Folate and colorectal neoplasia: relation between plasma and dietary markers of folate and adenoma recurrence

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ABSTRACT

Background: The results of epidemiologic studies indicate that higher intakes or blood concentrations of folate are associated with a lower risk of colorectal neoplasia; however, only one study assessed the role of homocysteine.

Objective: We assessed the relation between biochemical and dietary markers of folate status and colorectal adenoma recurrence.

Design: Analyses were conducted in 1014 men and women aged 40–80 y who had undergone removal of all colorectal polyps. Diet and supplement use were ascertained through a food-frequency questionnaire administered at study entry. Blood collected at baseline was used to measure plasma folate and homocysteine concentrations. Unconditional logistic regression was used to assess the odds of recurrence associated with the intakes of folate, methionine, and vitamins B-6 and B-12 and with plasma folate and homocysteine.

Results: Relative to subjects in the highest quartile of plasma homocysteine, those in the lowest quartile had an odds ratio (OR) of adenoma recurrence of 0.69 (95% CI: 0.47, 1.02; \( P \) for trend = 0.02) after adjustment for confounding factors. Lower odds of recurrence were shown for higher plasma folate (OR: 0.66; 95% CI: 0.46, 0.97) and higher total intakes (dietary plus supplemental) of folate (OR: 0.61; 0.42, 0.89) and vitamin B-6 (OR: 0.65; 0.45, 0.94). Slightly weaker and nonsignificant associations were shown for dietary folate, methionine, and total vitamin B-12.

Conclusions: A lower recurrence of colorectal adenomas was shown in subjects with higher intakes and plasma concentrations of folate. Additional markers involved in folate metabolism, including lower homocysteine and higher vitamin B-6 intake, were also associated with lower odds of recurrence.

KEY WORDS Colorectal adenoma, folate, homocysteine, methionine, vitamin B-6, vitamin B-12

INTRODUCTION

Colorectal cancer is the third most common lethal cancer in men and women and the second leading cause of cancer deaths in the United States (1). In most cases, colorectal cancer represents the final stage of a progressive, multistep, carcinogenic process of evolution that involves adenomatous polyps (2, 3). Cancer is hypothesized to arise as a result of excess DNA damage or of the inappropriate expression of critical genes. Folate and other co-factors, such as vitamins B-6 and B-12 and methionine, are involved in biological methylation and maintenance of intracellular folate pools for DNA synthesis; therefore, a folate deficiency might lead to cancer through disruption of these events (4, 5). Additional mechanisms have been proposed and are reviewed elsewhere (5).

An increasing body of epidemiologic evidence from prospective and retrospective observational studies supports the role of folic acid in reducing the risk of colorectal cancer. Eighteen studies of colon or colorectal cancer (6–23) and 7 studies of adenoma endpoints (24–27) have been reported. Overall, inverse associations with colon or colorectal cancer have been shown whether folate was assessed from the diet (7, 8, 10–13, 15–22) or in blood (12, 14, 15). Studies of adenoma prevalence also support an inverse association with folate intake (24–29); however, a study of adenoma recurrence showed no association between folate intake and recurrence after adjustment for additional nutrients including dietary fiber (30). Despite consistency in the reported associations, in approximately one-half of these studies, the relative risks were not statistically significant. Associations between colorectal neoplasia and vitamins B-6 and B-12 have also been published (8, 13, 21, 24).

Homocysteine, a sulfur-containing amino acid formed by the adenylation and subsequent demethylation of methionine, has been shown to be a sensitive indicator of folate intake (31–33) although homocysteine is influenced by additional factors (34). Increased homocysteine concentrations are thought to directly affect carcinogenesis by diminishing DNA methylation in critical tissues through a simultaneous increase in intracellular S-adenosylhomocysteine (5). Inclusion of homocysteine in the assessment of folate in carcinogenesis is important because we may be dealing with an issue of inadequate folate metabolism, which is indicated by the reduced function of enzymes involved
in homocysteine metabolism, rather than merely a state of folate deficiency. However, to our knowledge, only one study has been published on the association between serum homocysteine and colorectal cancer (15); the results of this study showed a positive, nonsignificant association.

Given the continued interest in the role of folate and the risk of colorectal neoplasia, we assessed the relation between several markers involved in folate metabolism (dietary and supplemental folate, intakes of methionine and vitamins B-6 and B-12, and plasma folate and homocysteine) and the risk of adenoma recurrence. We are unaware of a single published report of colorectal neoplasia that includes all these important factors.

SUBJECTS AND METHODS

Study population

Analyses were conducted in participants in the Wheat Bran Fiber trial, of which details were previously reported (35). The Wheat Bran Fiber intervention had no significant effect on adenoma recurrence (36), which allowed us to use data from all participants who provided a blood sample for folate and homocysteine analysis. Men and women aged 40–80 yr who had had at least one colorectal adenoma measuring ≥3 mm removed at colonoscopy within 3 mo before study entry were recruited between 1990 and 1995 from the Phoenix, AZ, metropolitan area. We excluded subjects with a personal history of inflammatory bowel disease or hereditary colon cancer syndromes as well as those with more than one first-degree family member affected with colorectal cancer. Participants (n = 1429) were randomly assigned to the trial, and 1304 (91.2%) completed the study by undergoing at least one colonoscopy after randomization. Adenoma recurrence was defined as the recurrence of at least one colorectal adenoma or a colon cancer occurrence anytime after randomization. The study was approved by the University of Arizona Human Subjects Committee.

Dietary and supplemental folate intake

We assessed dietary folate and supplement use from our 113-item Arizona Food-Frequency Questionnaire (AFFQ), which contained questions about diet during the previous year. Details regarding the development of the AFFQ (37) and assessment of its validity and reliability were reported previously (38). Nutrient data were generated by using data from the US Department of Agriculture’s Continuing Survey of Food Intake of Individuals, 1994–1996, and the Nutrient Database for Standard Reference, version 13. The latter database includes folic acid values before fortification of the food supply and was thus essential because all of our baseline AFFQs were administered before this mandate. The AFFQ also contains a section on vitamin and mineral supplement use that includes questions on brand, dose, and frequency of use. The vitamin and mineral supplement database contains over 180 nutritional supplement preparations. Using information on supplement use, we derived total intakes (diet plus supplements) of folate and vitamins B-6 and B-12.

Plasma folate and homocysteine

Blood was drawn from fasting participants and placed in tubes containing heparin. After collection, the samples were centrifuged for 10 min at 2000 rpm (model TJ-6 centrifuge; Beckman, Fullerton, CA) and 4 °C, and aliquots were stored at −80 °C.

Baseline samples were available for 1014 participants. Analyses of homocysteine and folate in plasma were conducted at the University of California, Los Angeles, 8–12 y after collection and storage. For homocysteine measurement, the reversed-phase HPLC method of Kuo et al (39) was used. HPLC analysis was carried out by using an Agilent Technology HPLC 1100 system (Agilent Technology, San Diego) connected to a Varian 9070 fluorescence detector (Varian Inc, Walnut Creek, CA). Thiols were separated by using a Bakerbond C18, 4.6 × 250-mm column (Mallinckrodt Baker, Phillipsburg, NJ) and gradient elution from 92% mobile phase A (0.1 mol KHP04/L, pH 2.0) and 8% mobile phase B (methanol) to 60% mobile phase A and 40% mobile phase B at 12 min with an equilibration time of 8 min between injections. For quality-control purposes, pooled plasma samples were analyzed with each batch of samples. The intraassay CV was 5.7%, and the interassay CV was 4.4%. Folate was analyzed by using the Bio-Rad Quantaphase II Radioassay kit (Bio-Rad, Hercules, CA). Plasma was mixed with 125I-folate in a solution containing dithiothreitol and cyanide. The mixture was boiled to inactivate endogenous binding proteins. The mixture was cooled and combined with immobilized, affinity-purified porcine intrinsic factor and folate-binding protein and incubated for 1 hr at room temperature. The endogenous and labeled vitamins competed for the limited number of binding sites on the basis of their relative concentrations. The reaction mixture was centrifuged at room temperature for 10 min at 1500 g and decanted. Labeled and unlabeled vitamins binding to the immobilized binding proteins were concentrated at the bottom of the tube in the form of a pellet. The radioactivity associated with the pellet was counted, and the folate concentration was determined from the standard curve. The intraassay CV ranged from 4.1% to 5.2%, and the interassay CV ranged from 4.1% to 8.7%.

Risk factor and covariate data

Self-administered questionnaires were used to obtain data on sociodemographic variables, family history of colorectal cancer in first-degree relatives, history of polyps before the baseline adenoma, aspirin use, and cigarette smoking. In addition, we considered baseline adenoma characteristics as potential confounding variables because they have been shown to be significant predictors of adenoma recurrence in this study population (40). Data on adenoma characteristics (ie, number, size, location, and histology) were obtained from medical records and pathology reports as previously reported (40).

Statistical analysis

Age- and sex-adjusted Pearson correlation coefficients were used to assess the relations between markers of folate. We first assessed the main effects of plasma folate and homocysteine, dietary and total (dietary plus supplement use) intakes of folate, and total intakes of vitamins B-6 and B-12 on adenoma recurrence. We used quartile cutoffs based on the total study population for these variables. Adenoma recurrence status (positive or negative) was used as the dependent variable. As described above, we considered a priori potential confounding variables that are suspected or established risk factors for colorectal neoplasia. Variables that were independently associated with both adenoma recurrence and baseline folate markers were initially included in the model. We then tested additional covariates in the model, including number of colonoscopies conducted during the
trial, which is a strong predictor of adenoma recurrence. Lastly, we considered all the folate markers and additional dietary factors, as additional confounders. Multivariate logistic regression was used to assess the importance of each independent variable in the etiology of adenoma recurrence (41). The final list of covariates was generated largely by statistical testing of the best-fit model. Trend tests were conducted by modeling the quartile-specific category for each participant, with the exception of plasma folate, which was treated as a continuous variable on the basis of results from fractional polynomials to determine the best transformation (41). All P values are 2 sided. STATA statistical software, version 7 (Stata Press, College Station, TX) was used to perform statistical analyses.

RESULTS

Baseline characteristics of the subjects according to plasma homocysteine and folate concentrations are presented in Table 1. Compared with the subjects in the lowest quartile of homocysteine concentrations, those in the highest quartile were significantly older and significantly more likely to be male and current smokers; the subjects in the highest quartile also had significantly lower dietary and total intakes of folate and total intakes of vitamin B-6 and significantly higher intakes of alcohol. In addition, multiple adenomas and adenomas with villous histology were significantly more common in the subjects in the highest quartile of homocysteine concentrations than in those in the lowest quartile. For plasma folate, the subjects in the highest quartile were significantly older, significantly more likely to be aspirin users, and significantly less likely to be current smokers than were those in the lowest quartile; the subjects in the highest quartile also had significantly higher dietary and total intakes of folate and total intakes of vitamin B-6 and significantly lower intakes of alcohol. The presence of multiple adenomas was significantly less common in the subjects in the highest quartile of folate concentrations than in those in the lowest quartile.

Age- and sex-adjusted Pearson correlation coefficients between factors involved in folate metabolism are shown in Table 2. High correlations were observed between dietary folate intake and both total folate intake and methionine intake, between total folate intake and total intakes of vitamins B-12 and B-6, and between total intakes of vitamins B-12 and B-6. Modest correlations were found between the remaining nutrient intakes. Plasma homocysteine was inversely correlated with plasma folate, total intakes of folate and vitamins B-12 and B-6, and methionine intake; however, many of these correlations were weak.

### Table 1

Baseline characteristics of the subjects according to plasma homocysteine and folate concentrations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Plasma homocysteine (μmol/L)</th>
<th>Plasma folate (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.0 (n = 254)</td>
<td>8.7 (n = 253)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>62.3 ± 9.0</td>
<td>64.6 ± 8.8</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>134 (52.8)</td>
<td>178 (70.4)</td>
</tr>
<tr>
<td>History of colorectal cancer in parent or sibling [n (%)]</td>
<td>39 (15.4)</td>
<td>31 (12.3)</td>
</tr>
<tr>
<td>Aspirin use [n (%)]</td>
<td>57 (22.4)</td>
<td>84 (33.2)</td>
</tr>
<tr>
<td>Current smoker [n (%)]</td>
<td>30 (11.8)</td>
<td>42 (16.6)</td>
</tr>
<tr>
<td>Previous polyps [n (%)]</td>
<td>84 (33.1)</td>
<td>76 (30.0)</td>
</tr>
<tr>
<td>No. colonoscopies</td>
<td>2.1 ± 0.9</td>
<td>2.0 ± 0.8</td>
</tr>
</tbody>
</table>

Notes:
- Median (all such values).
- P for trend < 0.05 (maximum-likelihood ordered logit estimation) for homocysteine and folate.
- P = ± SD (all such values).
- P for trend < 0.05 (maximum-likelihood ordered logit estimation) for homocysteine.
- History of colorectal cancer in parent or sibling.
- Regular use in the previous month.
- P for trend < 0.05 (maximum-likelihood ordered logit estimation) for folate.
- History of previous polyps before qualifying colonoscopy; data missing for 129 subjects.
- After qualifying procedure.
- Data missing for 13 subjects.
- Size of largest adenoma > 1 cm. Data missing for 13 subjects.
Plasma folate was positively and significantly correlated with both dietary and total intakes of folate and with intakes of the other dietary variables except methionine and alcohol. Alcohol was not correlated with either the dietary or the biochemical factors; this may have been due to the fact that alcohol consumption was very low in our study population (median: 1.3 g/d).

Results of multivariate logistic regression analyses for adenoma recurrence and markers of folate status are presented in Table 3. Compared with the subjects who had a plasma homocysteine concentration > 11.58 μmol/L, those with a concentration < 7.84 μmol/L had an odds ratio (OR) of 0.69; although the 95% CIs included 1, a significant trend across quartiles was shown (P = 0.02). Plasma folate was inversely and significantly associated with recurrence [OR: 0.66 (95% CI: 0.46, 0.97)] for the subjects in the highest quartile compared with those in the lowest quartile; P for trend = 0.04). The protective effect began in the second quartile, which corresponded to plasma concentrations that were indicative of folate deficiency (7.0 nmol/L) (42), and no further reduction in odds of recurrence was shown with increasing concentrations. Total folate intake was inversely associated with recurrence (OR: 0.61; 95% CI: 0.42, 0.89), whereas no significant association was shown for dietary folate intake. Intakes of vitamin B-12 and methionine were inversely, but not significantly, related to recurrence; however, relative to the subjects in the lowest quartile of vitamin B-6 intake, the odds of recurrence for the subjects in the highest quartile was 0.65 (95% CI: 0.45, 0.94; P for trend = 0.03). Inclusion of additional covariates, including simultaneous adjustment for all folate mark-
ers, in the multivariate model did not materially alter the overall results. It has been reported in some studies (13, 30) that adjustment for dietary fiber substantially attenuates the association between dietary folate and colorectal neoplasia. When we included dietary fiber in the multivariate models, the results did not materially change: the OR for the subjects in the highest quartile of total folate intake compared with those in the lowest quartile was 0.66 (95% CI: 0.43, 1.01; P for trend = 0.06).

Published reports (7, 17, 25, 27) suggest that the association between folate and colorectal neoplasia varies by sex. When we conducted secondary analyses stratified by sex, although our point estimates were less precise, the results suggested differences only for plasma folate and total folate intake. The odds of recurrence for the men and for the women in the highest quartile of plasma folate compared with those in the lowest quartile were 0.54 (95% CI: 0.34, 0.85) and 1.06 (95% CI: 0.53, 2.10), respectively; the corresponding results for total folate intake were 0.51 (95% CI: 0.32, 0.82) for the men and 0.87 (95% CI: 0.45, 1.68) for the women. However, in neither analysis was the interaction significant (P = 0.11 for plasma folate and sex, and P = 0.19 for total folate intake and sex).

DISCUSSION

The results of the present study indicate that biochemical and dietary factors involved in folate metabolism are involved in the etiology of colorectal adenoma recurrence. Except for plasma homocysteine, all the variables (dietary and biochemical) were inversely related to odds of recurrence; however, significant associations were shown only for plasma folate and total intakes of folate and vitamin B-6. The magnitude of the effect for significant associations was similar, with ORs ranging from 0.61 to 0.66.

We observed inverse associations between adenoma recurrence and total folate intake at intakes above those recommended (ie, the cutoff for the highest quartile was 664 µg/d, whereas the recommended dietary allowance is 400 µg/d) (34). Thus, higher-than-recommended intakes of folic acid may be necessary to confer a protective effect of this nutrient on colorectal carcinogenesis. The inverse association between folate intake and adenoma recurrence was stronger for total intake (ie, including supplemental sources) than for dietary intake. This may be the result of a higher overall intake among the subjects who reported taking multivitamin supplements than among those who did not or of the higher bioavailability of folate from supplements than from dietary sources (43). Furthermore, inverse associations were detected with intakes before fortification of the food supply in the United States, which became mandatory on 1 January 1998. Data on the association between vitamins B-6 and B-12 and the risk of colorectal neoplasia are sparse and inconsistent (8, 13, 21, 24). Our data showed a 35% lower odds of recurrence in the subjects in the highest quartile of vitamin B-6 intake than in those in the lowest quartile and an inverse, nonsignificant association between vitamin B-12 intake and recurrence. Contrary to published reports (20, 21, 25), the results of our study do not support an inverse association between dietary methionine and colorectal neoplasia. Furthermore, we were unable to assess the relation between alcohol intake and recurrence because alcohol consumption was relatively low in our population (median: 1.3 g/d), although positive associations have been detected at higher intakes (11, 17, 20).

As noted previously, few studies have assessed biochemical markers of folate in the etiology of colorectal neoplasia (12, 14, 15, 26), and only one study has assessed homocysteine (15). One additional study of 62 adenoma cases and 50 controls reported only mean concentration differences (44). A major limitation of these studies is their small sample size. To our knowledge, the present study is the largest to date to include biological markers. Plasma folate is regarded as a sensitive marker of dietary intake (45); however, unlike red blood cell folate, which reflects long-term exposure (42), plasma folate is thought to be a marker of recent exposure to folate. Bird et al (26) found similar associations with colorectal adenomas whether red blood cell folate or plasma folate was used. Glynn et al (12) assessed dietary and serum folate and colorectal cancer in a cohort study of male smokers and found no association with the biological marker. Contrary to these findings, we observed protective effects whether we used plasma folate or dietary sources of folate. This suggests that the lower odds of recurrence observed with total folate than with dietary folate was probably due to the intake of folate per se rather than to the intake of other compounds found in folate-rich foods or in multivitamins. Interestingly, the odds of adenoma recurrence were lowest in the subjects in the second quartile of plasma folate, which corresponds to the cutoff that defines deficiency (7 mmol/L) (42); higher concentrations were not associated with lower recurrence rates.

In the present study, a significant dose response was shown between plasma homocysteine and adenoma recurrence; the cutoff for the highest quartile of plasma homocysteine in our population was 11.58 µmol/L. Kato et al (15) showed that, relative to subjects with a homocysteine concentration ≤ 7.91 µmol/L, those with a concentration ≥ 12.2 µmol/L had an nonsignificant OR of 1.72. Thus, it is possible that stronger ORs exist at wider ranges of homocysteine concentrations. We were unable to assess the risk of adenoma recurrence associated with mild to moderate elevation of plasma homocysteine (> 14 mmol/L) (46) because such elevated concentrations were present in only 9% of the population. The importance of this biological marker is underscored by the results of the study by Kim et al (47), in which blood folate and homocysteine were correlated with colonic mucosal folate concentrations; the results showed that although all systemic markers correlated well with mucosal folate concentrations, homocysteine was shown to be the strongest correlate. As also observed in other studies (48, 49), the correlations between plasma homocysteine and dietary variables in our study were generally modest, which suggests that additional factors influence this biological marker. The fact that the correlations between dietary intake and plasma folate in our study were higher than those between dietary intake and plasma homocysteine also indicates that plasma folate is a more sensitive marker of folate intake than is homocysteine.

Despite the substantial amount of published data related to folate and colorectal carcinogenesis, the mechanisms responsible for this effect remain unclear. Plausible mechanisms described in the literature include the role of folate in one-carbon metabolism, DNA methylation, DNA synthesis, and cell proliferation (5, 50). It was recently proposed that an imbalance between biological methylation and nucleotide synthesis is key to understanding the mechanisms responsible for the role of folate in carcinogenesis (5). In addition, compared with higher intakes, lower folate intakes were shown to be associated with a signifi-
cantly higher risk of Ki-ras mutations (prominent in colorectal neoplasia) in adenomas (51) and carcinomas (52).

The homozygous mutant genotype (677CC→T) in the methyltetrahydrofolate reductase gene (MTHFR) results in elevated homocysteine concentrations and decreased plasma folate concentrations, and an adequate folate intake is required to decrease the elevated homocysteine concentrations (53, 54). Because of the low prevalence of the MTHFR polymorphism (10–15%), relatively large study populations are required to assess its interaction with folate status with a high degree of precision. The protective effect of folate against the risk of colon cancer has also been proposed to be stronger among subjects with a positive family history of colorectal cancer than among those without such history (55). Although we did not detect any confounding effect of family history in our analyses, we were unable to conduct an analysis stratified by this variable because of the low percentage of subjects with a positive history (17%). In future analyses, we will explore interactions between markers of folate status and both MTHFR and family history by combining data from the present study with that from our second trial, which was recently completed.

The strengths of the present study include its prospective design, high follow-up rates, control for confounding variables, inclusion of biochemical and dietary sources of folate, and the largest data source of plasma folate and homocysteine for the assessment of colorectal neoplasia risk. Our data on homocysteine are of particular importance given the lack of information on this important biological marker in the published literature. The limitations of our study include its inability to assess the role of alcohol consumption or MTHFR genotypes. In addition, there was insufficient power to assess the effect on the recurrence of large or advanced adenomas, and this effect has been shown to be an important consideration in relation to methyl-deficient diets (25).

In summary, the results of the present study of adenoma recurrence support the proposed hypothesis that folate from dietary, supplemental, and biochemical sources is protective. In addition, homocysteine is positively associated with recurrence, and B vitamins involved in folate metabolism confer a protective effect against recurrence, with the strongest association shown for vitamin B-6. Confirmation of our folate findings awaits the results of ongoing randomized trials of the effect of folic acid supplementation on the rate of adenoma recurrence.

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REFERENCES
