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Low Vitamin B_{12} Increases Risk of Gastric Cancer: A Prospective Study of One-Carbon Metabolism Nutrients and Risk of Upper Gastrointestinal Tract Cancer

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Abstract

Previous studies have found associations between one-carbon metabolism nutrients and risk of several cancers, but little is known regarding upper gastrointestinal tract (UGI) cancer. We analyzed pre-diagnostic serum concentrations of several one-carbon metabolism nutrients (vitamin B12, folate, vitamin B6, riboflavin, and homocysteine) in a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study of male smokers, which was undertaken in Finland between 1985 and 1988.

We conducted a nested case-control study including 127 non-cardia gastric adenocarcinoma (NCGA), 41 esophago-gastric junctional adenocarcinoma (EGJA), and 60 esophageal squamous cell carcinoma (ESCC) incident cases identified within ATBC. Controls were matched to cases on age, date of serum collection, and follow-up time. One-carbon nutrient concentrations were measured in fasting serum samples collected at baseline (up to 17 years prior to cancer diagnosis). Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression. Lower pre-diagnostic vitamin B12 concentrations at baseline were associated with a 5.8-fold increased risk of NCGA (95% CI = 2.7 to 12.6 for lowest compared to highest quartile, p-trend < 0.001). This association remained in participants who developed cancer more than 10

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years after blood collection, and after restricting the analysis to participants with clinically normal serum vitamin B12 (>300 pmol/L). In contrast, pepsinogen I, a known serologic marker of gastric atrophy, was not associated with NCGA in this population.

As vitamin B12 absorption requires intact gastric mucosa to produce acid and intrinsic factor, our findings suggest vitamin B12 as a possible serologic marker for the atrophic gastritis that precedes NCGA, one more strongly associated with subsequent NCGA than pepsinogen.

Keywords

esophageal cancer; gastric cancer; one-carbon metabolism; vitamin B12; gastric atrophy

INTRODUCTION

Cancers of the upper gastrointestinal (UGI) tract cause more than 1.1 million deaths per year worldwide[1]. *Helicobacter pylori (H. pylori)* is the strongest known risk factor for gastric cancer in Western countries[2]; however, as only a fraction of those infected with *H. pylori* develop clinically significant gastric disease and fewer still develop gastric cancer, other factors such as genetic predisposition and diet also likely play an etiologic role. Smoking[3], alcohol consumption[4], and dietary factors[5] are established risk factors for UGI cancer.

One-carbon metabolism nutrients could play an etiological role in UGI tract cancer through several mechanisms. One-carbon reactions generate purines and pyrimidines for DNA synthesis and repair, and generate the methyl groups needed for methylation of DNA, RNA and protein. Perturbing these crucial functions could promote carcinogenesis. Several B-vitamins are essential to the one-carbon metabolism pathway as cofactors and prosthetic groups: cobalamin (vitamin B12), folate (vitamin B9), pyridoxine (vitamin B6), and riboflavin (vitamin B2). Homocysteine is a metabolite of the pathway, wherein it is converted to methionine.

Several prospective studies report an association between dietary intake of one-carbon nutrients and UGI cancer[6–12]. However, the association between one-carbon nutrient serum concentrations and UGI cancer has been studied in only one cohort[13–15]. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, investigators reported a 0.79-fold decreased risk of gastric cancer per SD increase in serum vitamin B12, and a 0.78-fold decreased risk of gastric cancer per SD increase in serum vitamin B6[13, 15].

We measured serum concentrations of several one-carbon nutrients (vitamin B12, folate, vitamin B6, riboflavin, and homocysteine) in 253 UGI cancer cases:127 cases of non-cardia gastric adenocarcinoma (NCGA), 46 cases of esophago-gastric junctional adenocarcinoma (EGJA), 60 cases of esophageal squamous cell carcinoma (ESCC) and 326 controls, in a case-control study nested within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study.

METHODS

Study Cohort

The ATBC Study, a randomized controlled trial conducted in Finland, was designed to investigate whether daily supplementation with alpha-tocopherol, beta-carotene, or both, could reduce the risk of lung and other cancers[16, 17]. The study population included 29,133 male smokers (≥5 cigarettes/day), ages 50 to 69 years, from Finland who were recruited and randomized between 1985 and 1988. Participants were excluded from the trial if they had a history of a malignancy or other medical conditions that could limit long-term participation.

After the end of the trial in 1993, participants were followed as a cohort. Written informed consent was obtained from all individuals. The study was approved by the Special Studies IRB at the National Cancer Institute and by the IRBs at the National Public Health Institute in Finland.

Baseline Data collection (1985–1988)

Prior to randomization, participants completed questionnaires on general background characteristics (smoking habits, diet, lifestyle, education, medical history). During the baseline assessment (pre-randomization to alpha-tocopherol, beta-carotene supplements), participants provided a 12 hour fasting blood sample. Serum samples were stored at −70°C until analyzed. Participants completed a validated dietary assessment instrument, which assessed usual food consumption over the previous year[18]. The food frequency questionnaire was completed satisfactorily by 93% of study participants. Participants missing dietary data were included in analyses with a “missing” indicator variable. Excluding these participants from the study did not affect risk estimates (data not shown).

Case identification and control selection (Follow-up through 2002)

Cancer cases were identified through the Finnish Cancer Registry, which provides almost 100% ascertainment of cancer cases in the ATBC Cohort[19]. Cases were defined as incident tumors of the esophagus (ICD9 code 150), gastric cardia (ICD9 code 151.0), or incident gastric tumors not in the cardia (ICD9 code 151.1). ICDO codes were further used to classify histology. We classified EGJA patients according to the World Health Organization classification of tumors of the digestive system[20]: adenocarcinomas of the esophagus and gastric cardia. All cases were diagnosed between study baseline and April 30, 2002, with adequate serum. To be eligible for selection, controls had to be alive and cancer-free at the time of case diagnosis. 330 controls were matched to the 330 UGI selected cancer cases 1:1 on age at randomization (+/− 5 years) and date of blood draw (+/− 30 days). Of these, 326 controls and 253 cases (127 NCGA, 46 EGJA, 60 ESCC, 20 additional cases of mixed histology (carcinomas, carcinoid and sarcomas) were excluded from the site-specific analyses) had serum available for analysis. Here, we present results using all 326 controls, rather than only the 253 matched controls, because they add precision to our point estimates, though we note that our estimates were similar when we included only the 253 matched controls (Supplementary Table 1). The median follow-up time for participants was 13 years, the maximum follow-up was 17 years.

Laboratory analysis

Serum folate and vitamin B12 were determined by radioassay (Bio-Rad Laboratories). Pyridoxal 5′ phosphate (PLP), the biologically active form of vitamin B6, was determined by the tyrosine decarboxylase apoenzyme method[21]. Homocysteine and riboflavin were determined by high-performance liquid chromatography[22, 23].

Each batch contained case and matched control pairs placed adjacently but in random order, as well as six blinded quality control (QC) samples derived from a pool of serum. For the QC samples, the within-batch coefficients of variation were 6.1% for homocysteine, 5.3% for vitamin B6, 9.3% for folate, 6.8% for vitamin B12, and 5.8% for riboflavin (25 batches, 154 QC samples, 5–7 QC samples per batch).

H pylori seropositivity was measured by a multiplex serology assay that determined presence of serum antibodies to 15 *H. pylori* specific antigens. We defined overall *H. pylori* positivity as those seropositive to four or more antigens[24, 25]. Serum pepsinogen I was measured via radioimmunoassay[26–28]. A low serum pepsinogen I level was defined as 25 μg/L or less[29].

Statistical analysis

Statistical analyses were performed using Stata version 13.0 (Stata-Corp LP) and all p-values were two-sided. Serum nutrient quartiles were based on the distribution among the controls. We compared the distribution of selected characteristics for case and 1:1 matched control subjects using Wilcoxon rank-sum tests for continuous variables and Chi square test for categorical variables. Among controls, the associations between serum one-carbon nutrients (in quartiles) and selected characteristics were determined by the non-parametric tests for trend. Correlations between individual pairs of one-carbon nutrients were estimated using Spearman's rank order coefficient among the control subjects.

Odds ratios (OR) and 95% confidence intervals (CI) for the association between serum one-carbon nutrients and incident cancer were determined by conditional logistic regression with the highest quartile as referent. For continuous models, serum nutrient concentrations were scaled to ½ the interquartile range (a scale which is appropriate for data which is not normally distributed) of that nutrient in controls.

On inspection, the laboratory measurements of the one-carbon metabolites showed significant drift across analytic batches. To account for this analytic measurement drift we broke the original matching scheme (age and date of blood draw) and conditioned the logistic regression models on analytic batch. This approach produced similar results to matching by pair number, as cases and their matched controls were always included within the same batch.

Models were adjusted for age (years), BMI (body mass index, kg/m^2), number of cigarettes smoked per day, years of cigarette smoking, educational attainment (educated beyond elementary school, %), alcohol consumption (g/day), energy intake (kcal/day), fruit (g/day) and vegetable (g/day) consumption, *H. pylori* seropositivity, and low pepsinogen 1 status (PGI; ≤ 25 μg/L). Models were additionally adjusted for milk and meat consumption, and

ATBC assignment during preliminary analysis, though they were not included in final models because intake did not differ between cases and controls and addition of these elements to the models did not significantly change estimates.

Though case numbers were low for sub-group analysis, we examined possible effect modification by smoking dose (≥/< mean: 20 cigarettes/day), years of smoking (≥/< mean: 37 years), and alcohol consumption (<13 grams/day, 13–26 grams/day, ≥ 26 grams/day) using unconditional logistic regression.

For the NCGA/vitamin B12 disease association model, lag analysis was also performed: NCGA patients were classified according to whether they occurred within 2 years of baseline, within 10 years of baseline, or more than 10 years after baseline. We also performed a sensitivity analysis on this model, excluding vitamin B12 borderline and deficient participants (<300 pmol/L).

RESULTS

Baseline characteristics for cancer cases and controls are shown in Table 1. Compared to controls, NCGA cases had higher prevalence of *H. pylori* seropositivity and lower serum vitamin B12, folate, and vitamin B6. EGJA cases started smoking at an older age than controls. ESCC cases had lower BMI, higher alcohol consumption, and higher prevalence of *H. pylori* seropositivity and low pepsinogen I compared with controls.

Among all subjects (regardless of case/control status), 2% had deficient serum vitamin B12 (<150 pmol/L)[30]; 35% had borderline/below-sufficient vitamin B12 (<300 pmol/L)[31]; 33% had deficient serum folate (<7.5 nmol/L)[30]; 84% had borderline/below-sufficient serum folate (<12.0 nmol/L)[30]; 66% had deficient serum vitamin B6 (<30 pmol/mL)[30]. Homocysteine was elevated (>15 nmol/mL)[32] in 34% of subjects. There is no defined adequacy level for serum riboflavin.

Weak but statistically significant correlations were observed among the serum nutrients within controls (Table 2), including for vitamin B12, which was positively correlated with folate, vitamin B6, and riboflavin, and negatively correlated with homocysteine. The associations between serum one-carbon nutrient concentrations and baseline characteristics were analyzed in controls (Supplementary Table 2).

Table 3 presents odds ratios for the association between baseline serum one-carbon nutrient concentrations and NCGA, EGJA or ESCC. We present the results of three models: a crude model, a model fully adjusted for established risk-factors for UGI cancer, and a model additionally adjusted for *H. pylori* seropositivity and pepsinogen I status. Results from the three models were similar, although the confidence intervals for the associations reported in the additionally adjusted model are notably wider. This was likely due to the fact that our population had a very low proportion of *H. pylori* seronegativity, and low prevalence of low PG I status. The fully adjusted model was the most parsimonious. Compared to those with the highest (Q4) serum vitamin B12 concentration, participants with lower serum vitamin B12 had significantly elevated risk of NCGA (Table 3: $Q1_{vs}4$: OR: 5.77; 95% CI: 2.65,

12.56, P_{trend}<0.001). This association between low vitamin B12 and an increased risk of NCGA remained statistically significant across all three models.

Conversely, low serum folate concentrations were associated with a decreased risk of EGJA in the additionally adjusted model only ($Q1_{vs}4$: OR: 0.05; 95% CI: 0.00 to 0.72, P_{trend}=0.04), although the case numbers for EGJA are small (n=41) and the confidence intervals wide. None of the other one-carbon nutrients were associated with NCGA, EGJA or ESCC. Further, there was no evidence of interactions of any of the associations by smoking dose (<20 *vs.* ≥ 20 cigarettes/day), years of smoking (<37 *vs.* ≥ 37 years), or alcohol consumption (<13, 13–26, ≥ 26 grams/day).

The temporal nature of the associations between vitamin B12 and NCGA was explored by performing a lag analysis (Table 4). Due to small case numbers in some strata, we used a continuous model scaled to ½ the interquartile range in controls (73 pmol/L), rather than a quartile-based model. The magnitude of the association between serum vitamin B12 and NCGA appeared consistent over time, even after restricting the analysis to participants who developed cancer more than 10 years after blood collection.

Next, we performed a sensitivity analysis excluding cases and controls with vitamin B12 <300 pmol/L, thereby excluding both vitamin B12 deficient and borderline participants (Table 5). The association between lower (Q2 or Q3, relative to Q4) serum B12 concentrations and increased risk of NCGA remained with these deficient and borderline participants removed.

Finally, we compared the association between low serum vitamin B12 and NCGA, with the association between low serum pepsinogen I, a known serologic marker of gastric atrophy, and NCGA (Table 6). The association between low serum vitamin B12 and NCGA was significant across all time intervals analyzed, even when adjusting for low pepsinogen I. In contrast, in the ATBC population, there was no association between low pepsinogen I and NCGA.

DISCUSSION

We conducted a nested case-control study in the ATBC Study to examine the association between the serum concentrations of one-carbon nutrients and NCGA, EGJA, and ESCC. We observed a statistically significant 5.8-fold increase in risk of NCGA for subjects with lowest-quartile serum vitamin B12 (<291 pmol/L), relative to those with highest-quartile serum vitamin B12 (>436 pmol/L). This association remained statistically significant regardless of how soon after the blood draw the cancers occurred, and increased risk of NCGA persisted even when individuals with clinically deficient and borderline B12 concentrations (<300 pmol/L) were omitted from the analysis. Low serum folate concentrations were associated with a decreased risk of EGJA in one of our three risk models, though we note that these associations are based on relatively small numbers. There were no statistically significant associations between ESCC and serum concentrations of any of the one-carbon nutrients.

The relationship between UGI cancer and serum concentrations of one-carbon nutrients has previously been studied prospectively in only the EPIC cohort[13–15], wherein investigators found an association between low serum vitamin B12 and gastric cancer in cases with serologic evidence of gastric atrophy (n=44 cases, $Q4_{vs}1$ OR:0.08; 95% CI: 0.02, 0.38, $P_{trend}=0.002$)[15], and an association between low vitamin B6 and gastric cancer overall (n=235 cases, $Q4_{vs}1$ OR: 0.46; 95% CI: 0.26, 0.80, $P_{trend}=0.006$)[13]. Our results were similar – we also found a strikingly increased risk of gastric cancer, specifically NCGA, in subjects with lower serum vitamin B12 concentrations. While we did not observe any associations between UGI tract cancer and serum vitamin B6 concentration, median vitamin B6 was 59% lower in our study population than the EPIC study population, meaning our analysis might not have had the dynamic range to observe a similar association. Also, by contrast, the ATBC Study participants were (on average) younger than EPIC cohort members, they were all smokers and all males, meaning that there are many reasons to expect different serologic vitamin concentration profiles between the two studies.

That low serum vitamin B12 is associated with an increased risk of NCGA is mechanistically plausible. The prevailing model for intestinal type gastric cancer describes a progression from chronic superficial gastritis, to chronic atrophic gastritis, to intestinal metaplasia, dysplasia, and finally adenocarcinoma[33, 34]. Chronic atrophic gastritis decreases gastric acid secretion[35]. In parallel, vitamin B12 absorption requires an acid-producing gastric mucosa, to allow cleavage of vitamin B12 from its binding proteins in the stomach[36]. Therefore, any stimulus which induces chronic atrophic gastritis could potentially increase the risk of NCGA and simultaneously diminish vitamin B12 absorption, and therefore serum concentration. Accordingly, within our population, lower serum concentrations of vitamin B12 were associated with higher prevalence of low pepsinogen I status, a known serologic measure of gastric atrophy[27] (Supplementary Table 1). However, we find it intriguing that within this population, the association between vitamin B12 and NCGA appears stronger than that seen for pepsinogen I.

H. pylori infection is the strongest known initiator of the neoplastic sequence that leads to NCGA[37, 38]. *H. pylori* infection is also associated with food-bound vitamin B12 malabsorption[39–41] likely due to induction of atrophic gastritis and accompanying achlorhydria (increase in gastric pH). In turn, eradicating *H. pylori* infection has been shown to improve vitamin B12 status[39]. The prevalence of serologic positivity to *H. pylori* in our study population was very high (89% in controls and 99% in NCGA cases), thus *H. pylori* was likely the initiator that led to both poor vitamin B12 absorption, and ultimately NCGA, in our study population.

Pernicious anemia represents another potential link between vitamin B12 malabsorption and increased risk of NCGA[42]. Pernicious anemia is the end-stage of chronic autoimmune atrophic gastritis, in which antibodies to gastric parietal cells inhibit the secretion of intrinsic factor, eventually leading to macrocytic anemia due to diminished vitamin B12 absorption. It is possible that some subjects in our study had autoimmune atrophic gastritis, but without knowledge of their anti-parietal cell antibody status we cannot know for certain. However, given that the prevalence of undiagnosed pernicious anemia in people over 60 is only about 1.9%, and that exclusion of subjects with clinically deficient vitamin B12 (<150 pmol/mL,

the range expected in pernicious anemia) did not diminish the observed association of vitamin B12, it is unlikely that pernicious anemia is driving the association we observe.

The one prior study of serum folate with gastric cancer within the EPIC cohort found no association between serum folate concentrations and overall gastric cancer risk or cardia gastric cancer risk.[15] In the current study, we found an increased risk of EGJA with higher serum folate concentrations in just one model. While low folate has been shown to be protective against colorectal cancer in a population with low folate status,[43] with only 46 cases the apparent EGJA-folate association should be interpreted with caution.

Our study had some notable limitations. The ATBC Study was conducted in a cohort of male Finnish smokers, and therefore may not be generalizable to women or non-smokers. However, most studies report no significant difference in serum vitamin B12 concentrations in smokers compared to non-smokers[44]. Further limitations include the fact that assessment of the nutrients in this study occurred at a single point in time, so they may have changed over the course of the long follow-up period. The high prevalence of *H. pylori* in the population minimized our ability to adjust for it in our models, as did the low prevalence of low pepsinogen I status. Models including measures of *H. pylori* seropositivity and low pepsinogen I were therefore less stable than models without these variables, possibly due to homogeneity within the population.

A major strength of our study is that it is prospective: all serum one-carbon nutrients were measured prior to cancer diagnosis. The thorough baseline assessment of participants also allowed us to adjust for many confounders including *H. pylori*, pepsinogen I, alcohol consumption, cigarette smoking, and education and assess the relevance of several more. Further, the use of objective serum concentrations for the one-carbon factors, rather than self-reported nutrient intake, is likely a better indicator of absorbed and biologically active dose. This is particularly true of vitamin B12, for which more than 80% of deficiency states are due to malabsorption rather than insufficient dietary intake[36]. Our study also included a long follow-up time (17 years), and examination of the effect of nutrient concentrations over an extended period, allowing us to minimize the effects of potential preclinical disease at baseline on the exposure measure.

In summary, we observed an increased risk of NCGA with decreasing serum concentrations of vitamin B12. Further studies should explore the utility of vitamin B12 as a serologic marker of gastric atrophy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What's new?

B-vitamins are crucial for DNA methylation, synthesis and repair. We undertook a prospective analysis of circulating levels of B-vitamins in individuals that subsequently developed upper gastrointestinal cancer, and matched controls who did not. Low circulating vitamin B12 was associated with a 5-fold increase in risk of non-cardia gastric adenocarcinoma. Low vitamin B12 may be a marker of gastric atrophy. In this population, vitamin B12 is more strongly associated with gastric cancer than pepsinogen I, a commonly accepted serologic marker of gastric atrophy.

Table 1

Descriptive characteristics of non-cardia gastric adenocarcinoma (NCGA), esophago-gastric junctional adenocarcinoma (EGJA), and esophageal squamous cell carcinoma (ESCC) patients and controls subjects from the ATBC Study[1]

Variable	Control subjects	NCGA cases	*P*2	EGJA cases	*P*2	ESCC cases	*P*2
No. of Participants	326	127		46		60	
Median age at baseline (IQR)	57 (54, 61)	58 (54, 62)	0.24	59 (55, 63)	0.19	57 (54, 61)	0.87
Median body mass index[3], m/kg (IQR)	26.0 (24.0, 28.6)	25.5 (22.9, 28.2)	0.13	27 (24, 30)	0.90	24 (23, 28)	0.02
Median age started smoking (IQR)	19 (17, 20)	19 (17, 20)	0.39	20 (16, 23)	0.02	18 (17, 21)	0.66
Median years of smoking (IQR)	37 (32, 41)	38 (31, 43)	0.27	35 (30, 40)	0.61	39 (35, 42)	0.27
Median cigarettes smoked/day (IQR)	20 (15, 22)	20 (15, 25)	0.07	20 (15, 25)	0.71	20 (18, 25)	0.08
Median alcohol consumption, g/day (IQR)	10.8 (2.8, 25.0)	10.3 (1.4, 22.9)	0.94	9.6 (2.2, 21.8)	0.33	24.4 (10.7, 45.0)	0.01
Median energy intake, kcal (IQR)	2664 (2218, 3121)	2647 (2184, 3280)	0.75	2364 (2010, 2864)	0.16	2454 (2115, 3313)	0.97
Median vegetable intake, g/day (IQR)	714 (564, 889)	719 (529, 861)	0.95	678 (548, 875)	0.64	628 (460, 807)	0.10
Median fruit intake, g/day (IQR)	175 (98, 302)	189 (87, 326)	0.36	170 (88, 257)	0.46	132 (30, 254)	0.07
Post-elementary school education (n, %)	67 (21%)	13 (10%)	0.05	11 (24%)	0.81	9 (15%)	0.11
Serum Data							
Median vitamin B12, pmol/L (IQR)	349 (291, 438)	313 (258, 379)	0.0002	320 (277, 415)	0.08	327 (278, 416)	0.33
Median folate, nmol/L (IQR)	8.68 (6.84, 10.83)	8.18 (6.05, 10.10)	0.15	9.30 (6.80, 12.03)	0.45	9.28 (7.49, 12.32)	0.41
Median vitamin B6 (PLP), pmol/mL (IQR)	23.2 (17.0, 35.1)	21.9 (14.0, 33.1)	0.05	22.0 (13.8, 39.5)	0.74	26.7 (17.4, 47.6)	0.52
Median riboflavin, pmol/mL (IQR)	8.68 (6.71, 11.73)	8.0 (5.8, 10.8)	0.14	9.1 (6.2, 11.7)	0.11	9.1 (6.6, 11.8)	0.98
Median homocysteine, nmol/mL (IQR)	13.2 (11.0, 15.7)	14.1 (11.1, 17.1)	0.72	12.8 (11.2, 15.4)	0.66	13.4 (11.0, 16.6)	0.38
H. pylori seropositivity by multiplex[3] (n, %)	57 (89%)	114 (99%)	0.02	42 (95%)	0.57	54 (96%)	0.02
Pepsinogen I Low[4] (n, %)	33 (11%)	8 (13%)	0.41	3 (12%)	0.48	10 (28%)	0.04

[1] IQR = Interquartile Range.

[2] P-values are for each cancer type and their 1:1 matched controls. Wilcoxon rank-sum test for continuous variables; Chi-square test for categorical variables

[3] *H. pylori* status was assessed in 64 controls, 115 NCGA cases, 44 EGJA cases, and 56 ESCC cases

[4] Pepsinogen I status was assessed in 313 controls, 63 NCGA cases, 25 EGJA cases, and 36 ESCC cases; A low serum pepsinogen I level was defined as 25 μg/L or less

Table 2

Spearman correlation coefficients for serum one-carbon metabolites among controls in the ATBC Study (n=326)

	Vitamin B12	Folate	Vitamin B6 (PLP)	Riboflavin	Homocysteine
Vitamin B12	1.00				
Folate	0.23[1]	1.00			
Vitamin B6 (PLP)	0.11[2]	0.27[1]	1.00		
Riboflavin	0.24[1]	0.21[1]	0.21[1]	1.00	
Homocysteine	−0.14[2]	−0.32[1]	−0.12[2]	−0.05	1.00

[1] P<0.01

[2] P<0.05

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Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) for serum one-carbon nutrient concentration (quartiles[4] and continuous[5]) and the risk of non-cardia gastric adenocarcinoma (NCGA), esophago-gastric junctional carcinoma (EGJA), or esophageal squamous cell carcinoma (ESCC), in the ATBC Study

		Odds Ratio (95% Confidence Interval											
		NCGA (n = 127)				EGJA (n= 46)				ESCC (n= 60)			
		Ncases/Ncontrols	Unadjusted[1]	Fully adj.[2]	Additional adj.[3]	Ncases/Ncontrols	Unadjusted[1]	Fully adj.[2]	Additional adj.[3]	Ncases/Ncontrols	Unadjusted[1]	Fully adj.[2]	Additional adj.[3]
Vitamin B12 (pmol/L)	**Q1 (<291)**	54/81	5.67 (2.77, 12.05)	5.77 (2.65, 12.56)	7.24 (2.12, 24.73)	17/81	1.48 (0.64, 3.45)	0.90 (0.35, 2.34)	0.85 (0.12, 6.08)	19/81	1.39 (0.65, 3.00)	1.43 (0.62, 3.27)	1.01 (0.26, 3.87)
	Q2 (291–349)	29/82	3.00 (1.36, 6.64)	3.38 (1.50, 7.62)	4.03 (1.14, 14.19)	12/82	1.13 (0.46, 2.79)	0.88 (0.34, 2.31)	0.92 (0.14, 6.21)	15/82	0.96 (0.43, 2.16)	1.22 (0.51, 2.90)	0.71 (0.20, 2.54)
	Q3 (349–438)	34/81	3.47 (1.60, 7.53)	3.25 (1.46, 7.20)	3.11 (0.94, 10.32)	7/81	0.65 (0.23, 1.81)	0.57 (0.19, 1.72)	0.62 (0.07, 5.35)	12/81	0.84 (0.36, 1.92)	1.00 (0.42, 2.41)	0.84 (0.23, 3.14)
	Q4 (>438)	10/82	1.0 (ref)	1.0 (ref)	1.0 (ref)	10/82	1.0 (ref)	1.0 (ref)	1.0 (ref)	14/82	1.0 (ref)	1.0 (ref)	1.0 (ref)
	Ptrend		<0.001	<0.001	0.002		0.19	0.91	0.99		0.35	0.35	0.89
	Continuous	127/326	0.70 (0.6, 0.8)	0.69 (0.59, 0.81)	0.67 (0.51, 0.87)	46/326	0.91 (0.76, 1.09)	1.00 (0.82, 1.22)	1.05 (0.70, 1.56)	60/326	1.05 (0.99, 1.12)	1.05 (0.98, 1.12)	1.20 (1.02, 1.41)
Folate (nmol/L)	**Q1 (<6.8)**	40/81	1.60 (0.88, 2.90)	1.40 (0.73, 2.68)	0.74 (0.24, 2.23)	12/81	0.62 (0.27, 1.43)	0.60 (0.23, 1.57)	0.05 (0.00, 0.72)	11/81	0.53 (0.24, 1.18)	0.70 (0.28, 1.72)	0.32 (0.07, 1.34)
	Q2 (6.8–8.7)	40/80	1.72 (0.95, 3.12)	1.69 (0.89, 3.19)	1.21 (0.40, 3.68)	7/80	0.36 (0.14, 0.95)	0.28 (0.10, 0.80)	0.13 (0.02, 0.95)	14/80	0.64 (0.30, 1.35)	1.02 (0.45, 2.33)	0.35 (0.09, 1.46)
	Q3 (8.7–10.8)	21/83	0.91 (0.47, 1.77)	0.82 (0.41, 1.64)	0.56 (0.17, 1.84)	10/83	0.58 (0.25, 1.37)	0.47 (0.18, 1.25)	0.07 (0.01, 0.88)	12/83	0.47 (0.22, 1.03)	0.62 (0.27, 1.43)	0.36 (0.09, 1.48)
	Q4 (>10.8)	26/82	1.0 (ref)	1.0 (ref)	1.0 (ref)	17/82	1.0 (ref)	1.0 (ref)	1.0 (ref)	23/82	1.0 (ref)	1.0 (ref)	1.0 (ref)
	Ptrend		0.04	0.115	0.99		0.16	0.18	0.04		0.15	0.658	0.12
	Continuous	127/326	0.84 (0.73, 0.96)	0.86 (0.75, 0.99)	0.91 (0.71, 1.17)	46/326	1.20 (1.02, 1.41)	1.18 (0.99, 1.42)	1.75 (1.05, 2.92)	60/326	1.12 (0.96, 1.30)	1.06 (0.89, 1.25)	1.43 (1.10, 1.87)
Vitamin B6 (PLP; pmol/mL)	**Q1 (<16.9)**	45/81	1.74 (0.97, 3.09)	1.44 (0.77, 2.69)	1.20 (0.45, 3.21)	14/81	1.15 (0.49, 2.70)	1.54 (0.58, 4.10)	0.69 (0.07, 6.86)	15/81	0.71 (0.33, 1.52)	1.07 (0.46, 2.48)	2.77 (0.71, 10.86)
	Q2 (16.9–23.0)	26/82	0.95 (0.51, 1.79)	0.92 (0.48, 1.76)	0.66 (0.23, 1.88)	10/82	0.79 (0.32, 1.95)	0.75 (0.28, 2.01)	0.76 (0.10, 5.65)	9/82	0.44 (0.19, 1.04)	0.47 (0.18, 1.21)	0.78 (0.19, 3.19)
	Q3 (23.0–35.0)	29/81	1.07 (0.58, 1.98)	1.05 (0.56, 1.98)	0.84 (0.31, 2.26)	10/81	0.85 (0.35, 2.08)	0.93 (0.35, 2.44)	1.89 (0.32, 11.19)	16/81	0.81 (0.39, 1.69)	1.08 (0.49, 2.39)	1.15 (0.31, 4.18)
	Q4 (>35.0)	27/82	1.0(ref)	1.0(ref)	1.0(ref)	12/82	1.0(ref)	1.0 (ref)	1.0 (ref)	20/82	1.0 (ref)	1.0 (ref)	1.0 (ref)
	Ptrend		0.08	0.309	0.80		0.79	0.53	0.67		0.20	0.659	0.25
	Continuous	127/326	0.94 (0.88, 1.01)	0.95 (0.89, 1.01)	1.00 (0.90, 1.10)	46/326	0.97 (0.90, 1.05)	0.97 (0.88, 1.05)	0.99 (0.86, 1.13)		1.01 (0.96, 1.07)	0.99 (0.92, 1.06)	0.94 (0.82, 1.08)
Riboflavin (pmol/mL)	**Q1 (<6.7)**	44/81	1.92 (1.04, 3.54)	1.81 (0.94, 3.46)	1.09 (0.38, 3.12)	12/81	0.93 (0.37, 2.33)	0.99 (0.37, 2.66)	0.79 (0.10, 5.93)	15/81	1.03 (0.46, 2.31)	0.91 (0.38, 2.17)	0.68 (0.17, 2.79)
	Q2 (6.7–8.7)	30/82	1.30 (0.69, 2.43)	1.28 (0.66, 2.47)	1.07 (0.38, 3.05)	9/82	0.73 (0.29, 1.86)	0.64 (0.23, 1.78)	0.24 (0.03, 2.14)	14/82	0.99 (0.44, 2.21)	0.99 (0.41, 2.36)	0.87 (0.25, 3.02)
	Q3 (8.7–11.7)	29/81	1.26 (0.67, 2.36)	1.22 (0.64, 2.34)	0.87 (0.29, 2.61)	13/81	0.89 (0.38, 2.10)	0.92 (0.36, 2.34)	1.44 (0.19, 10.60)	16/81	1.19 (0.54, 2.63)	1.29 (0.56, 2.98)	1.34 (0.36, 4.96)
	Q4 (>11.7)	24/82	1.0(ref)	1.0 (ref)	1.0 (ref)	12/82	1.0 (ref)	1.0 (ref)	1.0 (ref)	15/82	1.0 (ref)	1.0 (ref)	1.0 (ref)
	Ptrend		0.04	0.079	0.77		0.77	0.816	0.51		0.94	0.705	0.49
	Continuous	127/326	0.95 (0.89, 1.02)	0.95 (0.89, 1.03)	1.01 (0.91, 1.12)	46/326	1.01 (0.94, 1.09)	1.02 (0.94, 1.10)	1.10 (0.92, 1.33)	60/326	1.00 (0.93, 1.06)	1.00 (0.94, 1.08)	1.12 (1.03, 1.20)
Homocysteine (nmol/mL)	**Q1 (<11.0)**	30/81	0.74 (0.41, 1.32)	0.86 (0.47, 1.58)	0.67 (0.25, 1.74)	10/81	1.16 (0.44, 3.04)	1.17 (0.41, 3.32)	1.75 (0.21, 14.79)	14/81	0.65 (0.29, 1.44)	0.74 (0.30, 1.80)	1.69 (0.42, 6.82)
	Q2 (11.0–13.2)	25/82	0.61 (0.34, 1.09)	0.74 (0.40, 1.37)	0.47 (0.17, 1.27)	16/82	1.68 (0.71, 3.96)	1.80 (0.68, 4.76)	1.18 (0.18, 7.83)	16/82	0.76 (0.36, 1.61)	0.98 (0.43, 2.23)	1.20 (0.33, 4.32)
	Q3 (13.2–15.6)	28/81	0.65 (0.37, 1.17)	0.78 (0.42, 1.42)	0.49 (0.18, 1.33)	9/81	0.98 (0.38, 2.52)	0.88 (0.31, 2.48)	0.40 (0.06, 2.65)	10/81	0.47 (0.20, 1.07)	0.74 (0.30, 1.79)	1.14 (0.29, 4.49)
	Q4 (>15.6)	44/82	1.0 (ref)	1.0 (ref)	1.0 (ref)	11/82	1.0 (ref)	1.0 (ref)	1.0 (ref)	20/82	1.0 (ref)	1.0 (ref)	1.0 (ref)
	Ptrend		0.25	0.573	0.41		0.49	0.452	0.46		0.41	0.629	0.49
	Continuous	127/326	1.02 (0.93, 1.11)	0.98 (0.89, 1.08)	1.03 (0.88, 1.21)	46/326	1.02 (0.89, 1.16)	1.03 (0.88, 1.19)	1.03 (0.74, 1.43)	60/326	1.11 (1.00, 1.22)	1.09 (0.98, 1.21)	1.16 (0.99, 1.36)

[1] Unadjusted = Crude odds ratios are calculated from conditional logistic regression models (conditioned on batch)

[2] Fully adj = Multivariable adjusted odds ratios are from conditional logistic regression models (conditioned on batch) adjusted for age, BMI, number of cigarettes smoked, years of cigarette smoking, educational attainment, alcohol consumption, energy intake, fruit, vegetables (not *H. pylori* or pepsinogen)

[3] Additional adj = Multivariable adjusted odds ratios are from conditional logistic regression models (conditioned on batch) adjusted for age, BMI, number of cigarettes smoked, years of cigarette smoking, educational attainment, alcohol consumption, energy intake, fruit, vegetables, as well as *H. pylori* positivity by multiplex and low pepsinogen.

[4] Cutpoints for quartiles were defined by all of the controls used in the study.

[5] Continuous models are scaled to ½ the interquartile range in controls

Table 4

Lag analysis (conditional logistic regression) of the association between vitamin B12 and risk of non-cardia gastric adenocarcinoma (NCGA), stratified by time to diagnosis since baseline

Time to NCGA	$n_{cases}/n_{controls}$	Odds Ratio[1] (95% CI)	*P*
All Cases	127/326	0.69 (0.59, 0.81)	<0.001
>2 years after baseline	101/326	0.66 (0.55, 0.79)	<0.001
<10 years after baseline	79/326	0.69 (0.57, 0.83)	<0.001
≥10 years after baseline	48/326	0.73 (0.58, 0.92)	0.009

[1] Multivariable adjusted odds ratios are from conditional logistic regression models (conditioned on batch) adjusted for age, BMI, number of cigarettes smoked, years of cigarette smoking, educational attainment, alcohol consumption, energy intake, fruit, and vegetables; B12 scaled to ½ the IQR in controls (73 pmol/L)

Table 5

Sensitivity analysis of the association between serum vitamin B12 (continuous and quartiles) and non-cardia gastric adenocarcinoma, excluding vitamin B12 borderline/deficient participants (OR, 95% CI)

	$n_{cases}/n_{controls}$	Odds Ratio (95% Confidence Interval)						
		Continuous[1, 2]	*P*	Q1[3]	Q2[3]	Q3[3]	Q4[3]	P_{trend}
All Participants	127/326	0.69 (0.59, 0.81)	<0.001	5.77 (2.65, 12.56)	3.38 (1.50, 7.62)	3.25 (1.46, 7.20)	1.0 (ref)	<0.001
Participants with normal B12 (>300 pmol/L)	66/232	0.68 (0.52, 0.90)	0.006	Excluded	3.52 (1.46, 8.58)	3.21 (1.40, 7.32)	1.0 (ref)	0.005

[1] Continuous model scaled to ½ the interquartile range in controls (73 pmol/L)

[2] Multivariable adjusted odds ratios are from conditional logistic regression models (conditioned by batch) adjusted for age, BMI, number of cigarettes smoked, years of cigarette smoking, educational attainment, alcohol consumption, energy intake, fruit, and vegetables.

[3] Cutpoints for quartiles were defined by all of the controls used in the study.

Table 6

Lag analysis of the association between low serum pepsinogen I (referent: >low serum pepsinogen/vitamin B12) or vitamin B12 concentrations by time to diagnosis since baseline and the risk of non-cardia gastric adenocarcinoma (NCGA)

		Pepsinogen I[2]				Vitamin B12[3]			
	Cases (Controls)	Crude[1]		Adjusted		Crude[1]		Adjusted	
Time to diagnosis relative to baseline		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
All Cases (controls)[4]	110 (316)	1.39 (0.59, 3.25)	0.45	1.05 (0.41, 2.73)	0.91	2.24 (1.26, 3.96)	0.01	2.45 (1.31, 4.59)	0.01
<10y	67 (316)	2.09 (0.61, 7.12)	0.24	1.18 (0.29, 4.83)	0.82	2.37 (1.00, 5.61)	0.05	2.26 (0.83, 6.18)	0.11
≥10y	43 (316)	0.97 (0.32, 2.96)	0.96	0.86 (0.25, 2.96)	0.81	2.12 (1.05, 4.26)	0.04	2.49 (1.15, 5.38)	0.02

[1] Crude odds ratios and 95% confidence intervals are calculated from conditional logistic regression models (conditioned on batch)

[2] A low serum pepsinogen I level was defined as 25 μg/L or less. Adjusted odds ratio and 95% confidence intervals were calculated by models adjusted for age at randomization, total years of smoking and total cigarettes per day, alcohol consumption (g/d), body mass index (kg/m^2), fruit intake (g/d), vegetable intake (g/d), post-primary school education, and vitamin B12 (Q1 vs Q2-4).

[3] A low vitamin B12 level is defined as Vitamin B12 <291 pmol/L, the first quartile in our analysis. Adjusted odds ratio and 95% confidence intervals were calculated by models adjusted for age at randomization, total years of smoking and total cigarettes per day, alcohol consumption (g/d), body mass index (kg/m^2), fruit intake (g/d), vegetable intake (g/d), post-primary school education, and low pepsinogen I (<25 μg/L)

[4] Subset of cases (n=110) and controls (n=316) who have both pepsinogen I and VitaminB12 measurements