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Plasma folate concentrations and colorectal cancer risk: a case-control study nested within the Shanghai Men's Health Study

Yumie Takata¹, Martha J. Shrubsole¹, Honglan Li², Qiuyin Cai¹, Jing Gao², Conrad Wagner³, Jie Wu¹, Wei Zheng¹, Yong-Bing Xiang², and Xiao-Ou Shu^{1,*}

¹Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN

²Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

³Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN

Abstract

Previous epidemiological studies of circulating folate concentration and colorectal cancer have reported inconsistent results. We evaluated associations of pre-diagnostic plasma folate concentration with colorectal cancer risk in a case-control study nested within the Shanghai Men's Health Study (2002–2010). Included herein are 288 cases who were diagnosed with incident colorectal cancer and 575 controls who were individually matched to cases on baseline characteristics. Folate concentrations in plasma were measured by microbiological assay. Multivariate conditional logistic regression was used to assess associations of plasma folate concentrations with colorectal cancer risk. Plasma folate was non-significantly but positively associated with colorectal cancer risk. Odds ratios (OR) and 95% confidence intervals (CI) were 1.38 (0.95–2.02) for the middle tertile of plasma folate concentrations and 1.33 (0.90–1.98) for the highest compared with the lowest tertile. The positive association reached statistical significance for the highest tertile of folate concentrations for men with late-stage colorectal cancer (OR=2.66; 95% CI=1.03–6.86) and for the middle tertile for cases diagnosed within the first 4 years after blood collection (OR=1.72; 95% CI=1.02–2.92) and for men in the high BMI group (OR=1.88; 95% CI=1.14–3.11). In our study population, where folic acid fortification of the food supply and vitamin supplement use are uncommon, plasma folate concentration was positively associated with colorectal cancer risk among men who may have had preneoplastic lesions. These findings need to be confirmed in studies with specific assessment of preneoplastic lesions and repeated measurements of folate level over time.

Keywords

folate; colorectal cancer; China; men

*To whom correspondence should be addressed: Professor Xiao-Ou Shu; Division of Epidemiology, Department of Medicine, Vanderbilt University Medical Center, 2525 West End Avenue, Suite 600 (IMPH) Nashville, TN 37203-1738; Tel: 615-936-0713; Fax: 615-936-8291; xiao-ou.shu@vanderbilt.edu.

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Introduction

Folate may affect colorectal carcinogenesis through DNA methylation, nucleotide synthesis, and DNA repair as part of one-carbon metabolism.^{1,2} Several previous studies have evaluated various measures of folate, including self-reported dietary intake, supplemental folic acid use, and circulating levels of folate, in relation to colorectal neoplasm risk. A large pooled analysis of 13 prospective cohort studies and two meta-analyses suggested an inverse association between self-reported folate intake and the risk of colon or colorectal cancer.^{3–5} On the other hand, a combined analysis of three intervention trials of folic acid supplementation (0.5 or 1.0 mg/day) for the prevention of colorectal adenoma recurrence found no effect on the risk of new adenoma after up to 3.5 years of folic acid supplement use.⁶ One of these trials, at 3–8 years after the initiation of supplementation, found a significant increased risk of multiple or advanced adenomas with folic acid supplementation of 1.0 mg per day.⁷ In contrast, a recent clinical trial of folic acid supplementation (1.0 mg/day) in China among individuals with no prior history of colorectal adenoma reported a protective effect of folic acid on risk for colorectal adenoma.⁸ A recent large meta-analysis of folic acid trials for the prevention of cardiovascular disease or colorectal adenoma recurrence found no effect on all-cancer or colorectal cancer risk.⁹ A recent meta-analysis of 8 nested case-control studies that used pre-diagnostic blood samples reported no association between circulating folate concentration and colorectal cancer.¹⁰ Altogether, current evidence on the associations of folate with colorectal cancer is mixed and may differ according to the presence/absence of colorectal adenoma or other preneoplastic lesions and differences in levels and sources of folate.^{11,12} It is noteworthy that the majority of previous studies of circulating folate and colorectal cancer were conducted in populations where the food supply is commonly fortified with B vitamins, including folic acid, during the entire study period or during some years of follow-up, and/or in which vitamin supplement use was relatively common.^{13–15} Few studies have been conducted in populations with no folic acid fortification and/or with infrequent use of vitamin supplements,^{16,17} such as China. Herein, we investigate associations between pre-diagnostic plasma folate concentrations and the risk of colorectal cancer in a case-control study nested within the prospective Shanghai Men's Health Study (SMHS).

Materials and methods

Study population

Study participants were selected from the SMHS, a population-based, prospective cohort study of 61,480 middle-aged and elderly men in Shanghai, China.¹⁸ These men were residing in urban areas of Shanghai, had no prior history of cancer, and were aged 40 to 74 years at the time of study recruitment, which occurred between 2002 and 2006 through home visits. During the home visits, trained interviewers administered the study questionnaire to collect data on participants' health conditions and lifestyle habits. For cohort follow-up and case ascertainment, participants' data files were linked to the Shanghai Vital Statistics Registry and Shanghai Cancer Registry databases annually, and in-person visits to participants' homes were made every 2–3 years. The response rates for the first and second in-person follow-up surveys were 97.6% and 93.6%, respectively. Only participants

who provided blood and urine samples at enrollment were eligible for the current study ($N=55,086$). Within this subset, 289 men were diagnosed with colorectal cancer (International Classification of Diseases, 9th Revision codes: 153 for colon cancer and 154 for rectal cancer)¹⁹ during follow-up through December 2010. Cancer diagnoses were verified by review of medical charts. Using incidence-density sampling methods, two controls were randomly selected for each case from among participants who were alive and had not been diagnosed with any type of cancer at the time of case diagnosis ($N=578$). Controls were matched to cases on age (± 2 years), time of blood sample collection (in the morning or afternoon), use of any vitamin supplement (information on specific vitamin supplements was not available) during the week before the baseline interview, time interval between last meal and the time of blood collection (± 2 hours), and date of blood collection (± 30 days). All participants provided written informed consent before the baseline interview was conducted. The study protocols were approved by the Institutional Review Boards of Vanderbilt University Medical Center and the Shanghai Cancer Institute.

Folate measurements and dietary assessments

Folate concentrations in plasma samples were measured microbiologically using *Lactobacillus casei* as described by Horne.^{20,21} The microorganism was grown in 96-well plates for 18 hours at 37°C in a folate-free culture medium with samples containing different amounts of folate. Growth response was measured by turbidity in a microplate reader at 540 nm. Standards and samples were grown in wells of the same microtiter plate. Six sets of three blinded quality control samples were measured along with the study samples, and the mean intra-assay and inter-assay coefficients of variation were 8.1% and 13.1%, respectively. Dietary intake of folate was assessed through data from an interviewer-administered food frequency questionnaire (FFQ), which assessed the frequency and amount (per *liang*, equivalent to 50 grams) of habitual intake for 81 food items or food groups during the year before the baseline interview. The FFQ was validated against multiple dietary recalls in a subset of SMHS participants.²² Intakes of total calories and nutrients were estimated based on the Chinese Food Composition Tables,²³ with the exception of vitamin B6, vitamin B12, and methionine, which were estimated based on the United States Department of Agriculture Food Composition Database, as described elsewhere.²⁴ The correlation between dietary folate intake assessed through the FFQ and plasma folate concentration measured in this study was 0.10. The correlation between folate intake derived from multiple 24-hour dietary recalls of foods covered by the FFQ and folate intakes derived directly from the FFQ was 0.47.

Statistical analysis

All statistical analyses were performed using SAS, Version 9.3 (Carey, NC). Participants with outlier values for plasma folate concentration (424.94 ng/mL) or C-reactive protein (CRP) level (118.96 mg/L) (two participants) and their matched set (two participants) were excluded, yielding 288 cases and 575 matched controls for the analysis. Plasma folate concentrations were categorized into tertiles based on the distribution among controls. Multivariate conditional logistic regression was used to assess associations between the tertiles of plasma folate concentration and risk of colorectal cancer. Potential confounders we considered included: education (four categories); income (four categories); occupation

(three categories); family history of any cancer or colorectal cancer; smoking history [in four categories of pack-years and according to status (never, past, or current)]; body mass index (BMI; <23, 23–25, or ≥25 kg/m², based on cut-offs suggested for Asians²⁵); waist-to-hip ratio (<0.90 or ≥0.90); regular alcohol consumption (none, <2 drinks/day, or ≥2 drinks/day; “regular” was defined as consuming beer, wine, or spirits, at least three times per week, for more than 6 months continuously); regular exercise participation (“regular” was defined as at least once per week continuously for at least three months within the five years before the baseline interview); regular use of multivitamin supplements; regular use of B vitamin-containing supplements [single B vitamin or multivitamin supplements; no information was available for specific B vitamin(s) taken by participants]; and intakes of total calories (Kcal/day), dietary fiber (g/day), total vegetables (g/day), total fruits (g/day), red meat (g/day), and dietary calcium (mg/day). Plasma CRP level (categorized into quartiles) was positively associated with colorectal cancer risk in this case-control study set²⁶; therefore, it was evaluated as a potential confounder and an effect modifier. Among these potential confounders, the final model included smoking status, income, and plasma CRP level (quartiles), because each of these variables changed the risk estimates for the association between plasma folate and colorectal cancer risk by at least 10%. To test for linear associations, we assigned the median value of plasma folate or dietary folate intake among controls to those in the respective tertile and modeled this variable as continuous in Cox regression models. To further elucidate potential non-linear associations, we conducted logistic regression with restricted cubic splines with four knots placed at the 5th, 35th, 65th, and 95th percentiles of plasma folate concentration and used a Wald test to determine statistical significance.

We conducted analyses stratified by the anatomical site of the cancer (colon or rectum), colorectal cancer stage (stage I/II or stage III/IV²⁷), and years between blood collection and colorectal cancer diagnosis (<4 years or ≥4 years). In addition, we conducted analyses stratified by factors that have previously been reported to affect or modify the association between folate intake and colorectal cancer risk^{14–16,28}: 1) regular alcohol consumption (no vs. yes), 2) smoking status (never vs. past/current smokers), 3) use of B vitamin-containing supplements (none vs. any), 4) use of multivitamin supplements (none vs. any), 5) dietary intakes of nutrients involved in one-carbon metabolism (i.e., riboflavin, vitamin B6, vitamin B12, folate, and methionine; low vs. high, determined by dichotomizing at the median), 6) plasma CRP concentration (low vs. high, determined by dichotomizing at the median), and 7) BMI category (low vs. high, determined by dichotomizing at the median). Finally, we conducted sensitivity analyses by excluding the top 1% of plasma folate concentrations (46.5 ng/mL or higher).

Results

There were 171 colon cancer cases and 117 rectal cancer cases. Among the cases, 94 cases were diagnosed with early-stage colorectal cancer (I and II), 68 with late-stage (III and IV), and 126 cases had no stage information available. Compared with controls, BMI was significantly higher among cases ($P=0.005$) and more cases used multivitamin supplements (10.8% among cases and 6.4% among controls, $P=0.03$). The median plasma folate concentrations were 6.89 ng/mL among cases and 6.81 ng/mL among controls (Table 1).

Among controls, the median folate concentration was higher among never smokers than among smokers (8.12 and 6.13 ng/mL, respectively). Similarly, among controls, those who had taken vitamin B-containing supplements had a higher median folate concentration than those who had not (10.53 and 6.66 ng/mL, respectively). In contrast, the median folate concentration was relatively similar between regular and non-regular alcohol consumers (6.7 and 6.9 ng/mL, respectively).

Overall, plasma folate concentration was associated with a non-significantly elevated risk of colorectal cancer. Odds ratios (OR) and 95% CIs were 1.38 (0.95–2.02) for the middle tertile and 1.33 (0.90–1.98) for the highest tertile of plasma folate concentrations compared with the lowest, with no significant linear trend (P -trend=0.15) (Table 2). Dietary folate intake, assessed through the FFQ, was not significantly associated with the risk of colorectal cancer (OR=1.28, 95% CI=0.87–1.89 for the middle tertile and OR=1.17, 95% CI=0.76–1.82 for the highest tertile of intake compared with the lowest; P -trend=0.29).

When we stratified by cancer stage, a significant positive association was found for the third tertile compared with the first tertile of plasma folate concentrations for late-stage colorectal cancer (OR=2.66, 95% CI=1.03–6.86) with a statistically significant trend (P -trend=0.04), but no association was observed for early-stage colorectal cancer (OR=1.15, 95% CI=0.56–2.38) (Table 3). A similar, although not statistically significant, positive association was observed for both colon and rectal cancers, when assessed separately (OR=1.46, 95% CI=0.88–2.44 for colon cancer, OR=1.16, 95% CI=0.61–2.23 for rectal cancer). The positive association for the middle tertile was statistically significant for cases diagnosed within the first 4 years after blood collection (OR=1.72, 95% CI=1.02–2.92). Among all participants, the test for non-linear association was not statistically significant (P =0.21).

Furthermore, the positive association between the middle tertile of plasma folate concentrations and colorectal cancer risk was statistically significant for the high BMI group (OR=1.88, 95% CI=1.14–3.11), but not for the low BMI group (OR=0.92, 95% CI=0.52–1.62) (Table 4). There was a statistically significant multiplicative interaction between plasma folate and BMI (P -interaction=0.04). Among non-users of multivitamin supplements, a positive, but not statistically significant, association was observed with increasing plasma folate concentration, whereas a non-significant inverse association was observed among multivitamin supplement users, although the test for multiplicative interaction was not significant (P -interaction=0.59). Furthermore, there was no interaction or clear trend for associations between plasma folate and colorectal cancer risk stratified by CRP level, regular alcohol consumption, smoking status, dietary folate intake from foods (Table 4), use of B-vitamin containing supplements, or dietary intakes of riboflavin, vitamin B6, vitamin B12, or methionine (data not shown). Based on our previous reports on inverse associations of total fruit and vegetable intake and tea consumption with colorectal cancer risk in analyses of the entire SMHS cohort,^{29,30} we further adjusted for each of these variables and found essentially no change in the association between plasma folate and colorectal cancer risk (data not shown). Sensitivity analyses conducted by excluding the top 1% of plasma folate concentrations slightly changed the risk estimates, but did not change the statistical significance or directions of the associations (data not shown).

Discussion

In this case-control study nested within the SMHS, we found no overall, statistically significant association between pre-diagnostic plasma folate concentration and risk of colorectal cancer. However, we observed significant positive associations for the highest tertile of folate concentrations among men with late-stage colorectal cancer and for the middle tertile of folate concentrations among men whose blood was collected within 4 years of the colorectal cancer diagnosis and who were in the high BMI group.

Our study does not support a protective effect for folate on colorectal cancer in a population where supplemental folic acid intake is low and the food supply is rarely fortified with this nutrient. This finding is consistent with one previous prospective study conducted in Sweden, where the food supply was not commonly fortified.¹⁶ Furthermore, the observed non-significant, positive association for the highest tertile of plasma folate concentrations compared with the lowest is generally consistent with two previous studies that found a similar association,^{16,31} although not with others.^{13,14,32,33} In our study, the positive association was predominantly seen for late-stage colorectal cancer, cases who were diagnosed within the first 4 years after blood collection, and those with high BMI. These results support the postulated growth-promoting effect of folate on later stages of colorectal carcinogenesis or among individuals with colorectal adenomas.^{11,12} This could be particularly applicable to our study population where colorectal cancer screening is uncommon; hence, cases in our study were more likely to be diagnosed at a later stage or to have had preneoplastic lesions at the time of blood collection than other populations with regular screening for colorectal cancer. This notion is supported by a finding from two large US prospective cohort studies where folate intake 12–16 years before diagnosis was associated with reduced risk.³⁴ Given the relatively short follow-up period of our cohort, the potential time-varying effect of folate on colorectal cancer can be evaluated only when our study accumulates a sufficient follow-up period. In addition, because our study is one of only a few to investigate interactions between plasma folate and BMI in relation to colorectal cancer risk^{15,31} and the first to report a significant interaction effect, this finding needs to be interpreted with caution and needs to be replicated in other populations.

Similar to our study finding, the National Health and Nutrition Examination Survey (NHANES)³⁵ has reported a significant positive association for the second quintile of serum folate compared with the first for all-cancer mortality (site-specific cancer mortality was not reported due to limited sample sizes), suggesting a potential non-linear association. In contrast, a recent meta-analysis of 8 nested case-control studies reported neither linear nor non-linear associations between pre-diagnostic blood folate concentration and colorectal cancer risk.¹⁰ However, that analysis did not stratify by the time interval between the blood draw and the diagnosis or by cancer stage. In our study, despite a significantly increased risk of colorectal cancer for the middle tertile of plasma folate concentrations, the test for non-linear association using restricted cubic splines was not significant. Therefore, the potential non-linear association observed in our study may be a result of the relatively unstable risk estimates due to the small sample size, which did not allow us to further investigate the effect of dose or timing of folate exposure on colorectal cancer. On the other hand, folate plays an important role in DNA methylation, synthesis, repair, and, ultimately,

carcinogenesis.² It is possible that folate's effects on carcinogenesis depend on the dose and timing of exposure to folate. Thus, this potential nonlinear association needs to be replicated in other epidemiological studies and investigated in experimental studies to elucidate the underlying biological mechanisms.

In comparing our study findings with previous findings, differences in folate assays and the implementation of folic acid fortification of the food supply in a given study population need to be addressed. With regard to folate assays, the majority of previous studies^{16,28,32,33} have used radioimmunoassay, whereas our study used microbiological assay, the gold standard for folate measurement.³⁶ NHANES has used both microbiological assay and radioimmunoassay to assess folate status, and close agreement between the two assays was reported, although concentrations appeared to differ.^{36,37} Of note, folate concentrations measured by radioimmunoassay are generally lower than those measured by microbiological assay.^{37,38} After considering assay differences, our median value appears to be relatively similar to those reported in previous studies, in which blood samples were collected prior to the folic acid fortification period.^{31,33} It is worth noting that the aforementioned meta-analysis of 8 studies reported a statistically significant inverse association for studies that used radioimmunoassay, but not for studies that used microbiological assay.¹⁰

In general, evidence on the association between folate and colorectal cancer risk is more consistent for studies that assessed self-reported folate intake than for studies that measured circulating folate.^{3,4} A pooled analysis of 13 cohort studies reported a statistically significant 13% lower risk of colon cancer for total folate intake of ≥ 560 $\mu\text{g/day}$ compared with <240 $\mu\text{g/day}$ (combined dietary intake and supplements),³ which generally corroborates meta-analysis results.^{4,5} However, when we considered dietary folate intake from foods, we found no statistically significant association of risk with folate intake. Furthermore, we found no clear difference in associations for plasma folate concentrations stratified by dietary folate intake. Two recent analyses from prospective cohort studies in the United States have assessed the association between folate intake at different time periods and colorectal cancer risk, covering the periods before and after folic acid fortification was initiated.^{34,39} One of the analyses reported a significant inverse association for intake at 12–16 years before diagnosis, but not for intakes within the last 12 years before diagnosis.³⁴ In contrast, another analysis observed a similar inverse association for folate intake assessed both before and after folate fortification, although only the association for post-fortification folate intake was borderline significant.³⁹ The post-fortification association covers an approximate time period of the last 12 years before colorectal cancer diagnosis. Thus, further investigation is warranted to clarify how the timing of the folate intake assessment affects its association with colorectal cancer risk.

The strengths of our study include the use of microbiological assay, which is considered to be the gold standard for measurement of plasma folate concentration.³⁶ In addition, we used prospectively-collected blood samples and exposure information in a well-characterized cohort study. Our study is the largest study of circulating folate concentration and colorectal cancer risk conducted among men in Asia ($N=288$). Given that the use of B vitamin-containing supplements and the fortification of the food supply with folic acid were uncommon during both study enrollment and the follow-up period in our study population,

the folate concentrations measured in the blood samples collected at baseline may reflect folate status during the follow-up period better than previous studies, in which fortification was initiated after the baseline blood sample collection.¹⁵ Nonetheless, our study collected only one blood sample per participant at baseline. It is possible that plasma folate level in free-living individuals could have changed over time. Given the prospective nature of our study, the misclassification of folate concentration is most likely non-differential, which may have attenuated the true association between folate concentration and colorectal cancer risk. Genetic polymorphisms in the *MTHFR* gene are known to influence plasma folate level.⁴⁰ However, genotyping information on *MTHFR* was available only for a limited case-control set of participants; thus, it was not included in the current analysis. Our study had limited power, especially for the analyses of non-linear associations and stratified and joint associations; thus, our findings from these analyses need to be interpreted with caution and need to be re-evaluated once more colorectal cancer cases have accrued and be replicated in other populations.

In summary, our results suggest that pre-diagnostic plasma folate concentration may be related to increased risk for colorectal cancer, particularly among individuals who are likely to have preneoplastic lesions. Our study adds to the evidence supporting the importance of considering the timing of folate assessment and preneoplastic lesions when investigating the folate and colorectal cancer association.

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Abbreviations used

BMI	body mass index
CI	confidence interval
CRP	C-reactive protein
FFQ	food frequency questionnaire
NHANES	National Health and Nutrition Examination Survey
OR	odds ratios
SMHS	Shanghai Men's Health Study

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Novelty and Impact

This study, conducted in a population where the predominant source of folate is from unfortified foods, adds to the evidence highlighting the importance of considering the timing of folate assessment and pre-existing colorectal conditions when investigating the folate and colorectal cancer association.

Table 1Characteristics of the study population by colorectal cancer status^{*}

	Cases	Controls	P-difference
Number	288	575	-
Age at baseline (years)	63.1 ± 8.7	63.4 ± 8.6	0.65
Smoking status			
Never smokers	105 (36.5%)	226 (39.3%)	
Past smokers	42 (14.5%)	110 (19.1%)	
Current smokers	141 (49.0%)	239 (41.6%)	0.08
Regular alcohol consumption			
None	196 (68.1%)	397 (69.0%)	
Moderate (<2 drinks/day)	54 (18.7%)	113 (19.7%)	
High (≥ 2 drinks/day)	38 (13.2%)	65 (11.3%)	0.71
Supplement use			
Multivitamin supplements	31 (10.8%)	37 (6.4%)	0.03
Single B vitamin supplements	17 (5.9%)	32 (5.6%)	0.84
NSAID use	38 (13.2%)	59 (10.3%)	0.20
Family history of colorectal cancer	8 (2.8%)	8 (1.4%)	0.15
Personal history of colorectal polyps	6 (2.1%)	6 (1.0%)	0.22
BMI (kg/m ²)	24.5 ± 3.4	23.8 ± 3.0	0.005
Regular exercise ^{**}	140 (48.6%)	309 (53.7%)	0.16
Total caloric intake (Kcal/day)	1915 ± 447	1864 ± 503	0.13
Riboflavin intake (mg/day)	0.95 ± 0.3	0.94 ± 0.3	0.54
Vitamin B6 intake (mg/day)	1.8 ± 0.6	1.7 ± 0.6	0.16
Folate intake (μg/day)	343 ± 113	343 ± 130	0.95
Vitamin B12 intake (μg/day)	2.55 ± 1.5	2.50 ± 1.5	0.66
Methionine intake (g/day)	1.7 ± 0.5	1.6 ± 0.6	0.14
Plasma CRP (mg/L)	2.07 ± 3.07	1.59 ± 3.57	0.04
Plasma folate (ng/mL)	10.0 ± 15.4	9.1 ± 7.7	0.34

* Shown as mean ± standard deviation or frequency

** Defined as at least once per week continuously for at least three months within the five years before the baseline interview

Table 2

Association between folate concentration or dietary folate intake and colorectal cancer risk

	Tertile 1	Tertile 2	Tertile 3	P-trend
Plasma folate *				
	(<5.59 ng/mL)	(5.59 – <8.50 ng/mL)	(8.50 ng/mL)	
Number of cases/controls	83/192	108/192	97/191	
OR (95% CI)	Reference	1.38 (0.95–2.02)	1.33 (0.90–1.98)	0.15
Dietary folate intake **				
	(<278.8 µg/day)	(278.8 – 396.6 µg/day)	(>396.6 µg/day)	
Number of cases/controls	83/192	103/192	102/191	
OR (95% CI)	Reference	1.28 (0.87–1.89)	1.17 (0.76–1.82)	0.29

* Adjusted for smoking status, income, and CRP category

** Adjusted for smoking status, income, CRP category, and total caloric intake

Table 3

Association of folate concentration and colorectal cancer risk stratified by tumor characteristics (tumor site and stage) and years from blood collection to diagnosis*

	Plasma folate			<i>P</i> -trend
	Tertile 1 <5.59 ng/mL	Tertile 2 5.59–8.50 ng/mL	Tertile 3 8.50 ng/mL	
By tumor site				
Colon				
Cases/controls	49/111	57/114	65/116	
OR (95% CI)	Reference	1.25 (0.75–2.07)	1.46 (0.88–2.44)	0.15
Rectum				
Cases/controls	34/81	51/78	32/75	
OR (95% CI)	Reference	1.55 (0.85–2.82)	1.16 (0.61–2.23)	0.61
By tumor stage				
Stage I or II				
Cases/controls	28/93	36/94	30/104	
OR (95% CI)	Reference	1.60 (0.79–3.25)	1.15 (0.56–2.38)	0.70
Stage III or IV				
Cases/controls	17/82	21/73	30/71	
OR (95% CI)	Reference	1.76 (0.72–4.29)	2.66 (1.03–6.86)	0.04
By years from blood collection to diagnosis				
4 years				
Cases/controls	44/108	61/100	58/117	
OR (95% CI)	Reference	1.72 (1.02–2.92)	1.28 (0.75–2.20)	0.34
>4 years				
Cases/controls	39/84	47/92	39/74	
OR (95% CI)	Reference	1.15 (0.66–2.02)	1.35 (0.74–2.45)	0.33

* Adjusted for smoking status, income, and CRP category; 29 cases missing tumor stage information and their matched controls were excluded

Table 4

Associations between folate concentration and colorectal cancer risk stratified by CRP, smoking status, alcohol consumption, dietary folate intake, multivitamin supplement use, and BMI*

	Plasma folate				
	Tertile 1 (<5.59 ng/mL)	Tertile 2 (5.59 – <8.50 ng/mL)	Tertile 3 (8.50 ng/mL)	P-trend	P-interaction
By CRP level					
Low CRP (<0.752 mg/L)					
Cases/controls	27/92	35/96	36/100		
OR (95% CI)	Reference	1.31 (0.73–2.34)	1.39 (0.74–2.59)	0.29	
High CRP (0.752 mg/L)					
No. of Cases/controls	56/100	73/96	61/91		
OR (95% CI)	Reference	1.38 (0.86–2.22)	1.22 (0.74–2.01)	0.42	0.89
By smoking status					
Never smokers					
Cases/controls	17/46	44/77	44/103		
OR (95% CI)	Reference	1.62 (0.81–3.24)	1.28 (0.65–2.54)	0.69	
Past/current smokers					
Cases/controls	66/146	64/115	53/88		
OR (95% CI)	Reference	1.26 (0.81–1.97)	1.44 (0.89–2.34)	0.13	0.59
By regular alcohol consumption					
No					
Cases/controls	55/126	75/126	66/140		
OR (95% CI)	Reference	1.48 (0.94–2.35)	1.28 (0.81–2.04)	0.31	
Yes					
Cases/controls	28/66	33/66	31/51		
OR (95% CI)	Reference	1.20 (0.63–2.29)	1.52 (0.76–3.04)	0.24	0.63
By dietary folate intake from foods					
Low folate intake (<372.2 µg/day)					
Cases/controls	39/98	51/102	43/88		
OR (95% CI)	Reference	1.27 (0.75–2.15)	1.27 (0.74–2.19)	0.37	
High folate intake (372.2 µg/day)					

Plasma folate					
	Tertile 1 (<5.59 ng/mL)	Tertile 2 (5.59 – <8.50 ng/mL)	Tertile 3 (8.50 ng/mL)	P-trend	P-interaction
Cases/controls	44/94	57/90	54/103		
OR (95% CI)	Reference	1.52 (0.89–2.57)	1.39 (0.81–2.37)	0.24	0.89
None		By multivitamin supplement use			
Cases/controls	79/189	106/187	72/162		
OR (95% CI)	Reference	1.41 (0.96–2.06)	1.20 (0.78–1.84)	0.31	
Any					
Cases/controls	4/3	2/5	25/29		
OR (95% CI)	Reference	0.44 (0.04–4.74)	0.95 (0.19–4.71)	0.87	0.59
BMI <23.9 kg/m ²		By BMI category			
Cases/controls	39/94	37/99	53/97		
OR (95% CI)	Reference	0.92 (0.52–1.62)	1.43 (0.82–2.49)	0.43	
BMI ≥23.9 kg/m ²					
Cases/controls	44/98	71/93	44/94		
OR (95% CI)	Reference	1.88 (1.14–3.11)	1.21 (0.70–2.08)	0.18	0.04

* Conditional logistic regression adjusted for smoking status (current smoking for interaction with smoking), income, and CRP category and further adjusted for total caloric intake for a joint association with dietary folate intake