cancer Lett* 2007.
 30. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5:1113–24.
 31. Riboli E, Kaaks R. European Prospective Investigation into Cancer and Nutrition. The EPIC Project: rationale and study design. *Int J Epidemiol* 1997;26 Suppl 1:S6–14.
 32. Carneiro F, Moutinho C, Pera G, et al. Pathology findings and validation of gastric and esophageal cancer cases in a European cohort (EPIC/EUR-GAST). *Scand J Gastroenterol* 2007;42:618–27.
 33. Midttun Ø, Hustad S, Solheim E, Schneede J, Ueland PM. Multianalyte quantification of vitamin B6 and B2 species in the nanomolar range in human plasma by liquid chromatography-tandem mass spectrometry. *Clin Chem* 2005;51:1206–16.
 34. Meyer K, Fredriksen A, Ueland PM. High-level multiplex genotyping of polymorphisms involved in folate or homocysteine metabolism by matrix-assisted laser desorption/ionization mass spectrometry. *Clin Chem* 2004;50:391–402.
 35. Koushik A, Kraft P, Fuchs CS, et al. Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2006;15:2408–17.
 36. Palli D, Masala G, Del Giudice G, et al. CagA+ *Helicobacter pylori* infection and gastric cancer risk in the EPIC-EURGAST study. *Int J Cancer* 2007;120:859–67.
 37. Foraker AB, Khantwal CM, Swaan PW. Current perspectives on the cellular uptake and trafficking of riboflavin. *Adv Drug Deliv Rev* 2003;55:1467–83.
 38. Merrill AH, Jr., McCormick DB. Affinity chromatographic purification and properties of flavokinase (ATP:riboflavin 5'-phosphotransferase) from rat liver. *J Biol Chem* 1980;255:1335–8.
 39. Yamada Y, Merrill AH, Jr., McCormick DB. Probable reaction mechanisms of flavokinase and FAD synthetase from rat liver. *Arch Biochem Biophys* 1990;278:125–30.
 40. Karthikeyan S, Zhou Q, Mseeh F, et al. Crystal structure of human riboflavin kinase reveals a β barrel fold and a novel active site arch. *Structure* 2003;11:265–73.
 41. Leklem J. Present knowledge in nutrition. pp. 174–83. Washington (DC): ILSI Press; 1996.
 42. Coburn SP. Modeling vitamin B6 metabolism. *Adv Food Nutr Res* 1996;40:107–32.
 43. Knol MJ, Vandenbroucke JP, Scott P, Egger M. What do case-control studies estimate? Survey of methods and assumptions in published case-control research. *Am J Epidemiol* 2008.
 44. Midttun Ø, Hustad S, Schneede J, Vollset SE, Ueland PM. Plasma vitamin B-6 forms and their relation to transsulfuration metabolites in a large, population-based study. *Am J Clin Nutr* 2007;86:131–8.
 45. Carmel R. Cobalamin, the stomach, and aging. *Am J Clin Nutr* 1997;66:750–9.
 46. Weck MN, Brenner H. Prevalence of chronic atrophic gastritis in different parts of the world. *Cancer Epidemiol Biomarkers Prev* 2006;15:1083–94.
 47. Bates CJ, Pentieva KD, Prentice A. An appraisal of vitamin B6 status indices and associated confounders, in young people aged 4–18 years and in people aged 65 years and over, in two national British surveys. *Public Health Nutr* 1999;2:529–35.
 48. Chiang EP, Smith DE, Selhub J, et al. Inflammation causes tissue-specific depletion of vitamin B6. *Arthritis Res Ther* 2005;7:R1254–62.

49. Gori AM, Sofi F, Marcucci R, et al. Association between homocysteine, vitamin B(6) concentrations and inflammation. *Clin Chem Lab Med* 2007;45:1728–36.
50. Kekki M, Samloff IM, Varis K, Ihamaki T. Serum pepsinogen I and serum gastrin in the screening of severe atrophic corpus gastritis. *Scand J Gastroenterol Suppl* 1991;186:109–16.
51. Hustad S, McKinley MC, McNulty H, et al. Riboflavin, flavin mononucleotide, and flavin adenine dinucleotide in human plasma and erythrocytes at baseline and after low-dose riboflavin supplementation. *Clin Chem* 2002;48:1571–7.
52. Powers HJ. Riboflavin (vitamin B-2) and health. *Am J Clin Nutr* 2003;77:1352–60.
53. Tuyns AJ, Kaaks R, Haelterman M, Riboli E. Diet and gastric cancer. A case-control study in Belgium. *Int J Cancer* 1992;51:1–6.
54. Gonzalez CA, Pera G, Agudo A, et al. Fruit and vegetable intake and the risk of stomach and oesophagus adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC-EUR-GAST). *Int J Cancer* 2006;118:2559–66.
55. IARC. Fruit and vegetables. IARC handbook of cancer prevention. Lyon (France): IARC Press; 2003.
56. Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr* 2003;78:559–69S.
57. Gonzalez CA, Jakszyn P, Pera G, et al. Meat intake and risk of stomach and esophageal adenocarcinoma within the European Prospective Investigation into Cancer and Nutrition (EPIC). *J Natl Cancer Inst* 2006;98:345–54.
58. Webster RP, Gawde MD, Bhattacharya RK. Modulation of carcinogen-induced DNA damage and repair enzyme activity by dietary riboflavin. *Cancer Lett* 1996;98:129–35.
59. Pangrekar J, Krishnaswamy K, Jagadeesan V. Effects of riboflavin deficiency and riboflavin administration on carcinogen-DNA binding. *Food Chem Toxicol* 1993;31:745–50.
60. Komatsu S, Yanaka N, Matsubara K, Kato N. Antitumor effect of vitamin B6 and its mechanisms. *Biochim Biophys Acta* 2003;1647:127–30.
61. Stabler SP, Lindenbaum J, Savage DG, Allen RH. Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. *Blood* 1993;81:3404–13.
62. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci U S A* 1997;94:3290–5.
63. Choi SW, Kim YI, Weitzel JN, Mason JB. Folate depletion impairs DNA excision repair in the colon of the rat. *Gut* 1998;43:93–9.
64. Huang YC, Chen W, Evans MA, Mitchell ME, Shultz TD. Vitamin B-6 requirement and status assessment of young women fed a high-protein diet with various levels of vitamin B-6. *Am J Clin Nutr* 1998;67:208–20.
65. Jarrard DF, Bova GS, Isaacs WB. DNA methylation, molecular genetic, and linkage studies in prostate cancer. *Prostate Suppl* 1996;6:36–44.
66. Jones PA, Buckley JD. The role of DNA methylation in cancer. *Adv Cancer Res* 1990;54:1–23.