One-carbon metabolism dietary factors and distal gastric cancer risk in Chinese women

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Abstract

Background—Previous studies on the association between one-carbon dietary factors and gastric cancer risk have been inconsistent.

Methods—We investigated this association using data from a prospective study, the Shanghai Women's Health Study (1997–2010), including 323 distal gastric cancer cases identified from 73,009 Chinese women. Hazard ratios and 95% confidence intervals were estimated using Cox proportional hazard regression after adjusting for confounders.

Results—Overall, no statistically significant association of gastric cancer was observed with dietary intake of folate, methionine, or B vitamins. However, when stratified by menopausal status, higher intake of riboflavin was associated with decreased gastric cancer risk in premenopausal women with HR of 0.35 (95% CI: 0.17, 0.73), 0.48 (0.24, 0.97), 0.28 (0.12, 0.65), and 0.23 (0.07, 0.91), respectively, for the quintile 2 to 5 intake groups compared to the lowest quintile intake (p for trend=0.02]. Among premenopausal women, highest intake of folate was associated with increased gastric cancer risk (HR: 2.62; 95% CI: 1.04, 6.59). There were no statistically significant associations observed among postmenopausal women.

Conclusions—These results suggest dietary factors involved in one-carbon metabolism are associated with gastric cancer risk among premenopausal women.

Impact—Riboflavin may be a protective factor and folate may be a risk factor for premenopausal gastric cancer.

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INTRODUCTION

Globally, gastric cancer is the second most frequent cause of cancer death, and the fourth most common cancer with over 738,000 gastric cancer deaths and over 1,000,000 new gastric cancer cases annually (1). Incidence varies greatly by geographic area, ethnicity, and gender which indicate the importance of lifestyle and environmental factors in gastric cancer etiology (2,3). *Helicobacter pylori* (*H. pylori*) is a known risk factor for gastric cancer, however, not all individuals with *H. pylori* infection will develop gastric cancer. Therefore, studies which evaluate other factors are still needed to understand gastric cancer risk.

One factor that varies greatly between populations is diet and some epidemiologic studies have suggested that intakes of fruits and vegetables, antioxidant nutrients such as vitamin C, retinol, and selected carotenoids may be inversely associated with gastric cancer risk, whereas salt and salt-preserved food may be adversely related to gastric cancer risk (4,5). The one-carbon metabolism pathway, which includes several dietary factors, may affect gastric cancer risk; the disruption of homeostasis in one-carbon (methyl group) metabolism affects the risk of other cancers (6–8). Disruption can occur as the result of dietary insufficiencies in the micronutrients involved in this pathway including folate, methionine, and B vitamins (riboflavin, B6 and B12). Folate and methionine are important dietary methyl donors and B vitamins also serve as cofactors in one-carbon metabolism.

A few epidemiologic studies have evaluated these one-carbon metabolism factors and results have been inconsistent with reports of overall null associations (9–13), decreased risk (14–21) and increased risk (15,16,21) as well as effect modification by genetic factors (20,22–24). Dietary folate, which has been proposed to affect carcinogenesis through either DNA synthesis or DNA methylation (6–8,25,26), has not been associated with gastric cancer risk in a meta-analysis (13) and some other recent studies (11,12,14,27). Nevertheless, the potential preventive effect of dietary folate and other one-carbon metabolism factors on gastric cancer risk is still suggested in several human or animal studies (23,24,27,28). Thus, we analyze data from a large-scale prospective study, the Shanghai Women's Health Study (SWHS), to investigate whether dietary folate, methionine, or B vitamins intakes are associated with gastric cancer risk in Chinese pre- and post-menopausal women, a population with a traditionally high risk of gastric cancer and a low use of vitamin supplements. Menopausal status has been shown to have a modifying effect on diet -cancer associations (29–31), and independently associated with gastric cancer risk in our previous study (32), and, therefore, is considered a potential effect modifier.

MATERIALS AND METHODS

Study design and participants

The SWHS is a population-based prospective study of 74,941 Chinese women aged 40–70 years at cohort entry. The study design and validated food frequency questionnaire (FFQ) have been described in previous publications (33,34). The baseline survey was administered from 1997 to 2000 in urban Shanghai. A total of 74,941 women were recruited from 81,170 eligible population individuals (response rate: 92.3%). The cohort was followed for cancer incidence and cause-specific mortality by using a combination of in-person follow-up surveys and annual record linkage to a population-based cancer registry, the Shanghai Cancer Registry, and official death certificates. During the follow-up survey, 99.8% of baseline participants was followed in the 1st wave (2000–2002), 98.7% in the 2nd wave (2003–2004), 96.7% in the 3rd wave (2004–2007), and 92.0% in 4th wave (2008–2010), with structured questionnaires. The study was approved by the institutional review boards of all institutions involved and written informed consent was obtained from all participants.

Dietary assessment and nutrients estimation

The FFQ included questions on 77 foods was used to collect information on frequency of consumption (daily, weekly, monthly, annually, and never) and amount consumed in *liang* (Chinese unit, 1 *liang*=50g) per unit of time. The daily intakes of total energy and nutrients, including folate, thiamine (vitamin B1), riboflavin (vitamin B2), and niacin (B3), were estimated by multiplying the amount of each food consumed by the nutrient content per gram of the food as obtained from the Chinese Food Composition Tables (35). Methionine, and vitamins B6 and B12 were derived using the US Department of Agriculture food composition tables as described previously (36). The FFQ used in the study was validated in a sample of 200 cohort participants against the averages of multiple 24 hour dietary recalls. The Pearson correlation coefficients for FFQ folate intake and plasma folate concentration was 0.52(29), and ranged from 0.56 to 0.59 for B vitamins intakes from the FFQ versus multiple 24 hour recalls (33). Total daily meat intake was calculated by combining red meat intake and poultry intake. The FFQ also solicited information on frequency of consumption of four salt-preserved foods (salted meat or preserved meat, salted fish, salted egg, and salted vegetables or preserved vegetables) using eight categories (< 5 times per year, 6–11 per year, 1–2 per month, 3–4 per month, 1–2 per week, 3–4 per week, 5–6 per week, and every day). Weekly salt-preserved food intake was derived by summing the median intake frequency of each food items and categorized as weekly or less than weekly intake.

Outcome ascertainment

Newly diagnosed gastric cancer cases were initially identified by data linkage with the Shanghai Cancer Registry, a population-based cancer registry which was established in 1963. Possible cancer cases identified by data linkage with the registry database or by in-person follow-up surveys that took place every 2–3 years were rechecked to eliminate any false positive matches by reviewing medical records from the diagnostic hospitals and home visiting. As of the end of 2010, 350 cases with incident primary gastric cancer were identified. Among the 350 gastric cancer cases, 323 cases were distal cancer, defined as gastric cancer in non-cardia or unspecified region which was coded with 151.1–151.9, based

on the International Classification of Disease 9th version (ICD-9). Deaths for any cause were ascertained by data linkage to death certificate registries with a confirmation by a home visit. Individuals with gastric cancer listed as a cause of death that was not confirmed through the registry data or medical record review (n=56) were treated as censored cases.

Statistical analysis

For this study, a total of 73,009 women, including 323 new distal gastric cancer cases, were included for the present analyses. Excluded from analysis, in order of exclusion, were 346 participants with gastrectomy history before baseline, 1,495 participants with a previous cancer history or were later identified by cancer registry data as having a cancer diagnosis prior to baseline, 50 participants who reported total energy intake of <500 or >4,000 kcal per day in the baseline FFQ, 14 participants missing menopausal status, and 27 gastric cancer cases in the cardia (151.0 of ICD-9).

The end of the study period was defined as the first of the following outcomes: first cancer diagnosis; any cause of death; or date of last follow-up. Comparisons of means or proportions of baseline characteristics between distal gastric cancer cases and non-cancer cases was performed using analysis of covariance or Cochran–Mantel–Haenszel tests adjusting for age and additionally adjusted for total energy intake for dietary factors. For each nutrient, quintiles of intake were derived using the distributions of intake among all cohort participants. In statistical models, the lowest quintile of intake was used as the reference group. Hazard ratios (HRs) and 95% confidence intervals (CIs) for distal gastric cancer associated with dietary intakes were estimated by Cox proportional hazards regression with age as a time scale. Other potential risk or preventive factors for gastric cancer in previous studies were evaluated for confounding and included as covariates in the model when evidence of confounding was present. These covariates included age at invitation, menopausal status, body mass index, total intake of energy (Kcal/day), peptic ulcer medication history, family history of gastric cancer, education level (< high school vs. high school), weekly salt-preserved food intake, and daily intakes of total meat, total vegetables and fruits (excluding watermelon), and sodium. Watermelon, although eaten seasonally, accounts for 44–52% of total fruit intake, and the quantification of this fruit in large quantities can be difficult to assess, potentially leading to exposure measurement error. Further, in the present study, intake of watermelon alone was not associated with gastric cancer risk (37). Inclusion of smoking status, alcohol drinking, and green tea drinking as covariates did not alter risk estimates and were not included as covariates in the final analyses. The median intake value of each intake quintile was used as a continuous variable in models testing for a linear trend across the intake levels. Stratified analysis was used to evaluate menopausal status as a potential effect modifier. All analyses were carried out with SAS statistical software (version 9.3; SAS institute, Inc., Cary, North Carolina).

RESULTS

Comparisons of general characteristics between distal gastric cancer cases and non-cases, adjusted for age at baseline, are shown in Table 1. Cases were significantly older, and more likely to be postmenopausal, and to have previously used peptic ulcer medicine than non-

cases. No significant differences were observed for income, education, familial cancer history, body mass index, total energy intake or for use of cigarettes, alcohol, aspirin, B vitamin complex, or multivitamin. Dietary intakes of vegetables and fruits, meat, sodium, or salted food were not associated with distal gastric cancer (Table 1).

Associations between B vitamins and methionine with distal gastric cancer risk are shown in Table 2. Overall, dietary intakes of these factors were not associated with risk of distal gastric cancer although folate intake had a suggestive non-significant increased risk. However, when stratified by menopausal status at baseline, premenopausal women with the highest folate intake had a statistically significant increased risk of distal gastric cancer (HR= 2.62, 95% CI: 1.04, 6.59 versus the 1st intake quintile) (Table 3). The increased risk among premenopausal women with the highest folate intake persisted after exclusion of gastric cancer cases diagnosed with the first and second years after baseline and age of onset was similar across low and high folate intakes(data not shown). Further, riboflavin intake was associated with a statistically significant decreased risk of distal gastric cancer in a dose-dependent manner (P_{trend}=0.02) among premenopausal women (HR= 0.35, 95% CI: 0.17, 0.73 for 2nd vs 1st quintile; HR= 0.49, 95% CI: 0.24, 0.99 for 3rd vs. 1st quintile; HR= 0.31, 95% CI: 0.13, 0.71 for 4th vs. 1st quintile; and HR= 0.23, 95% CI: 0.08, 0.73 for 5th vs. 1st quintile). The inverse association between riboflavin intake and distal gastric cancer risk in premenopausal women persisted after adjustment for additional potential confounders, such as chronic gastritis history and income level, and after exclusion of gastric cancer cases diagnosed with the first and second years after baseline (data not shown). No statistically significant associations were observed among post-menopausal women.

DISCUSSION

In the present large population-based cohort study, no significant overall associations between dietary intakes of one-carbon metabolism associated nutrients and distal gastric cancer were observed. However, among premenopausal women, riboflavin (B2) intake was inversely and significantly associated with risk of distal gastric cancer and folate intake was adversely and significantly associated with distal gastric cancer risk. These findings, to the best of our knowledge, are the first prospective evidence for a potential preventive effect of riboflavin and possible adverse effect of excess folate in gastric cancer.

In our study, high consumption of food folate may increase the risk of distal gastric cancer in premenopausal women. Although previous epidemiological studies have generally shown that low folate intake has been associated with increased risks for colon, breast, and pancreas cancers (11), our finding may be in line with the potential dual effects of folate on carcinogenesis (38,39). Folate may have a cancer preventive effect in early carcinogenesis, but may promote carcinogenesis in individuals who harbor early neoplasia or who have a very high intake (38–41) possibly by facilitating cellular proliferation (42,43). Inverse (16,19,23,24,27,44) or null associations (9–12,14) with folate intake have been reported for gastric cancer risk. However, prospective data are scarce, and only two cohort studies have evaluated the association between dietary folate and risk of gastric cancer (10,12) both of which had null findings. One study, the European Prospective Investigation into Cancer and Nutrition, also evaluated serum folate with gastric cancer risk and found no association (14).

A recent meta-analyses using pooled data on 49,621 participants from 13 folic acid intervention trials found that folic acid supplementation does not affect risk of gastric cancer (RR= 1.01, 95% CI: 0.58, 1.75)(45). We also found no overall association with folate intake in this Chinese population. We did, however, observe an increased risk for premenopausal women associated with the highest intake level. This association persisted after sensitivity analyses excluding the first two years of diagnosis, and participants with peptic ulcer medication use or chronic gastritis indicating excessive folate intake (> US daily recommended intake) may increase the risk of development of gastric cancer. Risk among premenopausal women needs to be further evaluated in additional studies.

Riboflavin is also involved in the folate-mediated one-carbon metabolism as a cofactor for the enzymes methyltetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR), thus, poor riboflavin status may lead to an elevated rate of DNA damage and altered methylation of DNA (46), both of which are important risk factors for cancer. Animal studies have founded that riboflavin deficiency can induce high DNA enzyme activity and increase DNA damage caused by the administration of carcinogens (47), increase carcinogen binding to DNA (48), and disrupt the integrity of the epithelium of various organs (49). Some carcinogens are metabolized by flavin-dependent enzymes, and riboflavin status may modify the effects of carcinogens (47). For these reasons, riboflavin deficiency may cause a variety of precancerous lesions in humans. Associations between cancer risk and riboflavin intake have been observed for esophageal cancer (50–52), cervical dysplasia (53), lung cancer (54,55), and colorectal cancer (30). Although an association between riboflavin and gastric cancer risk is plausible, findings from previous epidemiologic studies are inconsistent (9,15,16,19,52,56–61). The majority of these studies observed that dietary riboflavin intake (9,16,52,60,61), serum riboflavin level(56), and riboflavin supplementation (59) were not associated with gastric cancer risk. Two case-control studies in Western populations reported an increased gastric cancer risk associated with high intake of riboflavin (15,57). Our finding of an inverse association between intake and gastric cancer risk is consistent with two previous case-control studies conducted in Asian populations (19,58), including a previous study in Shanghai among both men and women (58). A possible explanation for the discrepancies between Asian and Western studies may be the intake level within the populations. The mean riboflavin intake in our studies was 0.8 mg/day which is lower than the US Recommended Dietary Allowance (RDA) of 1.1 mg/day for women (62). More than 81% of the participants had insufficient intake of dietary riboflavin. A genome-wide association study of gastric cancer identified a susceptibility locus for gastric cancer at C20orf54 at 20p13 which is responsible for transporting riboflavin and may modulate intestinal riboflavin absorption (63). Thus, it may be that riboflavin is only associated with risk of gastric cancer in the context of riboflavin deficiency or insufficiency due to intake or due to increased requirements due to genetic variation.

Another possible explanation for the discrepancies between the previous null findings of riboflavin and gastric cancer risk and the present findings may be differences in the age distributions of study subjects. To our knowledge, no previous study provided an analysis stratified by menopausal status- or by age. We found an inverse association in premenopausal women whose mean age (45 years) or age of gastric cancer diagnosis (57 years) were relatively younger than those of previous studies that showed null associations

(9,16,52,56). A Korean study with a similar age distribution (57 years) observed similar results as the present study (19). Thus, it is possible that the relationship between riboflavin intake and gastric cancer risk varies according to age or menopausal status. These findings need to be further evaluated in future studies.

A recent meta-analysis found that longer years of fertility and hormone replacement therapy use were related to reduced gastric cancer risk (64). On the other hand, tamoxifen treatment was associated with elevated risk. Although menopausal status was not linked to risk in the study, it was not assessed as an effect modifier. These findings indicate estrogen exposure was related to the risk of gastric cancer. We found that the associations between diet and gastric cancer risk differed by menopausal status(31). In addition, previous studies indicate there are large differences in gastric cancer risk between men and women. This difference may be explained by the presence of estrogen receptors in gastric tissue and cancers (65). Decreased sex hormone levels or the cumulative effect of other conventional risk factors for gastric cancer in postmenopausal women may explain the observed differences by menopausal status. But, the published literature is still insufficient to draw an inference about biological plausibility for why the associations of riboflavin and folate with gastric cancer risk were only observed among premenopausal women. In addition, we cannot eliminate the possibility that some of the observed associations may be spurious and the result of small samples sizes within some strata. Thus, further studies are necessary to understand the mechanism and confirm the findings.

Our study has some limitations. First, the status of *H. pylori* infection, one of the strongest risk factors for gastric cancer was not known; however, it is unlikely to substantially affect the findings as the prevalence of *H. pylori* infection in the Shanghai population is estimated to be 92% in women (66). Second, intakes of vitamin B6, vitamin B12, and methionine were estimated based on the United States Department of Agriculture Food Composition Database because these nutrients were not included in the Chinese Food Composition Tables. We have previously compared other vitamin levels between the two databases and found very high correlations (correlation coefficients>0.91) (29). Thus, potential misclassification of intake levels is likely minimal. Third, as noted previously, the timing of exposure, particularly for folate intake, may be important and we were not able to fully evaluate timing in this study. However, similar findings were observed in analyses where cases diagnosed within two years of baseline were excluded. Fourth, although thorough assessment of and adjustment for confounding was conducted, we cannot negate the potential for residual confounding.

Our study has several strengths. The current prospective cohort study is one of the largest to date to evaluate the association between dietary factors related to one-carbon metabolism and distal gastric cancer risk. Data for dietary intakes were collected by a comprehensive and validated FFQ (33). The prospective design and high participation rate (92.6%) both contribute to the likelihood of valid and generalizable study results. In addition, we were able to restrict analyses to distal gastric cancer. Distal gastric cancer differs from cardia gastric cancer, localized to near the gastro-esophageal junction, in etiology and epidemiological characteristics. Another strength of our study is that our study population is well suited to the study of B vitamins and gastric cancer because of high incidence of gastric

cancer (1), no vitamin fortification of the food supply, and low prevalences of alcohol drinking (less than 3%) and B vitamin supplement use (less than 5%).

In summary, although we found no overall associations between dietary intakes of one-carbon metabolism factors and gastric cancer risk, we did observe an inverse association of riboflavin intake among premenopausal women. Further, higher levels of folate intake may increase risk among this same subgroup. Thus, the findings from this study suggest roles for these two vitamins in premenopausal gastric cancer risk.

Acknowledgments

References

1. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127:2893–917. [PubMed: 21351269]

2. Glade MJ. Food, nutrition, and the prevention of cancer: a global perspective. American Institute for Cancer Research/World Cancer Research Fund, American Institute for Cancer Research, 1997. Nutrition. 1999; 15:523–6. [PubMed: 10378216]

3. Parkin DM. International variation. Oncogene. 2004; 23:6329–40. [PubMed: 15322508]

4. Liu C, Russell RM. Nutrition and gastric cancer risk: an update. Nutr Rev. 2008; 66:237–49. [PubMed: 18454810]

5. Tsugane S, Sasazuki S. Diet and the risk of gastric cancer: review of epidemiological evidence. Gastric Cancer. 2007; 10:75–83. [PubMed: 17577615]

6. Kim YI. Folate and cancer prevention: a new medical application of folate beyond hyperhomocysteinemia and neural tube defects. Nutr Rev. 1999; 57:314–21. [PubMed: 10575908]

7. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA. 1997; 94:3290–5. [PubMed: 9096386]

8. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr. 2000; 130:129–32. [PubMed: 10720158]

9. Pelucchi C, Tramacere I, Bertuccio P, Tavani A, Negri E, La Vecchia C. Dietary intake of selected micronutrients and gastric cancer risk: an Italian case-control study. Ann Oncol. 2009; 20:160–5. [PubMed: 18669867]

10. 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. [PubMed: 10679641]

11. Tavani A, Malerba S, Pelucchi C, Dal Maso L, Zucchetto A, Serraino D, et al. Dietary folates and cancer risk in a network of case-control studies. Ann Oncol. 2012; 23:2737–42. [PubMed: 22898036]

12. 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. [PubMed: 16835345]

13. 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. [PubMed: 17030196]

14. Vollset SE, Igland J, Jenab M, Fredriksen A, Meyer K, Eussen S, 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. [PubMed: 18006931]

15. 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. [PubMed: 9797127]

16. Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, et al. Nutrient intake and risk of subtypes of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev. 2001; 10:1055–62. [PubMed: 11588131]

17. Muñoz N, Plummer M, Vivas J, Moreno V, De Sanjosé S, Lopez G, et al. A case-control study of gastric cancer in Venezuela. Int J Cancer. 2001; 93:417–23. [PubMed: 11433408]

18. 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. [PubMed: 9305701]

19. Kim HJ, Kim MK, Chang WK, Choi HS, Choi BY, Lee SS. Effect of nutrient intake and Helicobacter pylori infection on gastric cancer in Korea: a case-control study. Nutr Cancer. 2005; 52:138–46. [PubMed: 16201845]

20. Boccia S, Hung R, Ricciardi G, Gianfagna F, Ebert MPA, Fang J-Y, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: a huge-GSEC review. Am J Epidemiol. 2008; 167:505–16. [PubMed: 18162478]

21. La Vecchia C, Negri E, Franceschi S, Decarli A. Case-control study on influence of methionine, nitrite, and salt on gastric carcinogenesis in northern Italy. Nutr Cancer. 1997; 27:65–8. [PubMed: 8970184]

22. Yoo J-Y, Kim S-Y, Hwang J-A, Hong S-H, Shin A, Choi IJ, et al. Association Study between Folate Pathway Gene Single Nucleotide Polymorphisms and Gastric Cancer in Koreans. Genomics Inform. 2012; 10:184–93. [PubMed: 23166529]

23. Galván-Portillo MV, Cantoral A, Oñate-Ocaña LF, Chen J, Herrera-Goepfert R, Torres-Sanchez L, et al. Gastric cancer in relation to the intake of nutrients involved in one-carbon metabolism among MTHFR 677 TT carriers. Eur J Nutr. 2009; 48:269–76. [PubMed: 19288150]

24. Gao S, Ding L-H, Wang J-W, Li C-B, Wang Z-Y. Diet folate, DNA methylation and polymorphisms in methylenetetrahydrofolate reductase in association with the susceptibility to gastric cancer. Asian Pac J Cancer Prev. 2013; 14:299–302. [PubMed: 23534741]

25. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res. 2001; 475:7–20. [PubMed: 11295149]

26. Kim YI, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, et al. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr. 1997; 65:46–52. [PubMed: 8988912]

27. Epplein M, Signorello LB, Zheng W, Cai Q, Hargreaves MK, Michel A, et al. Helicobacter pylori prevalence and circulating micronutrient levels in a low-income United States population. Cancer Prev Res (Phila). 2011; 4:871–8. [PubMed: 21436385]

28. Gonda TA, Kim Y-I, Salas MC, Gamble MV, Shibata W, Muthupalani S, et al. Folic acid increases global DNA methylation and reduces inflammation to prevent Helicobacter-associated gastric cancer in mice. Gastroenterology. 2012; 142:824–833. e7. [PubMed: 22248660]

29. Shrubsole MJ, Shu XO, Li H-L, Cai H, Yang G, Gao Y-T, et al. Dietary B vitamin and methionine intakes and breast cancer risk among Chinese women. Am J Epidemiol. 2011; 173:1171–82. [PubMed: 21447479]

30. Zschäbitz S, Cheng T-YD, Neuhouser ML, Zheng Y, Ray RM, Miller JW, et al. B vitamin intakes and incidence of colorectal cancer: results from the Women's Health Initiative Observational Study cohort. Am J Clin Nutr. 2013; 97:332–43. [PubMed: 23255571]

31. Kweon S-S, Shu X-O, Xiang Y, Cai H, Yang G, Ji B-T, et al. Intake of specific nonfermented soy foods may be inversely associated with risk of distal gastric cancer in a Chinese population. J Nutr. 2013; 143:1736–42. [PubMed: 23986366]

32. Green J, Roddam A, Pirie K, Kirichek O, Reeves G, Beral V, et al. Reproductive factors and risk of oesophageal and gastric cancer in the Million Women Study cohort. Br J Cancer. 2012; 106:210–6. [PubMed: 22127287]

33. Shu XO, Yang G, Jin F, Liu D, Kushi L, Wen W, et al. Validity and reproducibility of the food frequency questionnaire used in the Shanghai Women's Health Study. Eur J Clin Nutr. 2004; 58:17–23. [PubMed: 14679362]

34. Zheng W, Chow W-H, Yang G, Jin F, Rothman N, Blair A, et al. The Shanghai Women's Health Study: rationale, study design, and baseline characteristics. Am J Epidemiol. 2005; 162:1123–31. [PubMed: 16236996]

35. Yang, Y.; Wang, G.; Pan, X. China Food Composition Tables. Beijing University Medical Press;

36. Shrubsole MJ, Yang G, Gao Y-T, Chow WH, Shu XO, Cai Q, et al. Dietary B vitamin and methionine intakes and plasma folate are not associated with colorectal cancer risk in Chinese women. Cancer Epidemiol Biomarkers Prev. 2009; 18:1003–6. [PubMed: 19240230]

37. Epplein M, Shu X-O, Xiang Y-B, Chow W-H, Yang G, Li H-L, et al. Fruit and vegetable consumption and risk of distal gastric cancer in the Shanghai Women's and Men's Health studies. Am J Epidemiol. 2010; 172:397–406. [PubMed: 20647333]

38. Ulrich CM. Folate and cancer prevention--where to next? Counterpoint. Cancer Epidemiol Biomarkers Prev. 2008; 17:2226–30. [PubMed: 18768487]

39. Kim Y-I. Folic acid supplementation and cancer risk: point. Cancer Epidemiol Biomarkers Prev. 2008; 17:2220–5. [PubMed: 18768486]

40. Kim Y-I. Folate: a magic bullet or a double edged sword for colorectal cancer prevention? Gut. 2006; 55:1387–9. [PubMed: 16966698]

41. Mason JB. Folate consumption and cancer risk: a confirmation and some reassurance, but we're not out of the woods quite yet. Am J Clin Nutr. 2011; 94:965–6. [PubMed: 21900462]

42. Song J, Medline A, Mason JB, Gallinger S, Kim YI. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. Cancer Res. 2000; 60:5434–40. [PubMed: 11034085]

43. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA. 2007; 297:2351–9. [PubMed: 17551129]

44. La Vecchia C, Ferraroni M, D'Avanzo B, Decarli A, Franceschi S. Selected micronutrient intake and the risk of gastric cancer. Cancer Epidemiol Biomarkers Prev. 1994; 3:393–8. [PubMed: 7920206]

45. Vollset SE, Clarke R, Lewington S, Ebbing M, Halsey J, Lonn E, et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50,000 individuals. Lancet. 2013; 381:1029–36. [PubMed: 23352552]

46. Huang J, Vieira A. DNA methylation, riboswitches, and transcription factor activity: fundamental mechanisms of gene-nutrient interactions involving vitamins. Mol Biol Rep. 2006; 33:253–6. [PubMed: 17077989]

47. 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. [PubMed: 8556699]

48. Pangrekar J, Krishnaswamy K, Jagadeesan V. Effects of riboflavin deficiency and riboflavin administration on carcinogen-DNA binding. Food Chem Toxicol. 1993; 31:745–50. [PubMed: 8225133]

49. Foy H, Kondi A. The vulnerable esophagus: riboflavin deficiency and squamous cell dysplasia of the skin and the esophagus. J Natl Cancer Inst. 1984; 72:941–8. [PubMed: 6584669]

50. Van Rensburg SJ. Epidemiologic and dietary evidence for a specific nutritional predisposition to esophageal cancer. J Natl Cancer Inst. 1981; 67:243–51. [PubMed: 6943364]

51. Zou XN, Taylor PR, Mark SD, Chao A, Wang W, Dawsey SM, et al. Seasonal variation of food consumption and selected nutrient intake in Linxian, a high risk area for esophageal cancer in China. Int J Vitam Nutr Res. 2002; 72:375–82. [PubMed: 12596503]

52. Chen H, Ward MH, Graubard BI, Heineman EF, Markin RM, Potischman NA, et al. Dietary patterns and adenocarcinoma of the esophagus and distal stomach. Am J Clin Nutr. 2002; 75:137–44. [PubMed: 11756071]

53. Liu T, Soong SJ, Wilson NP, Craig CB, Cole P, Macaluso M, et al. A case control study of nutritional factors and cervical dysplasia. Cancer Epidemiol Biomarkers Prev. 1993; 2:525–30. [PubMed: 8268768]

54. Bassett JK, Hodge AM, English DR, Baglietto L, Hopper JL, Giles GG, et al. Dietary intake of B vitamins and methionine and risk of lung cancer. Eur J Clin Nutr. 2012; 66:182–7. [PubMed: 21878960]

55. Takata Y, Cai Q, Beeghly-Fadiel A, Li H, Shrubsole MJ, Ji B-T, et al. Dietary B vitamin and methionine intakes and lung cancer risk among female never smokers in China. Cancer Causes Control. 2012; 23:1965–75. [PubMed: 23065072]

56. Eussen SJPM, Vollset SE, Hustad S, Midttun Ø, Meyer K, Fredriksen A, et al. 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. Cancer Epidemiol Biomarkers Prev. 2010; 19:28–38. [PubMed: 20056620]

57. Risch HA, Jain M, Choi NW, Fodor JG, Pfeiffer CJ, Howe GR, et al. Dietary factors and the incidence of cancer of the stomach. Am J Epidemiol. 1985; 122:947–59. [PubMed: 2998182]

58. Ji BT, Chow WH, Yang G, McLaughlin JK, Zheng W, Shu XO, et al. Dietary habits and stomach cancer in Shanghai, China. Int J Cancer. 1998; 76:659–64. [PubMed: 9610722]

59. Wang GQ, Dawsey SM, Li JY, Taylor PR, Li B, Blot WJ, et al. Effects of vitamin/mineral supplementation on the prevalence of histological dysplasia and early cancer of the esophagus and stomach: results from the General Population Trial in Linxian, China. Cancer Epidemiol Biomarkers Prev. 1994; 3:161–6. [PubMed: 8049638]

60. Graham S, Haughey B, Marshall J, Brasure J, Zielezny M, Freudenheim J, et al. Diet in the epidemiology of gastric cancer. Nutr Cancer. 1990; 13:19–34. [PubMed: 2300492]

61. Zhang ZF, Kurtz RC, Yu GP, Sun M, Gargon N, Karpeh M Jr, et al. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. Nutr Cancer. 1997; 27:298–309. [PubMed: 9101561]

62. A Report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, D.C: The National Academies Press; 1998.

63. Wang L-D, Zhou F-Y, Li X-M, Sun L-D, Song X, Jin Y, et al. Genome-wide association study of esophageal squamous cell carcinoma in Chinese subjects identifies susceptibility loci at PLCE1 and C20orf54. Nat Genet. 2010; 42:759–63. [PubMed: 20729853]

64. Camargo MC, Goto Y, Zabaleta J, Morgan DR, Correa P, Rabkin CS. Sex hormones, hormonal interventions, and gastric cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2012; 21:20–38. [PubMed: 22028402]

65. Ryu W-S, Kim J-H, Jang Y-J, Park S-S, Um J-W, Park S-H, et al. Expression of estrogen receptors in gastric cancer and their clinical significance. J Surg Oncol. 2012; 106:456–61. [PubMed: 22422271]

66. Wong H-L, Rabkin CS, Shu X-O, Pfeiffer RM, Cai Q, Ji B-T, et al. Systemic cytokine levels and subsequent risk of gastric cancer in Chinese Women. Cancer Sci. 2011; 102:1911–5. [PubMed: 21740481]

Table 1

Baseline characteristics comparing distal gastric cancer cases and non-case participants, Shanghai Women's Health Study, 1997–2010.

	Non-cancer[a] (n=72,686)	Cancer[a] (n=323)	p-value[b]
Age at enrollment, y	52.5 ± 9.04	57.9 ± 9.01	<0.001
Post-menopausal	35404 (48.7)	237 (73.4)	0.03
Ever cigarette smoker	1990 (2.7)	15 (4.6)	0.47
Ever regular alcohol drinker	1631 (2.2)	8 (2.5)	0.97
Ever regular green tea drinker	21769 (29.9)	75 (23.2)	0.17
Aspirin or other NSAID use	1467 (2.0)	6 (1.9)	0.30
Vitamin B complex user	2794 (3.8)	14 (4.3)	0.67
Multi-vitamin user	5142 (7.1)	17 (5.3)	0.16
Income level			
Low	20022 (27.6)	112 (34.7)	0.42
Middle	28248 (38.9)	132 (40.9)	
High	24400 (33.6)	79 (24.5)	
Educational attainment (high school)	9834 (13.5)	35 (10.8)	0.29
Family history of gastric cancer	4226 (5.8)	27 (8.4)	0.06
Family history of any cancer	19247 (26.5)	91 (28.2)	0.34
Chronic gastritis history	14175 (19.5)	70 (21.7)	0.49
Peptic ulcer medication	2390 (3.3)	20 (5.9)	0.03
Body mass index, kg/m^2	24.0 ± 3.4	24.6 ± 3.5	0.57
Total calorie intake, kcal/d	1676 ± 399	1644 ± 402	0.59
Total vegetable and fruit intake, g/d	447 ± 245	406 ± 223	0.12[c]
Total meat intake, g/d	66.3 ± 44.8	60.0 ± 42.9	0.69[c]
Sodium intake, g/d	341 ± 153	315 ± 148	0.53[c]
Intake of salted/preserved foods 1 per week [c]	52386 (72.1)	226 (70.2)	0.36[c,d]

[a] Values are presented as means ± std for continuous variables and n (%) for categorical variables.

[b] All p-values are adjusted for age at invitation except the direct comparison of age at invitation

[c] Additionally adjusted for total energy intake

[d] 23 cases are missing

Table 2

Association between daily dietary intake quintiles of methionine and B vitamins and distal gastric cancer risk, Shanghai Women's Health Study, 1997–2010.

Intake quintile	Range	No. of cases	Minimally adjusted HR (95% CI) [a]	Fully adjusted HR (95% CI)[b]
Dietary Folate, *ug*/day				
Quintile 1	45–206	77	1.00 (ref.)	1.00 (ref.)
Quintile 2	206–253	60	0.90 (0.63–1.27)	0.97 (0.68–1.38)
Quintile 3	253–301	54	0.84 (0.59–1.22)	0.96 (0.66–1.42)
Quintile 4	301–366	70	1.12 (0.78–1.60)	1.34 (0.91–1.99)
Quintile 5	366–2048	62	1.04 (0.69–1.56)	1.42 (0.87–2.32)
P for trend			0.53	0.08
Methionine, g/day				
Quintile 1	0.3–1.1	79	1.00 (ref.)	1.00 (ref.)
Quintile 2	1.1–1.3	77	1.15(0.83–1.61)	1.22 (0.86–1.72)
Quintile 3	1.3–1.6	53	0.86 (0.58–1.26)	0.94 (0.61–1.45)
Quintile 4	1.6–1.9	55	0.96 (0.63–1.46)	1.10 (0.67–1.82)
Quintile 5	1.9–5.7	59	1.11 (0.67–1.82)	1.39 (0.70–2.76)
P for trend			0.88	0.76
Riboflavin, mg/day				
Quintile 1	0.1–0.6	91	1.00 (ref.)	1.00 (ref.)
Quintile 2	0.6–0.7	56	0.71 (0.50–1.00)	0.71 (0.50–1.02)
Quintile 3	0.7–0.9	74	0.98 (0.70–1.37)	0.99 (0.68–1.45)
Quintile 4	0.9–1.1	54	0.75 (0.51–1.10)	0.77 (0.48–1.22)
Quintile 5	1.1–3.8	48	0.69 (0.44–1.09)	0.71 (0.38–1.34)
P for trend			0.20	0.48
Niacin, mg/day				
Quintile 1	3.2–10.8	84	1.00 (ref.)	1.00 (ref.)
Quintile 2	10.8–12.7	67	0.91 (0.65–1.29)	1.00 (0.70–1.45)
Quintile 3	12.7–14.7	54	0.80 (0.53–1.19)	0.93 (0.60–1.45)
Quintile 4	14.7–17.3	59	0.91 (0.59–1.42)	1.16 (0.69–1.97)
Quintile 5	17.3–54.3	59	0.97 (0.55–1.71)	1.44 (0.69–3.00)
P for trend			0.82	0.48
Vitamin B6, mg/day				
Quintile 1	0.3–1.2	94	1.00 (ref.)	1.00 (ref.)
Quintile 2	1.2–1.5	58	0.69 (0.49–0.98)	0.73 (0.51–1.05)
Quintile 3	1.5–1.8	57	0.71 (0.50–1.02)	0.78 (0.52–1.19)
Quintile 4	1.8–2.1	62	0.79 (0.54–1.16)	0.91 (0.56–1.49)
Quintile 5	2.1–6.9	52	0.66 (0.41–1.06)	0.84 (0.41–1.69)
P for trend			0.19	0.81
Vitamin B12, *ug*/day				
Quintile 1	0.0–1.3	77	1.00 (ref.)	1.00 (ref.)
Quintile 2	1.3–2.0	80	1.26 (0.91–1.73)	1.29 (0.93–1.79)

Intake quintile	Range	No. of cases	Minimally adjusted HR (95% CI)[a]	Fully adjusted HR (95% CI)[b]
Quintile 3	2.0–2.6	59	1.04 (0.73–1.47)	1.09 (0.74–1.60)
Quintile 4	2.6–3.6	58	1.10 (0.77–1.59)	1.19 (0.78–1.82)
Quintile 5	3.6–34.2	49	1.04 (0.69–1.56)	1.14 (0.66–1.96)
P for trend			0.95	0.73

[a] Adjusted for age at invitation and total energy intake

[b] Adjusted for age at invitation, menopausal status, body mass index, total energy intake, peptic ulcer medication history and family history of gastric cancer, education level, total meat intake, all vegetables and fruits intake (except watermelon), sodium intake, and intake frequency of salted foods

Table 3

Association between daily dietary intake quintiles of methionine and B vitamins and gastric cancer risk stratified by menopausal status, Shanghai Women's Health Study, 1997–2010.

Intake quintile	Range	Premenopausal women		Postmenopausal women	
		No. of cases	Fully-adjusted HR (95% CI)[a]	No. of cases	Fully-adjusted HR (95% CI)[a]
Dietary Folate, ug/day					
Quintile 1	45–206	17	1.00 (ref.)	60	1.00 (ref.)
Quintile 2	206–253	19	1.20 (0.60–2.37)	41	0.88 (0.58–1.34)
Quintile 3	253–301	15	1.08 (0.50–2.32)	39	0.92 (0.59–1.44)
Quintile 4	301–366	10	0.80 (0.33–1.96)	60	1.50 (0.96–2.33)
Quintile 5	366–2048	25	2.62 (1.04–6.59)	37	1.06 (0.59–1.91)
P for trend			0.21		0.19
Methionine, g/day					
Quintile 1	0.3–1.1	18	1.00 (ref.)	61	1.00 (ref.)
Quintile 2	1.1–1.3	17	0.81 (0.40–1.64)	60	1.37 (0.92–2.04)
Quintile 3	1.3–1.6	17	0.75 (0.34–1.66)	36	0.98 (0.59–1.64)
Quintile 4	1.6–1.9	17	0.74 (0.29–1.88)	38	1.26 (0.69–2.29)
Quintile 5	1.9–5.7	17	0.76 (0.21–2.74)	42	1.75 (0.78–3.94)
P for trend			0.61		0.52
Riboflavin, mg/day					
Quintile 1	0.1–0.6	23	1.00 (ref.)	68	1.00 (ref.)
Quintile 2	0.6–0.7	12	0.35 (0.17–0.73)	44	0.90 (0.59–1.36)
Quintile 3	0.7–0.9	20	0.49 (0.24–0.99)	54	1.27 (0.81–1.99)
Quintile 4	0.9–1.1	15	0.31 (0.13–0.71)	39	1.08 (0.62–1.89)
Quintile 5	1.1–3.8	16	0.23 (0.08–0.73)	32	1.11 (0.52–2.36)
P for trend			0.02		0.53
Niacin, mg/day					
Quintile 1	3.7–10.8	18	1.00 (ref.)	68	1.00 (ref.)
Quintile 2	10.8–12.7	19	0.94 (0.46–1.90)	48	1.02 (0.67–1.56)
Quintile 3	12.7–14.7	13	0.63 (0.26–1.49)	41	1.08 (0.65–1.81)
Quintile 4	14.7–17.3	20	0.96 (0.36–2.54)	39	1.26 (0.67–2.36)

Intake quintile	Range	Premenopausal women		Postmenopausal women	
		No. of cases	Fully-adjusted HR (95% CI)[a]	No. of cases	Fully-adjusted HR (95% CI)[a]
Quintile 5	17.3–54.3	16	0.82 (0.20–3.33)	43	1.87 (0.79–4.42)
P for trend			0.78		0.28
Vitamin B6, mg/day					
Quintile 1	0.3–1.2	21	1.00 (ref.)	73	1.00 (ref.)
Quintile 2	1.2–1.5	13	0.48 (0.23–1.00)	45	0.84 (0.55–1.27)
Quintile 3	1.5–1.8	17	0.58 (0.27–1.26)	40	0.88 (0.54–1.45)
Quintile 4	1.8–2.1	20	0.62 (0.26–1.51)	42	1.07 (0.59–1.93)
Quintile 5	2.1–6.9	15	0.42 (0.11–1.52)	37	1.14 (0.49–2.63)
P for trend			0.38		0.73
Vitamin B12, *ug*/day					
Quintile 1	0.0–1.3	13	1.00 (ref.)	64	1.00 (ref.)
Quintile 2	1.3–2.0	21	1.20 (0.59–2.44)	59	1.28 (0.88–1.86)
Quintile 3	2.0–2.6	15	0.77 (0.35–1.70)	44	1.20 (0.77–1.86)
Quintile 4	2.6–3.6	20	0.93 (0.41–2.12)	38	1.25 (0.75–2.06)
Quintile 5	3.6–34.2	17	0.75 (0.27–2.06)	32	1.33 (0.70–2.54)
P for trend			0.45		0.43

[a] Adjusted for age at invitation, menopausal status, body mass index, total energy intake, peptic ulcer medication history and family history of gastric cancer, education level, total meat intake, all vegetables and fruits intake (except watermelon), sodium intake, and intake frequency of salted foods