Systematic Reviews and Meta- and Pooled Analyses

Quantifying the Dose-Response Relationship Between Circulating Folate Concentrations and Colorectal Cancer in Cohort Studies: A Meta-Analysis Based on a Flexible Meta-Regression Model

Shu-Chun Chuang*, **Matteo Rota, Marc J. Gunter, Anne Zeleniuch-Jacquotte, Simone J.P.M. Eussen, Stein Emil Vollset, Per Magne Ueland, Teresa Norat, Regina G. Ziegler, and Paolo Vineis**

* Correspondence to Dr. Shu-Chun Chuang, Division of Health Policy Translation, Institutes of Population Health Sciences, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County, 35053 Taiwan (e-mail: scchuang@nhri.org.tw).

Most epidemiologic studies on folate intake suggest that folate may be protective against colorectal cancer, but the results on circulating (plasma or serum) folate are mostly inconclusive. We conducted a meta-analysis of case-control studies nested within prospective studies on circulating folate and colorectal cancer risk by using flexible meta-regression models to test the linear and nonlinear dose-response relationships. A total of 8 publications (10 cohorts, representing 3,477 cases and 7,039 controls) were included in the meta-analysis. The linear and nonlinear models corresponded to relative risks of 0.96 (95% confidence interval (CI): 0.91, 1.02) and 0.99 (95% CI: 0.96, 1.02), respectively, per 10 nmol/L of circulating folate in contrast to the reference value. The pooled relative risks when comparing the highest with the lowest category were 0.80 (95% CI: 0.61, 0.99) for radioimmunoassay and 1.03 (95% CI: 0.83, 1.22) for microbiological assay. Overall, our analyses suggest a null association between circulating folate and colorectal cancer risk. The stronger association for the radioimmunoassay-based studies could reflect differences in cohorts and study designs rather than assay performance. Further investigations need to integrate more accurate measurements and flexible modeling to explore the effects of folate in the presence of genetic, lifestyle, dietary, and hormone-related factors.

circulating folate; colorectal cancer; dose-response relationship

Abbreviations: CI, confidence interval; CRC, colorectal cancer; PLP, pyridoxal 5′-phosphate; RR, relative risk; THF, tetrahydrofolic acid.

Worldwide, colorectal cancer (CRC) is the third most common cancer in men and the second most common in women, and in the developed countries it is the second most common cause of death from cancer (1). However, the etiology of CRC is poorly understood. Observational studies have shown that modifiable lifestyle factors, such as smoking, alcohol drinking, physical activity, obesity, and diet, are associated with CRC risk, but each seems to explain only a small proportion of the total CRC incidence (2).

Most epidemiological studies (prospective and retrospective) report an inverse linear association between folate intake and CRC (3). In 2007, the World Cancer Research Fund (London, United Kingdom) released a report stating that "there is limited evidence suggesting that foods containing folate protect against CRC" (4, p. 284). Folate plays a pivotal role in the 1-carbon metabolism pathway and has been hypothesized to be associated with CRC risk through at least 2 distinct mechanisms, including DNA hypomethylation and subsequent proto-oncogene activation and uracil misincorporation during DNA synthesis, leading to DNA instability (5).

However, the associations between folate and CRC have been inconsistent in prospective studies that use circulating (serum or plasma) folate (6–12) and in clinical trials of colorectal adenoma (13–17). For example, in the Aspirin/Folate Polyp Prevention Study (13), participants with recent histories of colorectal adenoma were randomized into groups that received 1 mg/day of folic acid or placebo. During 6 years of follow-up, the relative risks for any adenoma and advanced

adenoma were 1.13 (95% confidence interval (CI): 0.93, 1.37) and 1.67 (95% CI: 1.00, 2.80), respectively. Such associations may imply that folate is involved in CRC progression. Nevertheless, the other 3 large-scale trials showed null associations in general (14, 15, 17).

Whereas folate deficiency could increase cancer risk, folate supplementation could promote the progression of existing cancerous or precancerous lesions (18). Indirect evidence relating folate to CRC includes a reversal of the downward trend in CRC rates in the United States, Canada, and Chile after the introduction of mandatory folic acid fortification of flour (19, 20). On this basis, it was suggested that the cancer prevention effect of folate is time- and dose dependent.

Although there have been several review articles on folate intake and CRC risk, there has been no systematic evaluation of studies that have investigated the association of circulating folate with CRC development. Circulating folate is likely to be a valid marker that reflects folate intake and folate available to tissues (21); hence, the circulating form of folate may be a better indicator than intake for evaluating the association between folate and CRC; however, results from studies of circulating folate have been inconclusive.

There have been discussions on the interchangeability between radioimmunoassay and microbiological assay for folate. The radioimmunoassay is precise, but there are accuracy problems. Although the microbiological assay detects most biologically active folate species with comparable sensitivity, the radioimmunoassay binds different folate species

with different affinities (22). The differences in measurements could also contribute to the inconclusive observations.

Given that folate has been postulated to both reduce and increase the risk of CRC, the identification of U-shaped curves may be necessary. Because cancer development and treatment may influence the blood levels of biomarkers, we sought to address the question by using the results from case-control studies nested within prospective cohort studies of circulating folate.

MATERIALS AND METHODS

Search strategy and selection criteria

We conducted a literature search of the PubMed database until February 2012. The search was restricted to English-language articles and human studies and used the following search terms: "folate" and "colorectal cancer" in the abstract and title. We also checked the reference lists of articles retrieved from the PubMed search. Studies were included in the meta-analysis if they met the following criteria: case-control studies nested within a prospective cohort study; the exposure of interest was circulating (plasma or serum) levels of folate; and the outcome of interest was colorectal, colon, or rectal cancer. When several publications were available from the same study, the most recent publication, or the one including the largest number of subjects, was included. Figure 1 illustrates the study search and selection process.
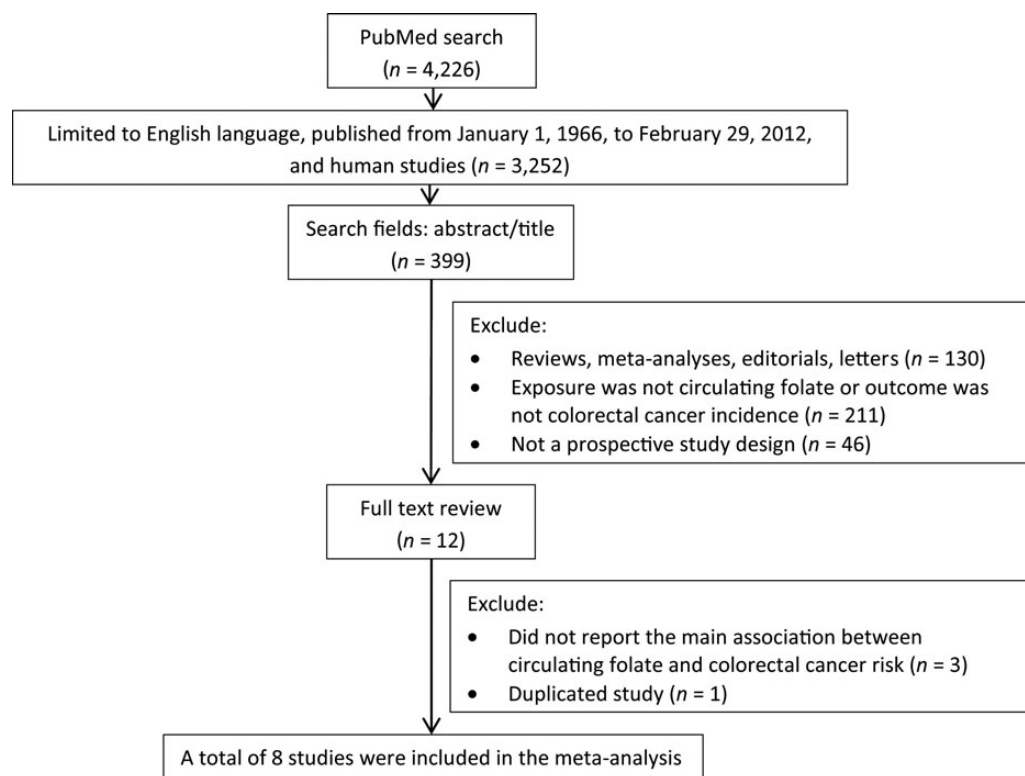

Figure 1. Flow chart of the study search and selection.

Table 1. Summary Characteristics of the 8 Nested Case-Control Studies on Circulating Folate Concentrations and CRC Risks Included in the Analyses

First Author, Year (Reference No.)	Cohort	Years at Recruitment	Sex	Age, years	Cohort Size	No. of Cases	No. of Controls	Biological Sample	Assay Type	Circulating Folate Range in Controls, nmol/L[a]	Percentile Range	Matching Variables	Conditional Logistic Regression Adjusted for
Eussen, 2010 (11)	EPIC	1992–1998	Female, male	25–70	520,000	1,367	2,325	Plasma	MA	4.9–34.0	5%, 95%	Age, sex, study centers, and date of blood collection	Smoking status, educational level, physical activity, fiber intake, red and processed meat intakes, alcohol consumption, and BMI[b]
Kato, 1999 (6)	NYUWHS	1985–1991	Female	35–65	15,785	105	523	Serum	RIA	12.2–31.0	25%, 75%[c]	Age, menopausal status at enrollment, date of enrollment, and date of subsequent blood donation[d]	Family history of CRC, beer intake, prior occult blood testing, and number of hours spent in sport activities in their early 30s
Le Marchand, 2009 (31)	MEC	2001–2006	Female, male	45–75	67,594	224	411	Plasma	RIA	23.8–63.7	25%, 75%	Sex, birth year, race/ethnicity, location, date of blood draw, time of blood draw, and hours of fasting prior to blood draw	Hours of moderate or vigorous physical activity, intake of processed meat, pack-years of smoking, BMI, ethanol intake, family history of CRC, and history of CRC screening
Lee, 2012 (12)	HPFS	1993–1995	Male	40–75	18,225	173	345	Plasma	RIA	5.4–32.0[e]	6%, 94%	Age, month, and year of blood collection	BMI, family history of CRC, physical activity, pack-years of smoking, aspirin use, height, history of endoscopy, and fasting status
Lee, 2012 (12)	NHS	1989–1990	Female	30–55	32,826	189	377	Plasma	RIA	7.2–49.5[e]	6%, 94%	Age, month and year of blood collection, and fasting status	BMI, family history of CRC, physical activity, pack-years of smoking, postmenopausal hormone use, aspirin use, height, and history of endoscopy

Table continues

Table 1. Continued

First Author, Year (Reference No.)	Cohort	Years at Recruitment	Sex	Age, years	Cohort Size	No. of Cases	No. of Controls	Biological Sample	Assay Type	Circulating Folate Range in Controls, nmol/L[a]	Percentile Range	Matching Variables	Conditional Logistic Regression Adjusted for
Lee, 2012 (12)	PHS	1982–1984	Male	40–84	14,916	240	408	Plasma	MA	4.2–30.1[e]	6%, 94%	Age and smoking status	BMI, physical activity, fasting status, and aspirin assignment
Otani, 2008 (8)	JPHC[f]	1990–1995	Female, male	40–69	133,323	375	750	Plasma	RIA	13.1–20.1 (men); 15.4–24.7 (women)	25%, 75%	Age, sex, date of blood drawn, time since last meal, and study location	Pack-years of smoking, alcohol consumption, BMI, physical exercise, any vitamin supplement use, and family history of CRC
Shrubsole, 2009 (10)	SWHS	1996–2000	Female	40–70	74,942	303	1,188	Plasma	MA	4.8–709.3	0%, 100%	Age at baseline, date and time of blood collection, interval since last meal, menopausal status, and antibiotic use in the past week	Educational attainment, baseline household income, smoking status, drinking status, physical activity, hormone replacement therapy, menopausal status, family history of CRC, BMI, NSAID use, use of a B-vitamin supplement, history of colorectal polyps, history of diabetes, and dietary intakes of energy, vegetables, fruits, red meats, and calcium
Van Guelpen, 2006 (7)	NSHDC[g]	1985–2002	Female, male	25–74	85,000	226	437	Plasma	RIA	5.8–11.1	25%, 75%	Age, sex, subcohort, date of health survey, and fasting status at sample donation	BMI[e], current smoking, recreational and occupational physical activity, and alcohol intake

Table continues

Table 1.   Continued

First Author, Year (Reference No.)	Cohort	Years at Recruitment	Sex	Age, years	Cohort Size	No. of Cases	No. of Controls	Biological Sample	Assay Type	Circulating Folate Range in Controls, nmol/L[a]	Percentile Range	Matching Variables	Conditional Logistic Regression Adjusted for
Weinstein, 2008 (9)	ATBC	1985–1988	Male	50–69	29,133	275	275	Serum	RIA	6.4–10.8	25%, 75%	Age at randomization and date of blood draw	BMI, occupational and leisure physical activity, and intakes of vitamin D and iron

Abbreviations: ATBC, Alpha-Tocopherol Beta-Carotene Cancer Prevention Study; BMI, body mass index; CRC, colorectal cancer; EPIC, European Prospective Investigation Into Cancer and Nutrition; HPFS: Health Professionals Follow-up Study; JPHC, Japan Public Health Center–Based Prospective Study; MA, microbiological assay; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; NSAID, nonsteroidal antiinflammatory drug; NSHDC, Northern Sweden Health and Disease Cohort; NYUWHS, New York University Women's Health Study; PHS, Physicians' Health Study; RIA, radioimmunoassay; SWHS, Shanghai Women's Health Study.

[a] All studies that reported ng/mL were converted into nmol/L by a factor of 7/3 (27).

[b] Body mass index is weight (kg)/height (m)².

[c] Cases and controls combined.

[d] Premenopausal subjects were also matched by day and phase of menstrual cycle at enrollment.

[e] The lower and upper bounds of the interquartile range of the lowest and highest quartiles, respectively.

[f] The JPHC comprised 2 cohorts, 1 of which recruited subjects aged 40–59 years in 1990 and collected samples between 1990 and 1992, and the other of which recruited subjects aged 40–69 years in 1993 and collected samples between 1993 and 1995 (8).

[g] The NSHDC comprised the following 3 cohorts: Monitoring Trends and Determinants in Cardiovascular Disease (MONICA), which recruited random subjects aged 25–74 years living in Västerbotten (n = 2,000) and Norrbotten (n = 2,500), Sweden, in 1986; the Västerbotten Intervention Program, which invited all subjects living in Västerbotten to a health survey when they were turning 40, 50, or 60 years of age in 1985; and the Managed Support Program, which invited all women living in Västerbotten and aged 50–70 years to undergo mammography in 1995 (7).

Data extraction

The following data were extracted from each study: the first author's last name; publication year; name of the cohort; years of recruitment and blood collection; participants' sex and age; sample sizes of cases, controls, and cohort sizes, both overall and in each exposure stratum (contacting corresponding authors if this information was missing); matching criteria; biological samples; circulating folate assay; range of circulating folate in the controls; variables adjusted for in the analyses; and the relative risk estimates with the corresponding 95% confidence intervals for each category of circulating folate levels.

Statistical analysis

We used a flexible meta-regression model, which provides the best fitting 2-term fractional-polynomial model, to test a linear or a nonlinear dose-response relationship between circulating folate concentrations and CRC risk. The statistical methods used for this analysis are described in detail elsewhere (23, 24). In brief, this approach takes into account the correlation within the same study among reported dose-specific log (relative risk) estimates due to the common reference group, the heterogeneity among studies, and the nonlinear trend component of the dose-response relationship. For each study, the midpoint/median level of circulating folate for each category, except the highest, and a level 1.2 times the lower cut point of the highest category were assigned to each corresponding relative risk estimate (25). The best fitting model is defined as the one with the smallest Akaike's Information Criterion. Because folate levels never reach null values, we investigated the relationship between folate levels and CRC risk on the basis of the contrast of each folate level with the reference category within a study (26). All circulating folate values were converted to nmol/L (27). The heterogeneity among the studies was tested with the Q statistic (28) by using the linear trend estimates.

The statistical analyses were repeated by converting the exposure levels using the equation provided by Fazili et al. (29) to improve the comparability of the measurements among studies. We also combined the study-specific relative risks, comparing the highest with the lowest category, with an assumption that the measurement error due to interlab variation in absolute concentrations would be less likely to influence a comparison based on study-specific quantiles. The degree of heterogeneity was estimated by using the I^2 statistic, which represents the percentage of total variation contributed by between-study variance (28).

RESULTS

A total of 399 publications were retrieved from the PubMed search. After careful filtering and checking, we identified 9 potentially relevant articles concerning circulating folate in relationship to the risk of CRC from case-control studies nested within prospective cohort studies. One publication (30) was excluded because of a later report with longer follow-up (9) from the same study population. One publication included results for 3 distinct cohorts (the Nurses' Health Study, the Health Professionals Follow-up Study, and the Physicians' Health Study). Therefore, the meta-analysis on the dose-response

relationship between circulating folate and CRC includes results for 10 cohorts from 8 publications (Table 1). Almost all publications appeared after 2006, but only 1 cohort in the United States, the Multiethnic Cohort Study, collected blood after 1998 (i.e., after the folic acid fortification policy was implemented). The 10 cohorts contributed 3,477 cases and 7,039 controls, and the European Prospective Investigation into Cancer and Nutrition cohort alone contributed more than one-third (35.8%) of the cases and controls.

Figure 2 presents the cohort-specific dose-risk functions. Because no heterogeneity among studies was detected ($Q = 8.53$, $P = 0.58$), a fixed-effect model was used in further analyses. Under a linear fixed-effect model, we observed a non–statistically significant weak inverse dose-response relationship between circulating folate concentrations and CRC risk (relative risk (RR) = $\exp(-0.00375 \cdot x)$, Akaike's Information Criterion = -58.4 (Figure 3A), where x = circulating folate in contrast to the reference value). A stronger, but still non–statistically significant inverse association was observed for the studies that used radioimmunoassay (RR = $\exp(-0.00599 \cdot x)$, Akaike's Information Criterion = -39.0 (Figure 3B), which corresponds to a relative risk of 0.94 (95% CI: 0.88, 1.01) per 10 nmol/L of circulating folate in contrast to the reference value).

Because of the potential reversal in the risk estimates at high folate levels, a nonlinear fixed-effect model was also fitted. The fixed-effect model with power terms p1 = 3 and p2 = 2 presented the lowest Akaike's Information Criterion value (-253.4) among the estimable second-order fractional polynomial models tested. The equation of the best fitting model was a relative risk of $\exp(-1.44\mathrm{E}-7 \cdot x^3 - 0.00011 \cdot x^2)$. The estimated summary relative risk for CRC obtained with the best-fitting fractional polynomial model was 0.99 (95% CI: 0.96, 1.02) per 10 nmol/L of circulating folate levels in contrast to the reference value. Interestingly, there seemed to be a reversal of the weak inverse association noted at lower concentrations at approximately 14 nmol/L (lowest RR = 0.92, 95% CI: 0.75, 1.13) if we restrict the analysis to the 3 studies that used the microbiological assay. However, the estimation was based on only 3 studies, and each of them had a limited range of circulating folate (<20 nmol/L), thus leading to imprecise estimates at high values.

In sensitivity analyses based on the linear models, the exclusion of the Multiethnic Cohort Study, which was conducted in the post–folic acid fortification era (RR = 0.98, 95% CI: 0.92, 1.05 per 10 nmol/L in contrast to the reference value), or the European Prospective Investigation into Cancer and Nutrition Study, which contributed the largest proportion of cases and controls (RR = 0.97, 95% CI: 0.91, 1.03 per 10 nmol/L), did not change the estimates.

After the correction of measurements from different assays by using the equation provided by Fazili et al. (29), we obtained results similar to those without the correction (per 10 nmol/L in contrast to the reference value, RR = 0.97, 95% CI: 0.93, 1.01 (Figure 3A); and RR = 0.99, 95% CI: 0.97, 1.02 (Figure 3C)).

The combined relative risk comparing the highest with the lowest category was 0.91 (95% CI: 0.77, 1.05) (Figure 4). There was a stronger and statistically significant inverse association between circulating folate and CRC risk in studies that used the radioimmunoassay (RR = 0.80, 95% CI: 0.61, 0.99).

DISCUSSION

The findings from our meta-analysis of nested case-control studies with prospectively collected blood specimens indicate a null association between circulating folate and CRC risk. Furthermore, there is no evidence that a very high circulating folate concentration is associated with increased CRC risk in the nonlinear model. Nevertheless, when analysis is restricted to studies that used microbiological assay, there seems to be an increased risk at high levels of circulating folate. However, this observation was based on few studies, and the ranges used for the estimation were usually limited. Further studies are warranted.

In most of the studies included in this meta-analysis, the highest values of circulating folate were lower than 40 nmol/L, except in the Multiethnic Cohort Study (31), which was the only study in our analyses that collected blood samples after folic acid fortification was initiated in the United States in 1998. In the nationally representative National Health and Nutrition Examination Survey of the US population, conducted during 1988–1994, only 7% of participants had serum folate concentrations higher than 45.3 nmol/L; however, the percentage had increased to 38% in the 1999–2000 survey (32).

If folate does play a role in CRC prevention, the observed null association of circulating folate levels with CRC could be caused by the relatively narrow range of folate concentrations within the single studies, as well as the relatively low highest absolute concentrations, to provide a meaningful risk estimate in the study populations that were not exposed to mandatory folic acid fortification policy. Further, the impact of the narrow range in our study is the imprecise risk estimates at high concentrations (e.g., what we observed in the studies that used microbiological assay). As a result, the interpretation at >20 nmol/L must be cautious. Inclusion of postfortification blood samples may be helpful to expand the range of circulating folate.

Moreover, folate is not stable in serum and plasma and is therefore easily affected by storage and processing factors. For example, circulating folate could have been degraded ~60% after being stored at −25°C for ~30 years (33). Further, microbiologically active folate was degraded in room temperature at a rate of 0.16% per hour in serum, 1.61% in ethylenediaminetetraacetic acid, 0.08% in heparin, and 0.11% in citrate plasma in the first 24 hours after collection (34). Such instability can lead to artificially low absolute levels, making accurate quantification of exposure difficult and substantially attenuating the association between folate status and study outcomes. We are not able to address the effect of degradation in the current meta-analysis because the folate degradation kinetics during blood processing and storage vary by study, possibly for individual samples within the same study, and by folate forms in the individual samples. Interestingly, the 2 studies (6, 31) that showed the strongest inverse association had relative shorter storage times (i.e., years since recruitment to publication). However, another 2 studies with similar storage times, the Northern Sweden Health and Disease Cohort (7), for which the mean follow-up was 4.2 years for the cases but the recruitment covered a long period (1985–2002), and the Shanghai Women's Health Study (10), showed no association between circulating folate and CRC risk.

Theoretically, the absolute folate concentrations also vary by assay method, with the microbiological assay producing
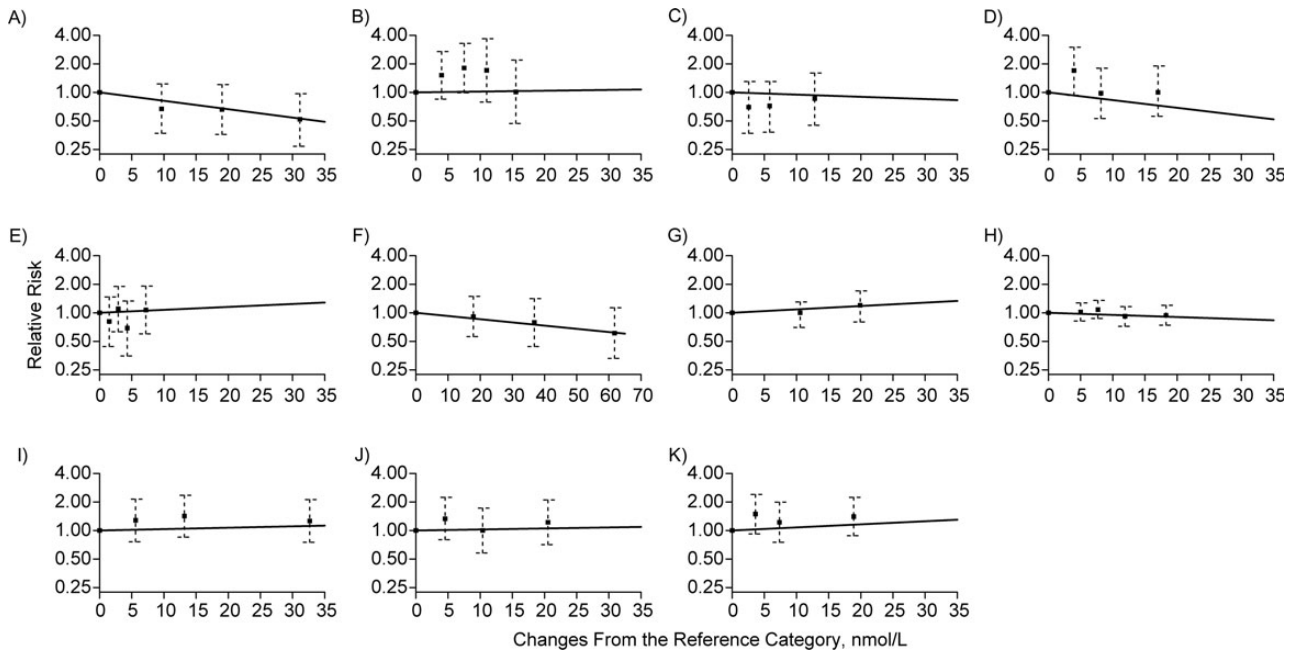
Figure 2. Black squares indicate relative risk estimates, and dotted lines are 95% confidence intervals. Lines indicate the predicted risk with the linear fixed-effects model. The vertical axis is on a log scale. The absolute concentrations (in nmol/L) used for the modeling at each category were as follows: A) 6, 16, 25, and 37 for Kato (6); B) 2, 6, 10, 13, and 18 for Van Guelpen (7); C) 11, 14, 17, and 24 for Otani (men) (8); D) 13, 17, 21, and 30 for Otani (women) (8); E) 6, 7, 9, 10, and 13 for Weinstein (9); F) 12, 29, 48, and 73 for Le Marchand (31); G) 11, 22, and 31 for Shrubsole (10); H) 4, 9, 12, 16, and 22 for Eussen (11); I) 9, 14, 22, and 41 for Lee (Nurses' Health Study) (12); J) 7, 11, 17, and 27 for Lee (Health Professionals Follow-up Study) (12); and K) 6, 10, 13, and 25 for Lee (Physicans' Health Study) (12).

higher readings than the radioimmunoassay. A major reason for this is that the radioimmunoassay only partially recovers 5-methyltetrahydrofolic acid (5CH$_3$THF), the main circulating

form of folate (29, 35). Because radioimmunoassay suffers from more severe measurement error, we expected stronger inverse association after correction for measurement error or
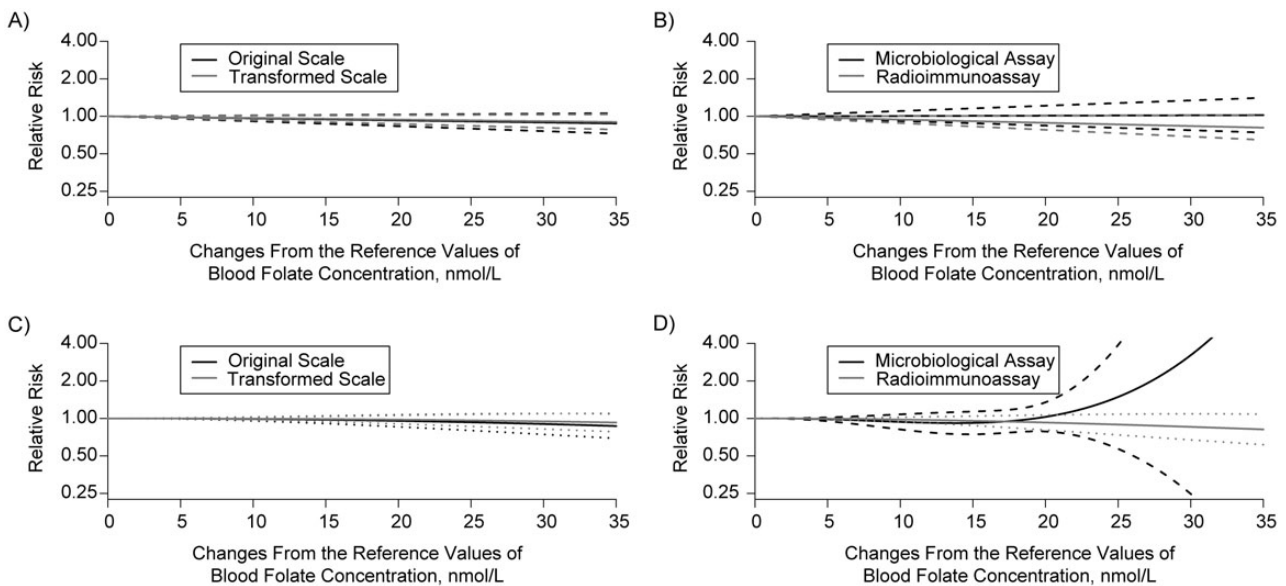
Figure 3. The dose-response relationships between circulating folate and colorectal cancer risk. A) Linear fixed-effect model, B) linear fixed-effect model by assay, C) nonlinear fixed-effect model, and D) nonlinear fixed-effect model by assay. The dashed lines represent 95% confidence intervals.
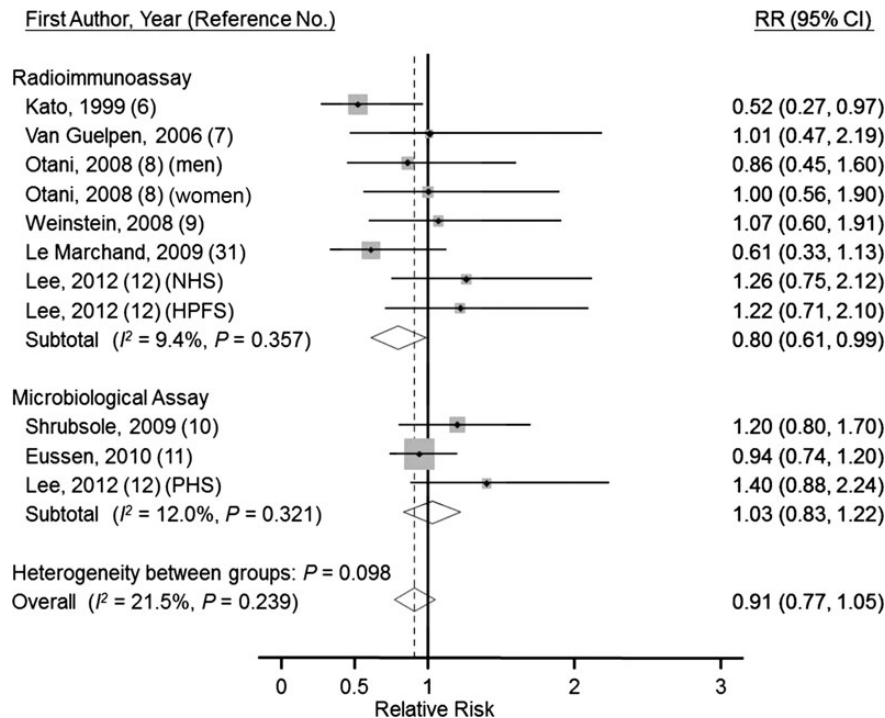
Figure 4. The association between circulating folate and colorectal cancer risk comparing the highest with the lowest category of circulating folate. The vertical dashed line represents the combined relative risk (RR). Solid diamonds are centered on the RR point estimates. The horizontal line through each diamond represents the 95% confidence interval (CI). The area of each gray square represents the relative weight of the study in the meta-analysis. The combined-effect estimate and its 95% CI are represented by a hollowed diamond and its horizontal tips. HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; PHS, Physicians' Health Study.

in studies that used the microbiological assay. We partially corrected the measurement error caused by assay method by adopting the equation provided by Fazili et al. (29) for calibrating the specific radioimmunoassay kit used in the National Health and Nutrition Examination Survey and repeated the meta-analyses. However, the correction did not change the results noticeably. A comparison of the summary relative risk associated with the highest category of folate status relative to the lowest category with a Forest plot provided the same conclusion (i.e., a null association) (Figure 4). Interestingly, we observed a statistically significant inverse association between circulating folate and CRC risk in the studies that measured folate by radioimmunoassay. Whereas the laboratory measurement error is more likely to be nondifferential, the grouping of continuous exposure data into categories may result in differential misclassification (36). Because the degradation rate can be different by folate species (33, 34), the selective underrecovery of 5CH$_3$THF by radioimmunoassay complicated the prediction of the direction of misclassification by assay.

It has been suggested that the methylenetetrahydrofolate reductase gene *677T* allele is associated with a reduced CRC risk (37). Methylenetetrahydrofolate reductase mediates the conversion of 5,10-methylenetetrahydrofolate (5,10-CH = THF), which is required for DNA synthesis and repair, into 5CH$_3$THF, which is involved in DNA methylation, and the enzyme activity decreases with the increase of the *T* allele of

the polymorphism (38). Therefore, some studies have suggested that the inverse association of the methylenetetrahydrofolate reductase gene *677T* allele and CRC risk could be caused by an increase of 5,10-CH = THF levels for DNA synthesis and repair (39, 40). The recently developed liquid chromatography–tandem mass spectrometry detects a spectrum of folate species (29) and may help to partition the effects from DNA synthesis/repair or methylation. In contrast to the null associations reported in studies that used circulating folate as the main exposure, a pooled analysis from 13 cohorts reported a statistically significant inverse association between folate intake and CRC risk with a linear dose-response relationship (41). Because previous dose-response controlled trials have shown increasing serum folate levels with folic acid dose (P_{trend} < 0.001) (21, 42), we expected a similar association for similar ranges of exposure. Interestingly, similar contradictory results were also reported for vitamin B6 intake and circulating levels of pyridoxal 5′-phosphate (PLP), which is the coenzyme form of vitamin B6 (43). That is, circulating PLP was inversely associated with CRC risk, but vitamin B6 intake was not associated (or weakly associated after excluding the influential study) with CRC. The arguments to explain these discrepancies include the fact that PLP is not identical to dietary vitamin B6 (44), and that low PLP may reflect low-grade inflammation instead of vitamin B6 deficiency (45). It is also possible that PLP and circulating folate confound their associations with

CRC because both of them play important roles in 1-carbon metabolism. It is unclear whether the effects are from folate alone, from vitamin B6/PLP, from their combination, or from other B vitamins in the 1-carbon metabolism.

Recently, a randomized, placebo-controlled trial of a combination supplement of folic acid, vitamin B6, and vitamin B12 showed neither beneficial effects nor increased risk for colorectal adenoma over 9 years of follow-up (17). The trial was conducted during the folic acid fortification era; baseline intakes of each micronutrient were high, with median intake of folate equivalent to the US recommended dietary allowance, and the daily dose of each micronutrient in the supplement was also high, with folic acid as high as 2.5 mg/day (46). The null results of the trial do not address the protective potential of folate in individuals with suboptimal intakes.

Additionally, the high intake of folate may imply high intakes of other nutrients, such as fiber. High intake of fiber is inversely associated with CRC (47). The inverse association of folate intake and CRC risk might reflect residual confounding by fiber intake. However, recent cohort studies with proper adjustment for fiber intake yielded similar inverse associations between folate intake and CRC risk (41, 48).

The etiology of CRC is complex and unlikely to be explained by a single factor. In clinical trials, researchers found that folic acid supplementation of 1 mg/day was not beneficial in adenoma patients with nutritionally adequate baseline folate status (15, 49). A potential explanation for this observation might be that an adenoma that develops in patients with moderate baseline folate status might actually go through a "folate-independent pathway" (15). Such an observation implies that the role of folate could vary according to the presence of other risk factors, such as smoking, alcohol consumption, diet, physical activity, and hormone-related factors. The CRC risk associated with folate might be different between people who smoke or drink alcohol heavily, or even between men and women.

In summary, our analyses suggest a null association between circulating folate concentrations and CRC risk. Interestingly, we observed a statistically significant inverse association between circulating folate and CRC risk in the studies that measured folate by radioimmunoassay, though the stronger association for the radioimmunoassay-based studies could reflect differences in cohorts and study designs rather than assay performance. Further investigations need to integrate more accurate measurements and modeling to explore the effects of folate in the presence of genetic, lifestyle, dietary, and hormone-related factors.

REFERENCES

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69–90.
2. Huxley RR, Ansary-Moghaddam A, Clifton P, et al. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. *Int J Cancer*. 2009;125(1):171–180.
3. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. *J Nutr*. 2002;132(8 suppl):2350S–2355S.
4. World Cancer Research Fund/American Institute for Cancer Research. *Colon and Rectum. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective*. Washington, DC: World Cancer Research Fund/American Institute for Cancer Research; 2007:280–288.
5. Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. *J Nutr Biochem*. 1999;10(2):66–88.
6. Kato I, Dnistrian AM, Schwartz M, et al. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. *Br J Cancer*. 1999;79(11-12):1917–1922.
7. Van Guelpen B, Hultdin J, Johansson I, et al. Low folate levels may protect against colorectal cancer. *Gut*. 2006;55(10):1461–1466.
8. Otani T, Iwasaki M, Sasazuki S, et al. Plasma folate and risk of colorectal cancer in a nested case-control study: the Japan Public Health Center–Based Prospective Study. *Cancer Causes Control*. 2008;19(1):67–74.
9. Weinstein SJ, Albanes D, Selhub J, et al. One-carbon metabolism biomarkers and risk of colon and rectal cancers. *Cancer Epidemiol Biomarkers Prev*. 2008;17(11):3233–3240.
10. Shrubsole MJ, Yang G, Gao YT, 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(3):1003–1006.
11. Eussen SJ, Vollset SE, Igland J, et al. Plasma folate, related genetic variants, and colorectal cancer risk in EPIC. *Cancer Epidemiol Biomarkers Prev*. 2010;19(5):1328–1340.
12. Lee JE, Wei EK, Fuchs CS, et al. Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal

cancer risk in three large nested case-control studies. *Cancer Causes Control*. 2012;23(4):537–545.

13. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA*. 2007;297(21):2351–2359.

14. Logan RF, Grainge MJ, Shepherd VC, et al. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology*. 2008;134(1):29–38.

15. Wu K, Platz EA, Willett WC, et al. A randomized trial on folic acid supplementation and risk of recurrent colorectal adenoma. *Am J Clin Nutr*. 2009;90(6):1623–1631.

16. Figueiredo JC, Mott LA, Giovannucci E, et al. Folic acid and prevention of colorectal adenomas: a combined analysis of randomized clinical trials. *Int J Cancer*. 2011;129(1):192–203.

17. Song Y, Manson JE, Lee IM, et al. Effect of combined folic acid, vitamin B(6), and vitamin B(12) on colorectal adenoma. *J Natl Cancer Inst*. 2012;104(20):1562–1575.

18. Kim YI. Folate and colorectal cancer: an evidence-based critical review. *Mol Nutr Food Res*. 2007;51(3):267–292.

19. Mason JB, Dickstein A, Jacques PF, 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(7):1325–1329.

20. Hirsch S, Sanchez H, Albala C, 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(4):436–439.

21. Caudill MA. Folate bioavailability: implications for establishing dietary recommendations and optimizing status. *Am J Clin Nutr*. 2010;91(5 suppl):1455S–1460S.

22. Shane B, Tamura T, Stokstad EL. Folate assay: a comparison of radioassay and microbiological methods. *Clin Chim Acta*. 1980;100(1):13–19.

23. Bagnardi V, Zambon A, Quatto P, et al. Flexible meta-regression functions for modeling aggregate dose-response data, with an application to alcohol and mortality. *Am J Epidemiol*. 2004;159(11):1077–1086.

24. Rota M, Bellocco R, Scotti L, et al. Random-effects meta-regression models for studying nonlinear dose-response relationship, with an application to alcohol and esophageal squamous cell carcinoma. *Stat Med*. 2010;29(26):2679–2687.

25. Berlin JA, Longnecker MP, Greenland S. Meta-analysis of epidemiologic dose-response data. *Epidemiology*. 1993;4(3):218–228.

26. Zittermann A, Iodice S, Pilz S, et al. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am J Clin Nutr*. 2012;95(1):91–100.

27. Rampersaud GC, Kauwell GP, Bailey LB. Folate: a key to optimizing health and reducing disease risk in the elderly. *J Am Coll Nutr*. 2003;22(1):1–8.

28. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327(7414):557–560.

29. Fazili Z, Pfeiffer CM, Zhang M. Comparison of serum folate species analyzed by LC-MS/MS with total folate measured by microbiologic assay and Bio-Rad radioassay. *Clin Chem*. 2007;53(4):781–784.

30. Glynn SA, Albanes D, Pietinen P, et al. Colorectal cancer and folate status: a nested case-control study among male smokers. *Cancer Epidemiol Biomarkers Prev*. 1996;5(7):487–494.

31. Le Marchand L, White KK, Nomura AM, et al. Plasma levels of B vitamins and colorectal cancer risk: the Multiethnic Cohort Study. *Cancer Epidemiol Biomarkers Prev*. 2009;18(8):2195–2201.

32. Pfeiffer CM, Caudill SP, Gunter EW, et al. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999–2000. *Am J Clin Nutr*. 2005;82(2):442–450.

33. Hannisdal R, Gislefoss RE, Grimsrud TK, et al. Analytical recovery of folate and its degradation products in human serum stored at −25 degrees C for up to 29 years. *J Nutr*. 2010;140(3):522–526.

34. Hannisdal R, Ueland PM, Eussen SJ, et al. Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. *J Nutr*. 2009;139(7):1415–1418.

35. Yetley EA, Pfeiffer CM, Phinney KW, et al. Biomarkers of folate status in NHANES: a roundtable summary. *Am J Clin Nutr*. 2011;94(1 suppl):303S–312S.

36. Rothman KJ, Greenland S, Lash TL. Validity in epidemiologic studies. In: Rothman KJ, Greenland S, Lash TL, eds. *Modern Epidemiology*. Philadelphia, PA: Lippincott Williams & Wilkins; 2008:128–147.

37. Taioli E, Garza MA, Ahn YO, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer: a HuGE-GSEC review. *Am J Epidemiol*. 2009;170(10):1207–1221.

38. Robitaille J, Hamner HC, Cogswell ME, et al. Does the MTHFR 677C→T variant affect the recommended dietary allowance for folate in the US population? *Am J Clin Nutr*. 2009;89(4):1269–1273.

39. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res*. 1996;56(21):4862–4864.

40. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res*. 1997;57(6):1098–1102.

41. Kim DH, Smith-Warner SA, Spiegelman D, et al. Pooled analyses of 13 prospective cohort studies on folate intake and colon cancer. *Cancer Causes Control*. 2010;21(11):1919–1930.

42. Anderson CA, Jee SH, Charleston J, et al. Effects of folic acid supplementation on serum folate and plasma homocysteine concentrations in older adults: a dose-response trial. *Am J Epidemiol*. 2010;172(8):932–941.

43. Larsson SC, Orsini N, Wolk A. Vitamin B6 and risk of colorectal cancer: a meta-analysis of prospective studies. *JAMA*. 2010;303(11):1077–1083.

44. Lu CC. Vitamin B6, blood PLP level, and risk of colorectal cancer. *JAMA*. 2010;303(22):2251–2252.

45. Leung EY, Roxburgh CS, Talwar D, et al. The relationships between plasma and red cell vitamin B2 and B6 concentrations and the systemic and local inflammatory responses in patients with colorectal cancer. *Nutr Cancer*. 2012;64(4):515–520.

46. Ziegler RG. Following up folate and its function in colorectal carcinogenesis. *J Natl Cancer Inst*. 2012;104(20):1525–1527.

47. Bingham S. The fibre-folate debate in colo-rectal cancer. *Proc Nutr Soc*. 2006;65(1):19–23.

48. Gibson TM, Weinstein SJ, Pfeiffer RM, et al. Pre- and postfortification intake of folate and risk of colorectal cancer in a large prospective cohort study in the United States. *Am J Clin Nutr*. 2011;94(4):1053–1062.

49. Figueiredo JC, Levine AJ, Grau MV, et al. Colorectal adenomas in a randomized folate trial: the role of baseline dietary and circulating folate levels. *Cancer Epidemiol Biomarkers Prev*. 2008;17(10):2625–2631.