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Plasma Folate, Vitamin B12, and Homocysteine and Cancers of the Esophagus, Stomach, and Liver in a Chinese Population

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Abstract

Evidence is accumulating regarding a role of micronutrients in folate metabolism in cancer risk. We investigated the associations of plasma folate, vitamin B12, and homocysteine with upper gastrointestinal (GI) cancers in a population-based case-control study in Taixing City, China. With informed consent, we recruited cases with cancers of esophagus (n = 218), stomach (n = 206), and liver (n = 204), and one common healthy control group (n = 405). A standardized epidemiologic questionnaire was used in face-to-face interviews, and blood samples were collected during interviews. We observed an inverse association between plasma folate levels and liver cancer. The adjusted odds ratio (aOR) was 0.46 [95% confidence interval (CI) = 0.24–0.88] comparing individuals in the highest quartile to those in the lowest. We found a positive association between plasma vitamin B12 levels and all three cancers. The aORs for those in the highest quartile were 2.80 (95% CI = 1.51–5.18) for esophageal cancer, 2.17 (1.21–3.89) for stomach cancer, and 9.97 (4.82–20.60) for liver cancer, comparing to those in the lowest quartile. We further observed interaction between plasma folate and vitamin B12 on these cancers. Our data indicated associations between plasma folate and vitamin B12 with upper GI cancers in Chinese population. Further research is warranted considering the debate over the necessity of food fortification.

INTRODUCTION

Upper gastrointestinal (GI) cancers of the stomach, liver, and esophagus are the fourth, sixth, and eighth most common cancers worldwide, respectively, based on GLOBOCAN 2012 estimates (1). About 2.19 million new cases of these 3 upper GI cancers were estimated to have occurred (15.5% of all cancers worldwide), and approximately 1.87 million people died from these cancers (22.8% of deaths from all cancers) in 2012 (1). The majority of these cancers (74%) occurred in less developed countries and China alone accounted for almost half (46.7%) of these cases (2).

Folate is a water-soluble B vitamin that plays an important role in DNA synthesis and methylation reactions by donating one-carbon units for cellular metabolism (3). Mandatory fortification of flour and grain products with folic acid, a synthetic form of folate, was introduced in North America in 1998 with an attempt to reduce the occurrence of neural tube defects (4). As of December 2014, 82 countries worldwide have legislation to mandate folic acid fortification in at least one industrially milled cereal grain (5). Although evidence has been accumulating for a protective role of folate in cancer development (6–8), recent studies also suggested a possible increased risk of cancer related to folic acid fortification (9,10), raising a debate on the necessity of food fortification and a need for more research on the role of folate and other related micronutrients on cancer prevention.

The World Cancer Research Fund concluded that there is limited evidence suggesting that folate protects against esophageal cancer and no conclusion was drawn for stomach or liver cancer (11) as there have been limited studies investigating the associations between dietary or plasma folate and upper GI cancers (7,12,13). In this study, we aimed to examine plasma folate and related micronutrients, including vitamin B12 and homocysteine, and cancers of the esophagus, stomach, and liver in a population-based case-control study conducted in China. We also explored potential interactions within these micronutrients and between these micronutrients and other environmental exposures on cancer susceptibility. Mandatory folic acid fortification is not adopted in China, which makes it an ideal site to study the role of blood folate and related micro-nutrients in carcinogenesis.

MATERIALS AND METHODS

Study Population

A population-based case-control study on cancers of the esophagus, stomach, and liver was conducted in Taixing City, Jiangsu Province, China in 2000 (14). Eligible cases were newly diagnosed esophageal, stomach, and liver cancer patients with pathologically or clinically confirmed diagnoses reported to the Taixing CDC Tumor Registry. Eligibility included having lived in Taixing for at least 10 yr, at least 20 yr old, in stable medical condition as determined by physicians, and willingness to participate. We recruited 218 esophageal, 206 stomach, and 204 liver cancer cases, which, respectively, represented 67%, 65%, and 57% of all new cancer patients. The control population was randomly selected from healthy individuals from the general population in Taixing who were also at least 20 yr of age and lived in Taixing for 10 or more years. Age, sex, and residential area (village or city block) were frequency matched between the esophageal, stomach, and liver cancer cases and controls in a 3:2 ratio. Among a total of 464 potential controls, 415 (89.4%) consented to participate. All study participants completed an epidemiologic questionnaire conducted by trained interviewers and donated an 8-ml blood specimen.

Laboratory Assays

The extraction of DNA from blood clots was carried out using a phenol-chloroform method. Enzyme-linked immunosorbant assays (ELISA) were performed to measure serum HBV surface antigen (HBsAg; Reagent Company of the Shanghai Hospital for Infectious Diseases, Shanghai, China), antihepatitis C virus (HCV) antibody (HCV IgG test, Shanghai Huamei Biological Company, Shanghai, China), and CagA-Helobacter pylori (H. *pylori*; H. Pylori IgG test, Reagent Company of the Shanghai Biotechnology Industry Park, Shanghai, China). Plasma aflatoxin B1 (AFB1)-albumin adduct levels were measured by ELISA assays as previously described (15), using free aflatoxin (Supelco) as a standard. Plasma levels of folate and vitamin B12 were measured using a competitive radioassay with ^{125}iodine-labeled folate and ^{57}cobalt-labeled vitamin B12 as tracers (Quantaphase II B12/folate radiobinding kit, Bio-Rad, Berkeley, CA). The mean within-run coefficient of variation (CV) was 10.9%, and the between-run CV was 16.6%. Plasma total homocysteine (tHcy) levels were measured using a commercially available chemiluminescent immunoassay system (IMMULITE 1000 Automated Analyzer; DPC, Los Angeles, CA). The mean within-run CV was 8.6%, and the mean between-run CV was 10.3%.

Statistical Analyses

Unconditional logistic regression analyses were conducted to estimate the associations of plasma levels of folate, vitamin B12, and tHcy with cancers of the esophagus, stomach, and liver, with crude and adjusted odds ratios (cORs and aORs, respectively) and 95% confidence intervals (CIs). Plasma levels of folate, vitamin B12, and tHcy were categorized according to the quartile distribution in the control group: 1) ≤8.9, 8.9–12.8, 12.8–17.7, >17.7 nmol/l for folate; 2) ≤154.2, 154.2–228.9, 228.9–324.1, >324.1 pmol/l for vitamin B12; and 3) ≤6.7, 6.7–9.5, 9.5–13.1, >13.1 μmol/l for tHcy. Potential confounding factors, including age (continuous), gender, education level (illiteracy, primary school, higher than middle school), body mass index (BMI; continuous), smoking pack-years (continuous), and alcohol drinking frequency (never, occasionally, often, everyday) were adjusted. In modeling one of the three plasma micronutrients, we adjusted for the other two plasma micronutrients categorized according to the quintile distributions in controls. In addition, we adjusted H. *pylori* infection (negative/positive) in stomach cancer analyses, and HBsAg status (negative/positive) and plasma AFB1-albumin adduct levels (quintiles) in liver cancer analyses.

Stratified analyses on environmental risk factors including smoking status, alcohol consumption, H. *pylori* infection (in stomach cancer analyses), HBsAg status (in liver cancer analyses), and plasma AFB1 levels (in liver cancer analyses), and statistical interactions between these 3 plasma micronutrients on both additive and multiplicative scales were conducted. Median levels in controls were used to dichotimize plasma levels of folate (12.8 nmol/l), vitamin B12 (228.9 pmol/l), tHcy (9.5 μmol/l), and AFB1-albumin adduct in both stratified and interaction analyses. Multiplicative interactions were assessed by including both the main effect variables and their product terms in logistic regression models. Two additive interaction measurements according to Knol et al. (16), relative excess risk due to interaction (RERI) and synergy index (SI), were calculated. The 95% CI of RERI and SI were estimated by the delta method (17, 18).

RESULTS

The comparisons of the distributions of selected demographic characteristics among cases of the three cancer types and controls are summarized in Table 1. In general, compared to the control group, cancer cases were more likely to be smokers, have lower BMI, and were less educated. Liver cancer cases had the highest male-to-female ratio (3.53). Stomach cancer cases were the oldest at diagnoses (mean age = 62.8 yr), followed by esophageal cancer cases (mean age = 60.6 yr), controls (mean age = 57.7 yr), and liver cancer cases (mean age = 53.9 yr). The proportion of HBsAg-positivity and anti-HCV-positivity in serum samples were higher in liver cancer patients than in controls. Liver cancer patients also had higher plasma AFB1 levels than controls. The median value of plasma folate levels was the highest among esophageal cancer cases (14.66 nmol/L), and it was the lowest among liver cancer cases (12.50 nmol/L). The measured plasma folate levels in our control population (median 12.76 nmol/L) were comparable with what have been observed in the United States before folic acid fortification (NHANES 1988–1994; median 12.50 nmol/L) (19). All cancer cases had substantially higherDmedian value of plasma vitamin B12 levels (300.46–382.77

pmol/L) than controls (228.8 pmol/L). Cancer cases also had higher median value of plasma tHcys levels (10.05–11.15 μmol/L) than controls (9.50 μmol/L).

The associations between plasma levels of folate, vitamin B12, and tHcy with cancers of the esophagus, stomach, and liver are presented in Table 2. There was weak or no linear relationship between these three plasma micronutrients (in quintile) (Pearson correlation coefficient r = 0.31, $P < 0.001$ between folate and vitamin B12; r = 0.046, $P = 0.14$ between folate and tHcy; r = 0.078, $P = 0.014$ between vitamin B12 and tHcy; data not shown). After the adjustment for potential confounding factors, as well as plasma levels of vitamin B12 and tHcy, we found an inverse association between plasma folate levels and liver cancer (P for trend 0.008). Individuals in the highest quartile of plasma folate levels were 54% less likely to have liver cancer than those in the lowest quartile (aOR = 0.46, 95% CI = 0.24–0.89). Plasma vitamin B12 levels were positively associated with all three cancers after the adjustment for potential confounding factors and plasma levels of folate and tHcy (P for trend ≤ 0.004). Compared to individuals in the lowest quartile of plasma vitamin B12 levels, those in the highest quartile had an aOR of 2.80 (95% CI = 1.51– 5.18) for esophageal cancer, 2.17 (95% CI = 1.21–3.89) for stomach cancer, and 9.90 (95% CI = 4.80–20.44) for liver cancer. We did not observe clear associations between plasma tHcy levels and esophageal, stomach, or liver cancer.

Potential statistical interactions between these three plasma micronutrients for the 3 cancers were shown in Table 3. There is potential statistical interaction between plasma levels of folate and vitamin B12 for all 3 cancers on both additive and multiplicative scales. Compared to individuals with lower levels of both plasma folate and vitamin B12, those with lower plasma folate levels and higher plasma vitamin B12 levels had the highest ORs for cancers; the ORs were 4.44 (95% CI 2.42–8.16) for esophageal cancer, 2.86 (95% CI = 1.58–5.18) for stomach cancer, and 8.81 (95% CI 4.56–17.04) for liver cancer.

In stratified analyses on other environmental risk factors, we detected heterogeneity of the associations between plasma vitamin B12 levels and liver cancer across the HBsAg strata (P for heterogeneity = 0.040), and between plasma folate levels and liver cancer across the strata of plasma AFB1-albumin adduct levels (P for heterogeneity = 0.002; Table 4). The positive association between plasma vitamin B12 levels and liver cancer was stronger among HBsAg-positive individuals (aOR = 9.54, 95% CI = 4.13–22.04) than among HBsAg-negative individuals (aOR association = 4.70, 95% CI 2.28–9.67). The inverse between plasma folate levels and liver cancer was observed among individuals with higher plasma AFB1 levels (aOR = 0.16, 95% CI = 0.07–0.37), but not among those with lower levels (aOR = 1.26, 95% CI = 0.62–2.57).

DISCUSSION

In this Southeastern Chinese high-risk population, we observed an inverse association between plasma folate levels and liver cancer, and this association appeared stronger among those with higher AFB1-albumin adducts levels. We found a positive association between plasma vitamin B12 levels and cancers of the esophagus, stomach, and liver. The association between B12 and liver cancer appeared more robust among chronic HBV carriers. In

addition, we reported for the first time a potential interaction between plasma levels of folate and B12 on these three cancers.

Our finding for the association between plasma folate and liver cancer is consistent with a hospital-based case-control study conducted in Taiwan reported that serum folate levels were inversely associated with hepatocellular carcinoma (HCC) (20), and of animal studies showing that dietary deficient in folate and other methyl donors can induce the development of liver cancer (21). The temporal sequence of pathological and molecular changes of folate/methyl-deficient diet induced hepatocarcinogenesis in rodents is very similar to the development of human HCCs (22). Folate and other micronutrients in one-carbon metabolism play an important role for the biosynthesis of *S*-adenosyl-L-methionine (SAM), the major methyl donor for cellular methylation reactions. DNA methylation is the major epigenetic mechanism for the control of gene expression and the maintenance of genome integrity. Global DNA hypomethylation is considered as a cause of oncogenesis (23). Rodent studies suggested that severe folate deficiency not only resulted in decreased SAM levels in the liver (24–26), but also increased uracil misincorporation followed by DNA strand breaks (27,28). Because folate also plays a fundamental role in purine and thymidylate synthesis, and a folate-deficient diet can lead to an overrepresentation of uracil in the nucleotide pool, which can result in reiterative repair of DNA strand breaks and abasic sites (29). James et al. (30) further indicated that lesion-containing DNA is less efficiently methylated than lesion-free DNA and that an increase in DNA strand breaks was followed by DNA hypomethylation. They also demonstrated that in promoter regions, the inappropriate binding of the DNA methyltransferase to unrepaired lesions or mispairs may promote local histone deacetylation, methylation, and regional hypermethylation associated with tumor suppressor gene silencing (30). In addition, a folate/methyl-deficient diet also resulted in altered lipid metabolism, oxidative stress, and other epigenetic alterations such as histone modification changes and microRNA alterations in hepatocarcinogenesis (22). Folate has also been shown to have antimutagenic effects on AFB1 in animal studies (31), which was supported by our observation that the inverse association between folate and liver cancer was only observed among those with higher AFB1 levels. We did not find a clear association between plasma folate and cancers of the esophagus or stomach in our current study. Although some studies indicated an association between dietary folate and these two cancers (7,12), there are no studies on blood folate status to the best of our knowledge. Blood folate status may also be associated with liver cancer prognosis. More advanced stage HCC patients had lower plasma/serum folate levels than those with an earlier stage of the disease (32,33). Although we do not have stage information, it is very likely that our group of liver cancer patients had less aggressive disease because those with more advanced disease might have been too ill during enrollment to participate or had died of cancer. Under the assumption that higher plasma folate is associated with better liver cancer prognosis, we may have underestimated the protective effect of folate if we were able to recruit all of the incident liver cancer patients.

Because we collected blood samples after disease diagnosis, the observed association might have been due to reverse causality. Recent literature suggested a dual role for folate in carcinogenesis. Folate may prevent tumor development before the existence of preneoplastic lesions, but increase tumorigenesis once preneoplastic lesions are established (34,35).

Therefore, lower plasma folate levels among liver cancer patients in our study may be a consequence of increased folate use by tumors (36). On the other hand, low folate status may be a consequence of general malnutrition caused by severe weight and appetite loss, which were frequently observed in several types of cancer patients, especially those with GI cancers (37,38). Reduced folate levels may also reflect underlying liver diseases among liver cancer patients because impaired liver function has been reported to accompany low folate status in patients with viral hepatitis, alcoholic liver disorders, and liver cirrhosis (39).

We observed a strong and consistent positive association between plasma vitamin B12 levels and all 3 upper GI cancers. Although whether the observed associations are causal remains speculative, vitamin B12 has been hypothesized to be one of the crucial parameters defining carcinogenicity of a methyl-deficient diet in animal models (40). Whereas a diet deficient in folate, choline, and methionine is sufficient to trigger hepatocarcinogenesis in rats without exposure to any known carcinogens (21,41–45), the effect of additional withdrawal of vitamin B12 is controversial (40). Wainfan and Poirier (46) found that livers in rats fed with a diet deficient in choline, methionine, folate, and vitamin B12 had decreased DNA methylation levels and altered gene expression. However, such severely methyl-deficient diet did not induce the development of liver cancer in rats in two other studies, with low incidence of HCC of 3.4% (47) and 0% (43). In epidemiologic studies, higher B12 levels have been associated with increased risk of prostate and lung cancer. In a meta-analysis, circulating B12 levels were associated with a 10% increased prostate cancer risk (pooled OR = 1.10, 95% CI = 1.01–1.19) for every 100 pmol/L increase (48). In the European Investigation into Cancer and Nutrition study, a higher risk for lung cancer was seen for elevated serum B12 (fourth vs. first quartile OR = 1.35, 95% CI = 1.00–1.82; *P* for trend = 0.04) (49). In a large cohort study in Denmark published recently, a strong association between elevated plasma vitamin B12 levels and the risk of several hematological and smoking- and alcohol-related cancers including liver cancer and gastric cancer was found (50). Elevated plasma vitamin B12 levels are therefore regarded as a marker for various types of cancers (50). These results are in line with our findings on the plasma levels of B12 and the risk of GI cancers.

The positive associations between B12 and cancer may also be due to reverse causality. Elevated plasma B12 levels have been reported in some cancer patients (51–53). The underlying mechanisms are poorly elucidated though the increased release of haptocorrin (HC; vitamin B12-binding protein) to the circulation was proposed as a plausible explanation (54). For liver cancer patients, an increased release of vitamin B12 from damaged hepatocytes and decreased uptake or the diminished clearance of HC by the liver may also be involved (53,55). In addition, patients with underlying liver diseases also have elevated plasma B12 levels (54,57). Vitamin B12 supplementation has been recommended for reducing side effects among cancer patients undergoing chemotherapy. Therefore, prior liver diseases and cancer treatment may confound our observed association. Although related information was not collected to investigate such confounding effect, the association between B12 and liver cancer was consistently seen across the stratum of chronic HBV infection, indicating the robustness of the association. Similarly, in the Danish cohort study, the risk of liver cancer associated with elevated B12 remained robust after the stratification on prior liver diseases, indicating that underlying liver diseases did not confound this

association (50). Therefore, although a clear causal relationship cannot be established, our study raises the importance of subsequent work on the role of plasma vitamin B12 on cancer etiology. As suggested by Arendt et al. (50) the mechanisms resulting in high vitamin B12 levels may be related to malignant pathogenesis.

We also observed a novel finding of a statistical interaction between plasma folate and vitamin B12 on all of the 3 upper GI cancers. Although the highest ORs for cancer were observed among individuals with lower folate and higher B12 levels, the lowest ORs were observed among those with both lower levels. Such finding seems to be consistent with the before-mentioned observation in rodent models that the additional removal of vitamin B12 from folate-/methyl-deficient diet may not effectively induce hepatocarcinogenesis (40). In other words, the cancer-inducing effect of folate deficiency may be of greater importance when vitamin B12 levels are high, which was suggested by our results. The interaction between folate and B12 has also been observed in one study on dietary folate intake and breast cancer (58). However, more studies are needed to confirm and elucidate such finding.

The major limitation in this study is confounding by indication because we collected blood samples after disease diagnosis. The observed associations might be caused by reverse causality, as discussed above. In addition, as esophageal, stomach, and liver cancers are fatal diseases, some cancer patients diagnosed at advanced stages were too ill to enroll. This may have resulted in the selection of patients with less aggressive cancers, which may have different etiologic indications. We also had limited sample size to detect interactions. However, given that there is limited prior research, our results are of importance.

In conclusion, the findings from this study suggest an inverse association between plasma folate levels and liver cancer, and positive associations between plasma vitamin B12 levels and cancers of the esophagus, stomach, and liver in a Chinese population where mandatory folic acid fortification is not implemented. Our data also indicated the presence of a potential interaction between plasma folate and vitamin B12 in these 3 cancers. Considering the practice of mandatory folic acid fortification in many countries, further research is warranted to investigate the associations of micronutrients involved in folate metabolism with cancer.

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136:E359–E386. [PubMed: 25220842]

2. Ferlay, J.; Soerjomataram, I.; Ervik, M.; Dikshit, R.; Eser, S., et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase no 11. International Agency for Research on Cancer; Lyon, France: 2013.
3. Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis. 2011; 34:101–109. [PubMed: 20544289]
4. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification—its history, effect, concerns, and future directions. Nutrients. 2011; 3:370–384. [PubMed: 22254102]
5. Food Fortification Initiative. [Accessed on 12/18/2014] http://www.ffinetwork.org/global_progress/index.php.
6. Kennedy DA, Stern SJ, Moretti M, Matok I, Sarkar M, et al. Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. Cancer Epidemiol. 2011; 35:2–10. [PubMed: 21177150]
7. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. Gastroenterology. 2006; 131:1271–1283. [PubMed: 17030196]
8. Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. Journal of the National Cancer Institute. 2007; 99:64–76. [PubMed: 17202114]
9. Hirsch S, Sanchez H, Albala C, de la Maza MP, Barrera G, et al. Colon cancer in Chile before and after the start of the flour fortification program with folic acid. Eur J Gastroenterol Hepatol. 2009; 21:436–439. [PubMed: 19190501]
10. Mason JB, Dickstein A, Jacques PF, Haggarty P, Selhub J, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev. 2007; 16:1325–1329. [PubMed: 17626997]
11. World Cancer Research Fund/American Institute for Cancer Research: Cancers. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. American Institute for Cancer Researc; Washington, DC: 2007. p. 244-318.
12. Liu YX, Wang B, Wan MH, Tang WF, Huang FK, et al. Meta-analysis of the relationship between the Metholenetetrahydrofolate reductase C677T genetic polymorphism, folate intake and esophageal cancer. Asian Pac J Cancer Prev. 2011; 12:247–252. [PubMed: 21517266]
13. Persson EC, Schwartz LM, Park Y, Trabert B, Hollenbeck AR, et al. Alcohol consumption, folate intake, hepatocellular carcinoma, and liver disease mortality. Cancer Epidemiol Biomarkers Prev. 2013; 22:415–421. [PubMed: 23307533]
14. Mu LN, Cao W, Zhang ZF, Cai L, Jiang QW, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms and the risk of primary hepatocellular carcinoma (HCC) in a Chinese population. Cancer Causes & Control: CCC. 2007; 18:665–675. [PubMed: 17503006]
15. Chen SY, Chen CJ, Tsai WY, Ahsan H, Liu TY, et al. Associations of plasma aflatoxin B1-albumin adduct level with plasma selenium level and genetic polymorphisms of glutathione S-transferase M1 and T1. Nutr Cancer. 2000; 38:179–185. [PubMed: 11525595]
16. Knol MJ, van der Tweel I, Grobbee DE, Numans ME, Geerlings MI. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. International Journal of Epidemiology. 2007; 36:1111–1118. [PubMed: 17726040]
17. Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. European Journal of Epidemiology. 2005; 20:575–579. [PubMed: 16119429]
18. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology. 1992; 3:452–456. [PubMed: 1391139]
19. Pfeiffer CM, Hughes JP, Durazo-Arvizu RA, Lacher DA, Sempos CT, et al. Changes in measurement procedure from a radioassay to a microbiologic assay necessitate adjustment of serum and RBC folate concentrations in the U.S. population from the NHANES 1988–2010. The Journal of Nutrition. 2012; 142:894–900. [PubMed: 22437557]
20. Wu MY, Kuo CS, Lin CY, Lu CL, Syu Huang RF. Lymphocytic mitochondrial DNA deletions, biochemical folate status and hepatocellular carcinoma susceptibility in a case-control study. Br J Nutr. 2009; 102:715–721. [PubMed: 19331700]

21. Henning SM, Swendseid ME. The role of folate, choline, and methionine in carcinogenesis induced by methyl-deficient diets. Advances in Experimental Medicine and Biology. 1996; 399:143–155. [PubMed: 8937554]
22. Pogribny IP, James SJ, Beland FA. Molecular alterations in hepatocarcinogenesis induced by dietary methyl deficiency. Molecular Nutrition & Food Research. 2012; 56:116–125. [PubMed: 22095781]
23. Das PM, Singal R. DNA methylation and cancer. J Clin Oncol. 2004; 22:4632–4642. [PubMed: 15542813]
24. Pogribny IP, Ross SA, Wise C, Pogribna M, Jones EA, et al. Irreversible global DNA hypomethylation as a key step in hepatocarcinogenesis induced by dietary methyl deficiency. Mutation Research. 2006; 593:80–87. [PubMed: 16144704]
25. Shivapurkar N, Poirier LA. Tissue levels of S-adenosylmethionine and S-adenosylhomocysteine in rats fed methyl-deficient, amino acid-defined diets for one to five weeks. Carcinogenesis. 1983; 4:1051–1057. [PubMed: 6872150]
26. Henning SM, McKee RW, Swendseid ME. Hepatic content of S-adenosylmethionine, S-adenosylhomocysteine and glutathione in rats receiving treatments modulating methyl donor availability. The Journal of Nutrition. 1989; 119:1478–1482. [PubMed: 2531221]
27. James SJ, Yin L. Diet-induced DNA damage and altered nucleotide metabolism in lymphocytes from methyl-donor-deficient rats. Carcinogenesis. 1989; 10:1209–1214. [PubMed: 2472230]
28. Pogribny IP, Muskhelishvili L, Miller BJ, James SJ. Presence and consequence of uracil in preneoplastic DNA from folate/methyl-deficient rats. Carcinogenesis. 1997; 18:2071–2076. [PubMed: 9395204]
29. Melnyk S, Pogribna M, Miller BJ, Basnakian AG, Pogribny IP, et al. Uracil misincorporation, DNA strand breaks, and gene amplification are associated with tumorigenic cell transformation in folate deficient/repleted Chinese hamster ovary cells. Cancer Letters. 1999; 146:35–44. [PubMed: 10656607]
30. James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, et al. Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. The Journal of Nutrition. 2003; 133:3740S–3747S. [PubMed: 14608108]
31. Bhattacharya RK, Francis AR, Shetty TK. Modifying role of dietary factors on the mutagenicity of aflatoxin B1: in vitro effect of vitamins. Mutation Research. 1987; 188:121–128. [PubMed: 3108659]
32. Kuo CS, Lin CY, Wu MY, Lu CL, Huang RF. Relationship between folate status and tumour progression in patients with hepatocellular carcinoma. The British Journal of Nutrition. 2008; 100:596–602. [PubMed: 18275623]
33. Lin CC, Yin MC. B vitamins deficiency and decreased anti-oxidative state in patients with liver cancer. Eur J Nutr. 2007; 46:293–299. [PubMed: 17571208]
34. Kim YI. Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies. Environ Mol Mutagen. 2004; 44:10–25. [PubMed: 15199543]
35. Kim YI. Folate and colorectal cancer: an evidence-based critical review. Mol Nutr Food Res. 2007; 51:267–292. [PubMed: 17295418]
36. Magnus EM. Folate activity in serum and red cells of patients with cancer. Cancer Res. 1967; 27:490–497. [PubMed: 6021921]
37. Nixon DW, Heymsfield SB, Cohen AE, Kutner MH, Ansley J, et al. Protein-calorie undernutrition in hospitalized cancer patients. Am J Med. 1980; 68:683–690. [PubMed: 6769330]
38. Bozzetti F, Migliavacca S, Scotti A, Bonalumi MG, Scarpa D, et al. Impact of cancer, type, site, stage and treatment on the nutritional status of patients. Ann Surg. 1982; 196:170–179. [PubMed: 7092367]
39. Wu A, Chanarin I, Slavin G, Levi AJ. Folate deficiency in the alcoholic—its relationship to clinical and haematological abnormalities, liver disease and folate stores. British Journal of Haematology. 1975; 29:469–478. [PubMed: 1191558]
40. Brunaud L, Alberto JM, Ayav A, Gerard P, Namour F, et al. Effects of vitamin B12 and folate deficiencies on DNA methylation and carcinogenesis in rat liver. Clinical Chemistry and Laboratory Medicine: CCLM/FESCC. 2003; 41:1012–1019.

41. Chandar N, Lombardi B. Liver cell proliferation and incidence of hepatocellular carcinomas in rats fed consecutively a choline-devoid and a choline-supplemented diet. Carcinogenesis. 1988; 9:259–263. [PubMed: 3338109]
42. Yokoyama S, Sells MA, Reddy TV, Lombardi B. Hepatocarcinogenic and promoting action of a choline-devoid diet in the rat. Cancer Research. 1985; 45:2834–2842. [PubMed: 2859112]
43. Mikol YB, Hoover KL, Creasia D, Poirier LA. Hepatocarcinogenesis in rats fed methyl-deficient, amino acid-defined diets. Carcinogenesis. 1983; 4:1619–1629. [PubMed: 6317218]
44. Ghoshal AK, Farber E. The induction of liver cancer by dietary deficiency of choline and methionine without added carcinogens. Carcinogenesis. 1984; 5:1367–1370. [PubMed: 6488458]
45. Nakae D, Yoshiji H, Mizumoto Y, Horiguchi K, Shiraiwa K, et al. High incidence of hepatocellular carcinomas induced by a choline deficient Lamino acid defined diet in rats. Cancer Research. 1992; 52:5042–5045. [PubMed: 1516060]
46. Wainfan E, Poirier LA. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. Cancer Research. 1992; 52:2071S–2077S. [PubMed: 1544143]
47. Hoover KL, Lynch PH, Poirier LA. Profound postinitiation enhancement by short-term severe methionine, choline, vitamin B12, and folate deficiency of hepatocarcinogenesis in F344 rats given a single low-dose diethylnitrosamine injection. Journal of the National Cancer Institute. 1984; 73:1327–1336. [PubMed: 6595443]
48. Collin SM, Metcalfe C, Refsum H, Lewis SJ, Zuccolo L, et al. Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: a case-control study, systematic review, and meta-analysis. Cancer Epidemiology, Biomarkers & Prevention. 2010; 19:1632–1642.
49. Johansson M, Relton C, Ueland PM, Vollset SE, Midttun Ø , et al. Serum B vitamin levels and risk of lung cancer. JAMA. 2010; 303:2377–2385. [PubMed: 20551408]
50. Arendt JF, Pedersen L, Nexo E, Sorensen HT. Elevated plasma vitamin B12 levels as a marker for cancer: a population-based cohort study. Journal of the National Cancer Institute. 2013; 105:1799–1805. [PubMed: 24249744]
51. Carmel R, Eisenberg L. Serum vitamin B12 and transcobalamin abnormalities in patients with cancer. Cancer. 1977; 40:1348–1353. [PubMed: 902243]
52. Fremont S, Champigneulle B, Gerard P, Felden F, Lambert D, et al. Blood transcobalamin levels in malignant hepatoma. Tumour Biol. 1991; 12:353–359. [PubMed: 1724707]
53. Wakatsuki Y, Inada M, Kudo H, Oshio G, Masuda T, et al. Immunological characterization and clinical implication of cobalamin binding protein in human gastric cancer. Cancer Research. 1989; 49:3122–3128. [PubMed: 2720670]
54. Arendt JF, Nexo E. Unexpected high plasma cobalamin : proposal for a diagnostic strategy. Clinical Chemistry and Laboratory Medicine: CCLM/FESCC. 2013; 51:489–496.
55. Boisson F, Fremont S, Migeon C, Nodari F, Droesch S, et al. Human haptocorrin in hepatocellular carcinoma. Cancer Detect Prev. 1999; 23:89–96. [PubMed: 10101589]
56. Ermens AA, Vlasveld LT, Lindemans J. Significance of elevated cobalamin (vitamin B12) levels in blood. Clin Biochem. 2003; 36:585–590. [PubMed: 14636871]
57. Chiche L, Jean R, Romain F, Roux F, Thomas G, et al. Clinical implications of high cobalamin blood levels for internal medicine. La Revue de medecine interne/fondee par la Societe nationale francaise de medecine interne. 2008; 29:187–194.
58. Lajous M, Lazcano-Ponce E, Hernandez-Avila M, Willett W, Romieu I. Folate, vitamin B(6), and vitamin B(12) intake and the risk of breast cancer among Mexican women. Cancer Epidemiology, Biomarkers & Prevention. 2006; 15:443–448.

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TABLE 1

Selected demographic characteristics among esophageal, stomach, and liver cancer cases and controls[†]

	Controls (*n* = 415)	Cases					
		Esophageal cancer (*n* = 218)		Stomach cancer (*n* = 206)		Liver cancer (*n* = 204)	
	(*n*,%)	(*n*,%)	*P* *	(*n*,%)	*P* *	(*n*,%)	*P* *
Age (continuous)	57.7±11.8	60.6±9.6	0.001	62.8±9.8	<0.001	53.9±13.0	<0.001
<45	60 (14.5)	7 (3.2)	<0.001	13 (6.3)	0.003	53 (26.5)	<0.001
45–54	98 (23.6)	66 (30.3)		38 (18.5)		63 (30.9)	
55–64	139 (33.5)	74 (33.9)		77 (37.4)		46 (22.6)	
≥65	118 (28.4)	71 (32.6)		78 (37.9)		42 (20.6)	
Gender							
Female	128 (30.8)	77 (35.3)	0.25	68 (33.0)	0.58	45 (22.1)	0.022
Male	287 (69.2)	141 (64.7)		138 (67.0)		159 (77.9)	
Plasma folate levels (nmol/1)	12.76 (8.90–17.66)	14.43 (9.73–24.85)	0.001	14.66 (9.34–23.24)	0.011	12.50 (9.39–22.15)	0.40
Plasma vitB 12 levels (pmol/1)	228.88 (154.23–324.06)	308.13 (220.12–424.58)	<0.001	300.46 (190.47–411.50)	<0.001	382.77 (254.37–571.15)	<0.001
Plasma tHcy levels (μmol/I)	9.50 (6.70–13.10)	11.15 (7.70–13.70)	0.016	10.05 (7.05–13.50)	0.40	10.55 (6.80–14.60)	0.21
BMI (continuous)	22.4±2.6	21.9±2.8	0.035	21.4±2.7	<0.001	21.5±2.7	<0.001
<18.5	18 (4.9)	24 (12.0)	0.014	25 (12.8)	0.002	23 (12.7)	0.003
18.5–25	296 (80.4)	154 (77.0)		155 (79.1)		143 (79.0)	
25–30	51 (13.9)	20 (10.0)		15 (7.7)		14 (7.7)	
≥30	3 (0.8)	2 (1.0)		1 (0.5)		1 (0.6)	
Education							
Illiteracy	73 (17.6)	83 (38.6)	<0.001	66 (32.0)	<0.001	44(21.6)	0.19
Primary school	142 (34.2)	101 (47.0)		107 (51.9)		77 (37.8)	
At least middle school	200 (48.2)	31 (14.4)		33 (16.0)		83 (40.7)	
Smoking							
Never	217 (55.9)	94 (46.8)	0.025	92 (46.9)	0.094	85 (46.2)	0.031
≤20	83 (21.4)	41 (20.4)		46 (23.5)		57 (31.0)	
>20	88 (22.7)	66 (32.8)		58 (29.6)		42 (22.8)	
Pack-years (continuous)	23.7±15.6	28.9±21.9	0.030	27.7±19.2	0.068	21.2±14.0	0.20
Alcohol drinking							

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	Controls (*n* = 415)	Cases					
		Esophageal cancer (*n* = 218)		Stomach cancer (*n* = 206)		Liver cancer (*n* = 204)	
	(*n*,%)	(*n*,%)	*P* *	(*n*,%)	*P* *	(*n*,%)	*P* *
Never	207(50.2)	116 (55.0)	0.015	111 (55.2)	0.70	87(45.3)	0.13
Occasionally	72(17.5)	18 (8.5)		31 (15.4)		29(15.1)	
Often	75(18.2)	37 (17.5)		32 (15.9)		51(26.6)	
Everyday	58(14.1)	40 (19.0)		27 (13.4)		25(13.0)	
H. *pylori* infection							
CagA+	251 (68.8)			130 (64.7)	0.32		
CagA	114 (31.2)			71 (35.3)			
HBV infection							
HBsAg +	102 (24.6)					132 (64.7)	<0.001
HBsAg −	312 (75.4)					72 (35.3)	
HCV infection							
Anti-HCV +	12 (2.9)					18 (9.0)	0.001
Anti-HCV −	403 (97.1)					183 (91.0)	
Plasma AFB1 adduct levels (quintile)							
1	75 (19.9)					26 (14.4)	0.054
2	76 (20.2)					36 (19.9)	
3	75 (19.9)					36 (19.9)	
4	76 (20.2)					28 (15.5)	
5	75 (19.9)					55 (30.4)	

†Values are presented as means ± SD for continuous variables, number (percentages) for categorical variables, and medians (25th–75th percentile) for plasma levels of folate, vitamin (vit) B12, and total homocysteine (tHcy). BMI = body mass index; HBV = hepatitis B virus; HCV = hepatitis C virus; HBsAg = HBV surface antigen; AFB1 = aflatoxin B1.

**P* values derived from Student *t*-tests for continuous variables, χ^2 tests or Fisher's exact tests for categorical variables, and Wilcoxon-Mann-Whitney test for the underlying distribution of plasma levels of folate, vitamin B12, and tHcy comparing cases to controls.

TABLE 2

Associations between plasma levels of folate, vitamin B12, and homocysteine and cancers of esophagus, stomach, and liver

	Case *n* (%)	Control *n* (%)	Crude OR (95% CI)	Adjusted OR[1] (95% CI)	Adjusted OR[2] (95% CI)
			Esophageal cancer		
Folate (nmol/l)					
≦8.90	43 (7.0)	102 (16.6)	1.00	1.00	1.00
8.90–12.76	41 (6.7)	102 (16.6)	0.95 (0.57, 1.59)	0.88 (0.50, 1.54)	0.72 (0.39, 1.31)
12.76–17.66	47 (7.7)	101 (16.4)	1.10 (0.67, 1.81)	0.99 (0.57, 1.71)	0.77 (0.43, 1.39)
>17.66	75 (12.2)	103 (16.8)	1.73 (1.09, 2.75)	1.58 (0.95, 2.64)	1.00 (0.57, 1.76)
P_{trend}*			0.012	0.051	0.80
Vitamin B12 (pmol/l)					
≦154.23	25 (4.1)	102 (16.7)	1.00	1.00	1.00
154.23–228.88	28 (4.6)	100 (16.3)	1.14 (0.62, 2.09)	1.00 (0.52, 1.93)	0.97 (0.49, 1.90)
228.88–324.06	60 (9.8)	102 (16.7)	2.40 (1.40, 4.12)	2.30 (1.27, 4.17)	2.16 (1.15, 4.04)
>324.06	93 (15.2)	102 (16.7)	3.72 (2.21, 6.26)	3.07 (1.73, 5.45)	2.80 (1.51, 5.18)
P_{trend}*			<0.001	<0.001	<0.001
Homocysteine (μmol/l)					
≦6.70	36 (5.9)	103 (16.7)	1.00	1.00	1.00
6.70–9.50	42 (6.8)	103 (16.7)	1.17 (0.69, 1.97)	1.31 (0.73, 2.33)	1.19 (0.65, 2.17)
9.50–13.10	67 (10.9)	101 (16.4)	1.90 (1.16, 3.10)	1.68 (0.97, 2.93)	1.47 (0.83, 2.60)
>13.10	61 (9.9)	102 (16.6)	1.71 (1.04, 2.81)	1.59 (0.91, 2.76)	1.54 (0.87, 2.72)
P_{trend}*			0.009	0.077	0.11
			Stomach cancer		
Folate (nmol/l)					
≦8.90	46 (7.6)	102 (16.7)	1.00	1.00	1.00
8.90–12.76	41 (6.7)	102 (16.7)	0.89 (0.54, 1.47)	1.05 (0.59, 1.84)	0.94 (0.52, 1.70)
12.76–17.66	41 (6.7)	101 (16.6)	0.90 (0.54, 1.49)	1.07 (0.60, 1.90)	0.93 (0.51, 1.69)
>17.66	73 (12.0)	103 (16.9)	1.57 (0.99, 2.49)	1.73 (1.01, 2.95)	1.26 (0.70, 2.27)
P_{trend}*			0.044	0.041	0.42
Vitamin B12 (pmol/l)					
≦154.23	37 (6.1)	102 (16.8)	1.00	1.00	1.00
154.23–228.88	33 (5.4)	100 (16.5)	0.91 (0.53, 1.57)	0.94 (0.51, 1.73)	0.90 (0.49, 1.68)
228.88–324.06	43 (7.1)	102 (16.8)	1.16 (0.69, 1.95)	1.16 (0.64, 2.08)	1.09 (0.60, 2.00)
>324.06	87 (14.4)	102 (16.8)	2.35 (1.47, 3.77)	2.50 (1.44, 4.31)	2.17 (1.21, 3.89)
P_{trend}*			<0.001	<0.001	0.004
Homocysteine (μmol/l)					
≦6.70	47 (7.7)	103 (16.9)	1.00	1.00	1.00
6.70–9.50	49 (8.0)	103 (16.9)	1.04 (0.64, 1.69)	1.35 (0.77, 2.35)	1.16 (0.65, 2.07)
9.50–13.10	48 (7.9)	101 (16.6)	1.04 (0.64, 1.69)	1.09 (0.62, 1.90)	0.95 (0.53, 1.70)
>13.10	56 (9.2)	102 (16.7)	1.20 (0.75, 1.93)	1.25 (0.73, 2.17)	1.12 (0.64, 1.96)

	Case *n* (%)	Control *n* (%)	Crude OR (95% CI)	Adjusted OR[1] (95% CI)	Adjusted OR[2] (95% CI)
P_{trend}*			0.46	0.60	0.88
			Liver cancer		
Folate (nmol/l)					
≦8.90	45 (7.5)	102 (16.9)	1.00	1.00	1.00
8.90–12.76	55 (9.1)	102 (16.9)	1.22 (0.76, 1.98)	1.18 (0.68, 2.05)	0.98 (0.53, 1.83)
12.76–17.66	38 (6.3)	101 (16.7)	0.85 (0.51, 1.42)	0.91 (0.50, 1.63)	0.64 (0.33, 1.24)
>17.66	58 (9.6)	103 (17.1)	1.28 (0.79, 2.05)	1.05 (0.60, 1.83)	0.46 (0.24, 0.89)
P_{trend}*		0.58	0.91	0.008	
Vitamin B12 (pmol/l)					
≦154.23	17 (2.8)	102 (16.9)	1.00	1.00	1.00
154.23–228.88	18 (3.0)	100 (16.6)	1.08 (0.53, 2.21)	1.19 (0.53, 2.68)	1.37 (0.59, 3.16)
228.88–324.06	38 (6.3)	102 (16.9)	2.24 (1.19, 4.21)	3.09 (1.53, 6.25)	4.27 (2.00, 9.10)
>324.06	124 (20.6)	102 (16.9)	7.29 (4.10, 12.98)	6.65 (3.49, 12.66)	9.90 (4.80, 20.44)
P_{trend}*		<0.001	<0.001	<0.001	
Homocysteine (μmol/l)					
≦6.70	49 (8.1)	103 (17.0)	1.00	1.00	1.00
6.70–9.50	37 (6.1)	103 (17.0)	0.76 (0.45, 1.25)	0.72 (0.40, 1.31)	0.67 (0.35, 1.27)
9.50–13.10	48 (7.9)	101 (16.6)	1.00 (0.62, 1.62)	0.94 (0.53, 1.67)	0.71 (0.38, 1.33)
>13.10	64 (10.5)	102 (16.8)	1.32 (0.83, 2.09)	1.56 (0.91, 2.70)	1.21 (0.67, 2.19)
P_{trend}*		0.13	0.067	0.45	

[1]Odds ratios (ORs) adjusted for age, gender, BMI, education, smoking pack-years, alcohol drinking frequency, H. *pylori* infection (in stomach cancer analyses), hepatitis B virus surface antigen (in liver cancer analyses), and plasma aflatoxin B1 levels (in liver cancer analyses);

[2]ORs further adjusted for the other two plasma micronutrients in quintile distribution.

*P value for chi-square test for trend.

TABLE 3

Interactions between plasma levels of folate, vitamin B12, and homocysteine on cancers of esophagus, stomach, and liver

Plasma micronutrients		Esophageal cancer		Stomach cancer		Liver cancer	
		Ca/Co	Adjusted OR* (95% CI)	Ca/Co	Adjusted OR* (95% CI)	Ca/Co	Adjusted OR* (95% CI)
Folate (nmol/l)	Vitamin B12 (pmol/l)						
≤12.76	≤228.88	26/133	1.00	38/133	1.00	23/133	1.00
≤12.76	>228.88	58/70	4.44 (2.42, 8.16)	48/70	2.86 (1.58, 5.18)	76/70	8.81 (4.56, 17.04)
>12.76	≤228.88	27/69	2.15 (1.10, 4.23)	32/69	2.07 (1.09, 3.91)	12/69	1.36 (0.56, 3.26)
>12.76	>228.88	95/134	3.18 (1.84, 5.49)	82/134	2.31 (1.37, 3.91)	84/134	3.89 (2.11, 7.17)
Interaction							
	Additive†	RERI=	−2.42 (−5.20, 0.37)		−1.62 (−3.66, 0.43)		−5.29 (−10.38, −0.19)
		SI=	0.47 (0.25, 0.89)		0.45 (0.21, 0.98)		0.35 (0.19, 0.66)
	Multiplicative	ROR=	0.33 (0.14, 0.77)		0.39 (0.17, 0.89)		0.32 (0.12, 0.90)
Folate (nmol/l)	tHcy (μmol/l)						
≤12.76	≤9.50	47/103	1.00	52/103	1.00	41/103	1.00
≤12.76	>9.50	75/101	1.20 (0.67, 2.17)	62/101	0.83 (0.46, 1.49)	55/101	1.14 (0.61, 2.12)
>12.76	≤9.50	31/102	0.94 (0.51, 1.73)	44/102	0.96 (0.54, 1.74)	45/102	0.55 (0.29, 1.07)
>12.76	>9.50	53/102	1.46 (0.82, 2.59)	42/102	1.10 (0.62, 1.95)	55/102	0.62 (0.32, 1.19)
Interaction							
	Additive†	RERI=	−0.33 (−1.34, 0.68)		0.05 (−1.01, 1.10)		0.13 (−1.19, 1.46)
		SI=	0.45 (0.05, 3.87)		1.07 (0.23, 5.07)		1.15 (0.28, 4.65)
	Multiplicative	ROR=	1.29 (0.59, 2.82)		1.37 (0.62, 3.03)		0.98 (0.42, 2.32)
Vitamin B12 (pmol/l)	tHcy (μmol/l)						
≤228.88	≤9.50	19/109	1.00	33/109	1.00	19/109	1.00
≤228.88	>9.50	34/93	1.80 (0.89, 3.61)	36/93	0.97 (0.52, 1.81)	16/93	1.12 (0.49, 2.53)
>228.88	≤9.50	59/96	3.24 (1.65, 6.36)	62/96	1.68 (0.93, 3.04)	66/96	5.07 (2.49, 10.30)
>228.88	>9.50	94/108	4.02 (2.10, 7.72)	68/108	1.61 (0.90, 2.88)	96/108	6.55 (3.29, 13.04)
Interaction							
	Additive†	RERI=	−0.02 (−1.99, 1.95)		−0.05 (−1.09, 1.00)		1.36 (−1.62, 4.34)
		SI=	1.00 (0.52, 1.90)		0.93 (0.19, 4.57)		1.33 (0.70, 2.52)

Plasma micronutrients		Esophageal cancer		Stomach cancer		Liver cancer	
		Ca/Co	Adjusted OR* (95% CI)	Ca/Co	Adjusted OR* (95% CI)	Ca/Co	Adjusted OR* (95% CI)
	Multiplicative	ROR=	0.69 (0.30, 1.60)		0.98 (0.44, 2.18)		1.16 (0.45, 2.99)

*Odds ratios (ORs) adjusted for age, gender, alcohol drinking frequency, body mass index, education, plasma total homocysteine (tHcy) levels in quintile distribution, *H. pylori* infection (in stomach cancer analyses), hepatitis B virus surface antigen (in liver cancer analyses), and plasma aflatoxin B1levels (in liver cancer analyses).

†The stratum with the lowest OR was assigned as the reference group in additive interaction analyses.

TABLE 4

Associations between plasma levels of folate, vitamin B12, and homocysteine (higher vs. lower) and cancers of esophagus, stomach, and liver, stratified on smoking, alcohol drinking, H. *pylori* infection (stomach cancer), hepatitis B virus (HBV) infection (liver cancer), and plasma aflatoxin B1 (AFB1) levels (liver cancer)

	Esophageal cancer			Stomach cancer			Liver cancer		
Stratum	**Adjusted OR* (95% CI)**	**Adjusted OR* (95% CI)**	***P*†**	**Adjusted OR* (95% CI)**	**Adjusted OR* (95% CI)**	***P*†**	**Adjusted OR* (95% CI)**	**Adjusted OR* (95% CI)**	***P*†**
Smoking	Never-smokers	Ever-smokers		Never-smokers	Ever-smokers		Never-smokers	Ever-smokers	
Plasma folate	0.92 (0.49, 1.73)	1.25 (0.72, 2.17)	0.21	1.41 (0.75, 2.69)	0.87 (0.49, 1.58)	0.49	0.55 (0.27, 1.10)	0.46 (0.24, 0.93)	0.84
Plasma VB12	2.71 (1.35, 5.44)	2.52 (1.40, 4.53)	0.61	1.49 (0.77, 2.87)	1.85 (1.02, 3.33)	0.77	5.97 (2.58, 13.83)	8.07 (3.81, 17.09)	0.55
Plasma tHcy	1.32 (0.73, 2.39)	1.32 (0.75, 2.32)	0.95	1.20 (0.64, 2.23)	0.85 (0.48, 1.51)	0.51	1.05 (0.53, 2.05)	1.17 (0.60, 2.27)	0.97
Alcohol drinking	Non-drinkers	Drinkers		Non-drinkers	Drinkers		Non-drinkers	Drinkers	
Plasma folate	1.17 (0.66, 2.07)	1.01 (0.55, 1.85)	0.85	1.29 (0.71, 2.34)	0.79 (0.42, 1.49)	0.35	0.51 (0.25, 1.02)	0.53 (0.28, 1.02)	0.83
Plasma VB12	2.58 (1.38, 4.80)	2.34 (1.24, 4.45)	0.92	1.32 (0.72, 2.44)	2.36 (1.21, 4.57)	0.47	6.66 (2.90, 15.28)	6.35 (3.07, 13.11)	0.99
Plasma tHcy	1.42 (0.82, 2.46)	1.46 (0.78, 2.73)	0.92	1.03 (0.59, 1.80)	1.11 (0.59, 2.10)	0.83	1.21 (0.63, 2.35)	1.15 (0.60, 2.20)	0.83
H. *pylori*/HBV infection				H. *pylori* CagA−	H. *pylori* CagA±		HBsAg	HBsAg ±	
Plasma folate				1.55 (0.91, 2.64)	0.66 (0.30, 1.43)	0.23	0.44 (0.22, 0.87)	0.83 (0.41, 1.65)	0.060
Plasma VB12				1.31 (0.77, 2.23)	3.27 (1.45, 7.35)	0.15	4.70 (2.28, 9.67)	9.54 (4.13, 22.04)	0.040
Plasma tHcy				0.97 (0.58, 1.62)	0.81 (0.40, 1.66)	0.79	1.14 (0.61, 2.12)	1.44 (0.70, 2.95)	1.0
Plasma AFB1 levels							Lower	Higher	
Plasma folate							1.26 (0.62, 2.57)	0.16 (0.07, 0.37)	0.002
Plasma VB12							5.06 (2.23, 11.49)	10.59 (4.22, 26.55)	0.97
Plasma tHcy							1.47 (0.71, 3.02)	1.46 (0.73, 2.96)	0.78

VB12 = vitamin B12; tHcy = total homocysteine.

†*P* for heterogeneity.

*Odds ratios (ORs) adjusted for age, gender, alcohol drinking frequency, body mass index, education, the other two plasma micronutrients in quintile distribution, H. *pylori* infection (in stomach cancer analyses), hepatitis B virus surface antigen (HBsAg; in liver cancer analyses), and plasma aflatoxin B1 (AFB1) levels (in liver cancer analyses).