Colorectal Adenomas in a Randomized Folate Trial: The Role of Baseline Dietary and Circulating Folate Levels

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Abstract

The Aspirin/Folate Polyp Prevention Study is a randomized, placebo-controlled trial of aspirin use and folic acid supplementation and incidence of colorectal adenomas in individuals with a history of these lesions. The trial showed that folic acid supplementation does not prevent the occurrence of new adenomas and may increase risk. We extend these results by investigating whether the effect of folic acid treatment differed by baseline dietary and circulating folate levels. Diet and supplement use were ascertained at baseline through a food-frequency questionnaire; a blood sample was used to determine plasma and RBC folate levels. Individuals were followed for 3 years (first follow-up) and subsequently for an additional 3 to 5 years (second follow up). We used generalized linear regression to estimate risk ratios and 95% confidence limits as measures of association. There was little evidence that baseline dietary and total folate intake, and plasma and RBC folate modified the association between folic acid treatment and risk of any adenomas or advanced lesions. However, there was a protective association of the highest tertile of dietary and total intake as well as circulating folate with risk of any adenomas among those in the placebo group but no association among individuals in the folic acid group. Our findings support the idea that although moderate doses of folate may be protective compared with deficiency, at some point of sufficiency, supplementation provides no additional benefit. (Cancer Epidemiol Biomarkers Prev 2008;17(10):2625–31)

Introduction

Extensive epidemiologic data have supported a chemopreventive effect of folate on risk of colorectal adenoma and cancer (1). However, in several animal studies, folic acid supplementation has enhanced the development and progression of existing premalignant and malignant lesions (2). Also, in our randomized clinical trial of folic acid supplementation of 1 mg/day with and without aspirin on the risk of incident colorectal adenomas, there was no reduction in risk overall but a significant increased risk for advanced lesions and multiple adenomas among individuals randomized to the folic acid treatment group after an average of 6 years follow-up (3). In addition, an ecologic analysis of folic acid supplementation in the United States and Canada reported data suggesting a temporal association between fortification and an increase in colorectal cancer rates (4).

Several editorials have been published to highlight the evidence from animal and human studies on the potential adverse consequences of folic acid supplementation (2, 5-8). It is becoming apparent that the timing and dose of folate exposure may be critical; high levels may promote carcinogenesis in the presence of pre-existing neoplastic lesions (8). Therefore, there is concern about the safety of chronic high intakes of folate (5), especially when there are a growing number of cancer survivors who each may harbor residual transformed cells (9).

In this study, we extend the findings of our randomized clinical trial (3) by investigating whether the association between folic acid treatment and adenomas differs by baseline dietary and circulating folate levels. We hypothesized that individuals with high dietary intake or circulating levels of folate may have a greater risk of new adenomas if supplemented with folic acid compared with those with lower dietary folate intake or circulating levels. Second, based on previous observational studies, we hypothesized that there may be a protective association of higher dietary or circulating

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folates with risk among individuals in the placebo group but not in the folic acid treatment group.

Materials and Methods

Study Design. The Aspirin/Folate Polyp Prevention Study is a randomized, double-blind, placebo-controlled trial of the efficacy of oral aspirin, folic acid, or both to prevent colorectal adenomas in patients with a history of adenomas at nine clinical centers (10). The trial had a three-by-two factorial design, comparing 81 and 325 mg/d aspirin with placebo and comparing 1 mg/d folic acid with placebo. Originally, the trial was designed to investigate only aspirin, but shortly after enrollment began, it was expanded to examine folic acid (100 individuals were randomized before the folic acid component was initiated). The study protocol was approved by the Institutional Review Board at all clinical centers and written informed consent was obtained from all study participants. The findings regarding aspirin and folate have been reported (3, 10).

Study Population—Randomization, Interventions, and Follow-up. Potential participants were recruited between July 1994 and March 1998. Eligible individuals had at least one of the following: 1 or more histologically confirmed adenomas removed within 3 mo before recruitment, 1 or more histologically confirmed adenomas removed within 16 mo before recruitment and a lifetime history of 2 or more confirmed adenomas, or a histologically confirmed adenoma at least 1 cm in diameter removed within 16 mo before recruitment. After completion of a 3-mo aspirin run-in period, compliant individuals who wished to continue participating were randomized in a 1:1 ratio to 1 mg per d of folic acid or placebo within strata defined by study center, sex, and age (60 y or younger versus older than 60 y). The period of treatment and follow-up was originally planned to be 3 y (first follow-up interval); however, because of concern that longer exposure to folic acid might be required to observe an antineoplastic effect, participants were asked to continue protocol folate treatment for a second colonoscopic surveillance cycle (usually 3 or 5 y; second follow-up interval). Thus, two surveillance intervals were included in this analysis. When a surveillance colonoscopy was not done at the end of the first follow-up interval, we used the last examination at least 1 year after randomization, on or before September 28, 2001, to delineate the end of the first follow-up interval. The second follow-up interval was defined as the time from the end of the first interval through the next surveillance colonoscopy on or before December 31, 2006; however, folic acid treatment ended October 1, 2004, because of funding constraints.

Data Collection

Questionnaires—Risk Factors and Diet. All participants completed a questionnaire regarding personal characteristics, medical history, and life-style habits. Dietary information was collected using the Block food frequency questionnaire administered to participants at baseline and at first follow-up. The validity and reliability of the food frequency questionnaire has been described previously (11). Questions assessed the average consumption of a food item during the past year. Brand and type of multivitamin supplement use were collected. Daily nutrient intakes were calculated by multiplying the frequency response by the nutrient content of the specified portion size using a comprehensive database. Total alcohol intake per day was calculated as the sum of alcohol content from beer, wine, and liquor.

Measurement of Plasma and RBC Folate. Blood samples were obtained from subjects at baseline and at first follow-up from nonfasting participants into 7-mL EDTA Vacutainer brand tubes. Plasma levels of folate were determined by a microbiological assay using a colistin sulfate–resistant strain of *Lactobacillus leichmannii* (12). EDTA samples with low (<2 nmol/L) or no folate, attributable to inhibition of bacterial growth by antibiotics, were reanalyzed with a method based on measurement of folate as *p*-aminobenzoylglutamate equivalents (13). RBC folate was determined by the ACS:180 folate assay, a competitive immunoassay using direct chemiluminescent technology (Bayer Corporation). Plasma folate was conducted at BEVITAL AS.

Study Outcomes. Adenoma occurrence was determined by colonoscopy and pathology review. All important medical events reported by participants were verified with medical record review. Records for all large bowel procedures (endoscopy or surgery) were obtained. Slides for all tissue removed from the bowel were obtained and sent to a single study pathologist for uniform review. Lesions were classified as neoplastic (adenomatous, including sessile serrated adenomas) or non-neoplastic.

The primary study outcome was the occurrence of one or more colorectal adenomas detected during each of the two follow-up intervals. A secondary outcome was advanced lesions, defined as invasive carcinoma or adenomas with at least 25% villous component, high-grade dysplasia, or estimated adenoma size of 1 cm or greater (as determined by the endoscopist).

Statistical Methods. Spearman's rank correlation coefficient was used to calculate correlations among baseline measures of folate status: dietary folate intake, total folate intake (diet plus supplements), plasma folate levels, and RBC folate levels. Overdispersed generalized linear models for the Poisson family as an approximation to the binomial family were used to compute crude and adjusted risk ratios to assess the risk of at least one new adenoma. We computed risk ratios for folic acid treatment versus placebo within strata (i.e., tertiles) defined by baseline levels of dietary, total intake, and plasma and RBC folate. For nutrient analysis, we computed quartiles from the residuals of the regression of the logarithm of folate intake on the logarithm of kilocalories and used the logarithm of caloric intake to adjust for energy intake. Covariates included in the models were age, sex, center, aspirin treatment group, baseline multivitamin use, and duration of follow-up. In addition, for dietary measures, we adjusted for log calories. We included multivitamin use in our final models as an indirect adjustment for other nutrients/vitamins and unknown life-style factors, although exclusion of this variable from adjusted models did not substantially change any of the results. For plasma measures, we also adjusted for time from blood draw to measurement (y), but because there was no significant change in the estimates of risk, we did not include this

variable in final models. Models for the second follow-up interval were also adjusted for the following covariates: alcohol consumption; smoking status; body mass index; family history of colorectal cancer; B_2, B_6, and B_{12}, and red meat consumption, but no significant evidence of confounding was observed. We used interaction terms and Wald tests (with or without adjustment for other variables) to test for heterogeneity by baseline folate levels. Similarly, to investigate whether fortification of the food supply with folic acid significantly affected these results, we stratified the population recruited before and after December 31, 1996 (mid point between years folic acid was introduced in the United States and Canada) but observed no evidence of effect modification and therefore present results on all participants.

We used the same approach to determine the association between tertiles of baseline dietary, total intake, plasma and RBC folate, and adenoma risk. We obtained risk ratio estimates stratified by randomized folic acid treatment group, and used interaction terms and Wald tests to test for evidence of interaction. When investigating the risk of advanced lesions, we dichotomized levels of dietary, total, and, plasma and RBC folate at the median, due to our limited sample size. We also examined the role of dietary and total folate intake levels at first follow-up and risk of any adenoma or advanced lesions in the second follow-up cycle, but our results did not differ substantially from results examining the association with baseline measures (data not shown).

All analyses of study folate treatment were conducted according to the principle of intention to treat. Two-sided P values of <0.05 were considered statistically significant. Stata (version 9.2) was used for all analyses.

Results

Characteristics of Study Participants. Among the 1,021 individuals randomized to folate treatment, 987 had a follow-up exam during the first follow-up interval and, of these, 427 (43.3%) had a recurrence of one or more colorectal adenoma. There were 926 individuals who continued in this clinical trial for a second follow-up interval; 729 (71.4%) continued treatment. Overall, 762 had a surveillance colonoscopy during the second follow-up interval and 295 (39%) had a recurrence. Individuals who completed the first follow-up interval did not differ significantly from those who completed the second follow-up on selected personal characteristics (Table 1).

Dietary and circulating folates were significantly correlated at baseline and also at first follow-up (ρ values range, 0.16-0.64; $P < 0.0001$). Plasma folate levels increased significantly from baseline to first follow-up in the placebo group (mean $\pm$ SD, 23.7 $\pm$ 17.0 versus 29.9 $\pm$ 14.3 nmol/L; $P < 0.0001$) and much more markedly with folic acid treatment (mean $\pm$ SD, 24.1 $\pm$ 18.2 versus 74.5 $\pm$ 35.8 nmol/L; $P < 0.0001$).

Association of Folic Acid Treatment and Risk of Colorectal Adenomas Stratified by Baseline Dietary, Total Intake, and Plasma and RBC Folate Levels. Table 2 shows the association of randomized folic acid treatment with adenoma risk, stratified by levels of the baseline folate measures/indices. During the first follow-up interval, folic acid treatment was not associated with risk of any adenoma among subjects with low levels of dietary, total intake, plasma, and RBC folate (Table 2). However, there were small, nonsignificant increased risks associated with folic acid treatment at the highest tertile levels of baseline total intake and high plasma folate levels. The difference between the folic acid relative ratio (RR) at the lowest [RR, 0.85; 95% confidence interval (95% CI), 0.67-1.09] and highest tertile (RR, 1.46; 95% CI, 1.12-1.89) of baseline total folate intake reached statistically significance ($P_{interaction} = 0.01$). For advanced lesions, the RRs for study folic acid treatment were statistically similar across tertiles of folate status. During the second follow-up interval, the folic acid RRs did not differ by baseline folate status. When we stratified by baseline multivitamin use, results were broadly similar among multivitamin users and nonusers (data not shown).

Association of Baseline Dietary/Total Intake and Plasma/RBC Folate and Risk of Adenomas Stratified by Folic Acid Treatment Group. Among subjects randomized to folic acid, there were no suggestions that baseline folate status was associated with risk of all adenomas (Table 3). In contrast, among those randomized to placebo, there were indications of inverse associations for all measures of folate status, and this was statistically significant for total folate intake (RR for third tertile versus first, 0.69; 95% CI, 0.51-0.94; $P_{trend} = 0.01$) and for plasma folate levels (RR for third tertile versus first, 0.72; 95% CI, 0.54-0.97; $P_{trend} = 0.03$). However, there was statistically significant evidence for heterogeneity between the treatment groups only for total folate intake ($P = 0.01$).

The patterns in the second follow-up interval were not the same as in the first interval. During this later period, there was a significant trend of increasing risk with increasing RBC folate among placebo subjects ($P = 0.02$), and a borderline increasing trend with dietary folate among those randomized to folic acid ($P = 0.05$). There was no indication of significant heterogeneity by randomized treatment group.

For advanced adenomas, we found no evidence of that baseline folate intake or circulating levels were significantly associated with risk and no evidence that these associations were modified by folic acid treatment group (data not shown). We observed similar findings for baseline folate status and risk of any adenoma and advanced lesions when stratified by baseline multivitamin use.

Discussion

In this report, we extend the analyses from our earlier report (3) regarding the effect of folic acid supplementation on risk of colorectal adenomas, by putting those results into the context of the baseline folate status of the subjects. We found little indication that folic acid treatment prevented the occurrence of adenomas among individuals with lower folate status and only weak indications that any adverse effect of supplementation was enhanced among those with higher folate status. During the first 3 years of the study, there was observational evidence of an inverse association of folate

Table 1. Selected characteristics of the study participants

Characteristic	First follow-up	Second follow-up
No of participants	987	776
Age at baseline (mean ± SD), y	57.4 ± 9.5	57.5 ± 9.2
Male sex, no	629 (63.7%)	497 (64.1%)
Body mass index (mean ± SD), kg/m^2	27.4 ± 4.6	27.3 ± 4.2
Current cigarette smoker, no (%)	142 (14.4%)	104 (13.5%)
Colorectal cancer in first-degree relative, no (%)	304 (38.1%)	249 (39.8%)
Aspirin treatment group, no (%)	657 (66.6%)	515 (66.4%)
Folate treatment group, no (%)	501 (50.8%)	403 (51.9%)
Alcohol (drinks per day), no (%)		
None	299 (31.6%)	225 (30.0%)
1 or less	449 (47.5%)	357 (47.7%)
2 or more	198 (20.9%)	167 (22.3%)
Plasma homocysteine (mean ± SD), μmol/L	9.8 ± 2.9	9.8 ± 2.9
Baseline plasma folate (mean ± SD), nmol/L	23.7 ± 17.4	23.2 ± 17.2
Baseline RBC folate (mean ± SD), ng/mL	322.2 ± 155.9	324.3 ± 155.9
Baseline dietary folate (mean ± SD), mcg/d	322.2 ± 155.9	324.3 ± 155.9
Baseline total folate intake (mean ± SD), mcg/d	458.6 ± 253.0	458.3 ± 246.3
Multivitamin use, no (%)	354 (35.9%)	276 (35.6%)
Adenoma characteristics (at baseline)*		
Number (mean ± SD)	1.57 ± 1.01	1.59 ± 1.01
Large adenomas (>1 cm), no (%)	219 (22.2%)	166 (21.4%)
Villous histology, no (%)	135 (13.7%)	103 (13.3%)
Proximal location, no (%)	452 (55.8%)	370 (47.7%)

NOTE: Counts do not necessarily add to the total sum due to missing data.
*Using standard definitions by Polyp Prevention Study Group (3, 10).

status with risk of all adenomas among placebo subjects but no suggestion of a similar pattern among those randomized to folic acid. During the later follow-up, there were no suggestions of similar patterns. Our findings for advanced lesions were inconsistent, possibly due to our more limited statistical power for that end point.

Folates are hypothesized to have a dual role in colorectal carcinogenesis. They function as major carriers of the one-carbon groups needed for methylation reactions and nucleotide synthesis (14, 15). Low-folate status may induce DNA hypomethylation, which can affect maintenance of DNA integrity and stability and expression of oncogenes and tumor suppressor genes (16). Indeed, high folate levels have been strongly inversely associated with risk of colorectal neoplasia in several epidemiologic studies (1). Of note is that epidemiologic studies showing an inverse association of high folates on colorectal adenoma (17-19) or cancer risk (20, 21) have been largely conducted since the late 1990s, when fortification of cereal grains with folic acid in the United States and Canada was introduced. As a result of this fortification, there was a significant increase in circulating levels of plasma folate in the general population (22, 23). Some subsequent studies showed inverse associations of dietary folate and cancer risk only among nonmultivitamin users (24, 25), a finding suggests folate may not be protective in populations that are relatively folate-replete.

In line with these observational studies, we observed suggestive, but nonsignificant evidence that higher levels of dietary folate intake and circulating folates may be inversely associated with risk of any adenoma among individuals in the placebo group during 3 years of follow-up. There was no evidence of a beneficial effect of high dietary or circulating folates among individuals randomized to the folic acid group with an indication, at least during the first follow-up period, of a potential harmful effect. However, these relationships were not apparent during the second follow-up up to 6 years, possibly because, at this point, more individuals were folate replete due to fortification of the food supply. Indeed, we observed in our data evidence that plasma levels of folate increased modestly from baseline to first follow-up in the placebo group, probably because of the fortification of the North American food supply with folic acid. These findings support the idea that although folate at moderate doses may be protective compared with deficiency, at some point of sufficiency, there may be no additional benefit with increasing intake. Interestingly, Song et al. (26) showed in a Apc^{Min} mouse model that dietary folate supplementation reduced the number of ileal polyps and colonic aberrant crypt foci at 3 months; however, at later time points (6 months), folate supplementation seemed to increase the number of ileal polyps. Although the mechanism for this finding is not entirely understood, the authors suggested that folate deficiency may have caused the regression of established polyps at later time points.

Additional data provide evidence of the critical importance of the timing of folate exposure. In a Apc+/− Msh2−/− mouse model, modest doses of folate supplementation given after the formation of neoplastic foci seemed to increase the development of new foci (27). These data support the idea that folate under certain circumstances may act to promote carcinogenesis. There are plausible biological reasons why high levels of folate may promote colorectal carcinogenesis. Neoplastic cells have a relatively high rate of proliferation (28) and an upregulation of folate receptors (29) compared with normal issue. Folates play a key role in the supply of nucleotides and thereby may facilitate tumor growth (14, 15). Furthermore, folic acid is a pharmaceutical fully oxidized, monoglutamyl form of folate. Folic acid is converted to the active dihydro and tetrahydro forms by dihydrofolate reductase. But although small oral doses of folic acid

are efficiently reduced, intakes above 400 μg can saturate the ability of dihydrofolate reductase to convert folic acid (30). As a result, unmetabolized folic acid may be detected in blood after folic acid supplementation at high doses (30). Unmetabolized folic acid has recently been associated with reduced natural killer cell cytotoxicity (31). Natural killer cells are part of the nonspecific immune response elicited as a first-line defense mechanism against pathogens or carcinogenic cells (32). The potential toxicity ascribed to folic acid, as opposed to natural forms of folate, is conjecture at this point.

In this study, we provide only inconsistent evidence that the associations of folic acid supplementation with risk of any adenoma or advanced lesions were modified by baseline dietary and circulating folate levels. This finding provides weak evidence in support of our initial hypothesis that individuals with high dietary intake or circulating levels of folate may have a greater risk of new adenomas if supplemented with folic acid compared with those with lower dietary folate intake or circulating levels. Possible explanations include the lack of statistical power or a threshold effect. Powers et al. (33) provide data from a double-blind randomized placebo-controlled intervention study of folate and riboflavin supplementation in healthy colorectal adenoma patients to show that unlike plasma folate, colon mucosal folate exhibits an upper threshold in response to a moderate folic acid supplementation. Their data suggest than 1,200 μg of folic acid per day for 45 days does not elicit any additional significant increase in colon folate over 400 μg of folic acid per day. This finding may reflect a regulation of folate uptake by colonocytes. For example, cellular uptake may be limited by the level of folylpolyglutamate synthetase, which catalyzes folate polyglutamation and thereby retention of folate (34).

This study has several limitations. The generalizability of our results may be limited as all participants in this clinical trial were volunteers who had a previous history of at least one colorectal adenoma. We had a limited sample size to investigate risk of advanced lesions, a

Table 2. Folic acid treatment at 1mg/d and risk of any adenomas and advanced lesions by baseline dietary, total intake, plasma and RBC folate levels

	First follow-up			*P**	Second Follow-up			*P**
	Placebo (*n* = 486)	Folic acid (*n* = 501)	RR (95% CI)		Placebo (*n* = 373)	Folic acid (*n* = 403)	RR (95% CI)	
Dietary folate intake †								
Any adenoma								
T1: 63.9-246.1 mcg ‡	74 (47.1%)	67 (43.2%)	0.91 (0.71-1.17)	0.13	40 (34.5%)	43 (34.7%)	0.99 (0.70-1.41)	0.67
T2: 246.5-352.9 mcg ‡	55 (36.9%)	85 (50.3%)	1.30 (1.01-1.69)		44 (35.8%)	53 (38.1%)	1.04 (0.76-1.44)	
T3: 353.1-1,285.6 mcg ‡	70 (43.8%)	64 (40.5%)	0.98 (0.76-1.27)		52 (42.6%)	58 (46.0%)	1.21 (0.89-1.65)	
Advanced lesions								
T1: 63.9-246.1 mcg ‡	15 (9.6%)	13 (8.4%)	0.88 (0.43-1.80)	0.43	10 (8.6%)	11 (8.9%)	0.89 (0.38-2.11)	0.21
T2: 246.5-352.9 mcg ‡	12 (8.1%)	24 (14.2%)	1.69 (0.87-3.28)		11 (8.9%)	13 (9.4%)	1.01 (0.46-2.21)	
T3: 353.1-1,285.6 mcg ‡	15 (9.4%)	19 (12.0%)	1.34 (0.70-2.58)		8 (6.6%)	17 (13.5%)	2.38 (1.03-5.48)	
Total folate intake †								
Any adenoma								
T1: 67.3-303.9 mcg*	78 (50.7%)	73 (45.1%)	0.85 (0.67-1.09)	*0.01*	40 (35.1%)	45 (34.9%)	0.97 (0.69-1.36)	*0.71*
T2: 304.5-551.3 mcg*	62 (42.5%)	72 (42.1%)	0.99 (0.76-1.28)		45 (37.2%)	59 (43.1%)	1.18 (0.86-1.61)	
T3: 552.0- 1,807.8 mcg*	59 (35.5%)	71 (47.7%)	1.46 (1.12-1.89)		51 (40.5%)	50 (40.7%)	1.09 (0.79-1.50)	
Advanced lesions								
T1: 67.3-303.9 mcg*	15 (9.7%)	20 (12.4%)	1.30 (0.68-2.48)	*0.94*	9 (7.9%)	9 (7.0%)	0.87 (0.35-2.17)	*0.58*
T2: 304.5-551.3 mcg*	14 (9.6%)	21 (12.3%)	1.17 (0.61-2.25)		9 (7.4%)	16 (11.7%)	1.43 (0.63-3.23)	
T3: 552.0-1,807.8 mcg*	13 (7.8%)	15 (10.1%)	1.39 (0.68-2.85)		11 (8.7%)	16 (13.0%)	1.61 (0.74-3.48)	
Plasma folate§								
Any adenoma								
T1: 2.4-13.6 nmol/L	70 (46.1%)	70 (47.0%)	0.98 (0.76-1.27)	*0.40*	46 (38.3%)	55 (43.7%)	1.17 (0.86-1.60)	*0.77*
T2: 13.6-26.7 nmol/L	62 (42.8%)	69 (44.0%)	1.02 (0.78-1.33)		45 (38.1%)	50 (37.6%)	1.01 (0.73-1.40)	
T3: 26.7-159.9 nmol/L	55 (36.9%)	66 (43.7%)	1.26 (0.58-2.42)		45 (38.8%)	45 (38.5%)	1.02 (0.73-1.43)	
Advanced lesions								
T1: 2.4-13.6 nmol/L	13 (8.6%)	15 (10.1%)	1.18 (0.58-2.42)	*0.35*	11 (9.2%)	15 (11.9%)	1.31 (0.61-2.80)	*0.93*
T2: 13.6-26.7 nmol/L	13 (9.0%)	15 (9.6%)	1.02 (0.50-2.10)		7 (5.9%)	15 (11.3%)	1.52 (0.63-3.69)	
T3: 26.7-159.9 nmol/L	11 (7.4%)	22 (14.6%)	2.06 (1.02-4.16)		11 (9.5%)	12 (10.3%)	1.21 (0.53-2.76)	
RBC folate ‡								
Any adenomas								
T1: 64.9-338.0 ng/mL	68 (45.3%)	81 (45.0%)	0.95 (0.74-1.21)	*0.60*	32 (29.6%)	56 (39.2%)	1.31 (0.92-1.85)	*0.50*
T2: 339.0-449.0 ng/mL	73 (42.4%)	68 (43.6%)	1.06 (0.82-1.36)		53 (39.6%)	49 (36.8%)	1.00 (0.73-1.37)	
T3: 450.0-1,133.0 ng/mL	64 (39.5%)	71 (43.6%)	1.14 (0.88-1.47)		56 (43.4%)	55 (44.0%)	1.04 (0.77-1.40)	
Advanced lesions								
T1: 64.9-338.0 ng/mL	10 (6.7%)	18 (10.0%)	1.45 (0.69-3.05)	*0.92*	7 (6.5%)	17 (11.9%)	1.74 (0.73-4.14)	*0.79*
T2: 339.0-449.0 ng/mL	11 (6.4%)	13 (8.3%)	1.37 (0.63-2.96)		7 (5.2%)	10 (7.5%)	1.57 (0.61-4.01)	
T3: 450.0-1,133.0 ng/mL	21 (13.0%)	26 (16.0%)	1.21 (0.70-2.11)		15 (11.6%)	18 (14.4%)	1.20 (0.61-2.37)	

NOTE: Counts do not necessarily add to the total sum due to missing data.

*P value for heterogeneity.

†Adjusted for age, sex, center, duration of follow-up, aspirin treatment group, multivitamin use, and log calories.

‡Ranges are based on 2000 calories/d.

§Adjusted for age, sex, center, duration of follow-up, multivitamin use, aspirin treatment group.

Table 3. Association of baseline dietary, total intake, plasma and RBC folate levels, and risk of any colorectal adenomas by folic acid treatment group

	First Follow-up				Second Follow-up			
	Placebo		Folic acid		Placebo		Folic acid	
	# events/ total*	RR (95% CI)	# events/ total*	RR (95% CI)	# events/ total*	RR (95% CI)	# events/ total*	RR (95% CI)
Dietary folate intake†								
T1: 63.9-246.1 mcg‡	74/157	1.00	67/155	1.00	40/116	1.00	43/124	1.00
T2: 246.5-352.9 mcg‡	55/149	0.79 (0.61-1.03)	85/169	1.13 (0.88-1.44)	44/123	1.03 (0.73-1.46)	53/139	1.09 (0.78-1.51)
T3: 353.1-1,285.6 mcg‡	70/160	0.87 (0.67-1.11)	64/158	0.93 (0.72-1.21)	52/122	1.12 (0.80-1.57)	58/126	1.37 (0.99-1.89)
P_{trend}		*0.26*		*0.60*		*0.51*		*0.05*
$P_{heterogeneity}$			*0.13*				*0.67*	
Total folate intake†								
T1: 67.3-303.9 mcg‡	78/154	1.00	73/162	1.00	40/114	1.00	45/129	1.00
T2: 304.5-551.3 mcg‡	62/146	0.79 (0.61-1.03)	72/171	0.92 (0.72-1.18)	45/121	1.02 (0.73-1.45)	59/137	1.25 (0.91-1.71)
T3: 552.0-1,807.8 mcg‡	59/166	0.69 (0.51-0.94)	71/149	1.18 (0.88-1.59)	51/126	1.08 (0.74-1.59)	50/123	1.22 (0.83-1.78)
P_{trend}		*0.01*		*0.41*		*0.60*		*0.25*
$P_{heterogeneity}$			*0.01*				*0.71*	
Plasma folate§								
T1: 2.4-13.6 nmol/L	70/152	1.00	70/149	1.00	46/120	1.00	55/126	1.00
T2: 13.6-26.7 nmol/L	62/145	0.86 (0.66-1.12)	69/157	0.89 (0.69-1.15)	45/118	0.99 (0.71-1.38)	50/133	0.85 (0.62-1.16)
T3: 26.7-159.9 nmol/L	55/149	0.72 (0.54-0.97)	66/151	0.92 (0.70-1.21)	45/116	1.04 (0.73-1.50)	45/117	0.91 (0.65-1.28)
P_{trend}		*0.03*		*0.54*		*0.84*		*0.52*
$P_{heterogeneity}$			*0.40*				*0.77*	
RBC folate§								
T1: 64.9-338.0 ng/mL	68/150	1.00	81/180	1.00	32/108	1.00	56/143	1.00
T2: 339.0-449.0 ng/mL	73/172	0.88 (0.68-1.14)	68/156	0.99 (0.77-1.26)	53/134	1.30 (0.91-1.86)	49/133	1.00 (0.73-1.36)
T3: 450.0-1,133.0 ng/mL	64/162	0.84 (0.63-1.11)	71/163	1.01 (0.78-1.30)	56/129	1.55 (1.08-2.24)	55/125	1.24 (0.90-1.69)
P_{trend}		*0.21*		*0.96*		*0.02*		*0.20*
$P_{heterogeneity}$			*0.60*				*0.50*	

NOTE: Counts do not necessarily add to the total sum due to missing data.
*Number of individuals with a new adenoma occurrence/total number of individuals.
†Adjusted for age, sex, center, duration of follow-up, aspirin treatment group, multivitamin use, and log calories.
‡Ranges are based on 2000 calories/d.
§Adjusted for age, sex, center, duration of follow-up, aspirin treatment group, and multivitamin use.

clinically important end point. We used a validated semiquantitative food frequency questionnaire, but self-reported dietary instruments are still subject to measurement error. In addition, the majority of the subjects were folate-replete as a result of fortification of the food supply during the recruitment/follow-up time intervals. In comparison to serum and RBC folate levels determined in two National Health and Nutrition Examination Surveys, one prefortification and one postfortification, our mean baseline levels are more similar to postfortification levels (serum, 26.9 nmol/L; RBC, 590 nmol/L; ref. 35). Lastly, although the second follow-up interval was analyzed under the intention to treat principle, only 71% of subjects actually continued their randomization treatment. Furthermore, because individuals in the second-up could select to be in folate treatment arm, this is essentially a cohort study and potentially subject to bias. To address concerns regarding interval validity, we adjusted our models for several potential confounders and observed no significant difference in estimates of risk. To address concerns about external validity, we compared individuals who completed the first follow-up to those that elected to continue and completed the second follow-up and observed no substantial differences.

Strengths of this study include the systematic collection of risk factor and dietary information at baseline and follow-up intervals as well as outcomes at two follow-up intervals. We also measured both dietary folate (diet only and total intake including supplements) and circulating levels (plasma and RBC), which allowed us to explore differences in the distribution of folates across measurements (i.e., dietary, total, and circulating). Plasma folate is predominately 5-MTHF monoglutamate, whereas RBC folate is mostly 5-MTHF polyglutamate but can sometimes contain formylated folates (36). Supplemental folate is pterolglutamic acid (folic acid), which is fully oxidized and bioavailable, and dietary folate is a mixture of polyglutamated folates, 5-MTHF and 5-formylTHF in combination with certain food constituents (such as antioxidants) that can influence folate bioavailability (37). For the most part, we observe consistent findings for dietary, total intake, and circulating levels of folate.

In addition, because of the prospective design, recall or selection biases are unlikely to explain our findings. In addition, inclusion of only individuals with a clear colonoscopy in this prospective clinical trial allowed us to assess the effect of folates on incident rather than prevalent adenomas, and thereby to make clear the temporal relationships between intake and adenoma occurrence. Furthermore, the high follow-up rates in this study (3) minimize the concern that differential rates of follow-up affected our results. Uniform, blinded follow-up also prevented differential ascertainment of end points according to folate intake. Furthermore, the randomized assignment of folic acid substantially reduces the possibility of confounding.

In this study, we provide no evidence that folic acid supplementation is beneficial in reducing risk of new adenomas even among individuals with low baseline folates. In addition, we show suggestive evidence that the protective association of higher folate status is limited to individuals who are not randomized to folic acid treatment group. These results add to the growing literature regarding the potential multimodal effects of folic acid supplementation.

Disclosure of Potential Conflicts of Interest

Wyeth provided study agents for the Aspirin/Folate Polyp Prevention Trial.

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