Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2008 October ; 17(10): 2625–2631. doi: 10.1158/1055-9965.EPI-08-0382.

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 red blood cell (RBC) folate levels. Individuals were followed for 3 years (1st follow up) and subsequently for an additional 3-5 years (2nd 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 while moderate doses of folate may be protective compared to deficiency, at some point of sufficiency supplementation provides no additional benefit.

Keywords

Folate; dietary; plasma; folic acid; supplementation; colorectal adenomas; clinical trial

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 pre-malignant 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 six 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 to those with lower dietary folate intake or circulating levels. Secondly, based on previous observational studies, we hypothesized that there may be a protective association of higher dietary or circulating folates with risk among individuals in the placebo group, but not in the folic acid treatment group.

Methods

Study Design

The Aspirin/Folate Polyp Prevention Study (AFPPS) 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 mg/day and 325 mg/day aspirin with placebo and comparing 1 mg/day folic acid with placebo. Originally, the trial was designed to investigate only aspirin, but shortly after enrolment 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: one or more histologically confirmed adenomas removed within 3 months before recruitment, one or more histologically confirmed adenomas removed within 16 months before recruitment and a lifetime history of two or more confirmed adenomas, or a histologically confirmed adenoma at least 1 cm in diameter removed within 16 months before recruitment. After completion of a 3-month aspirin run-in period, compliant individuals who wished to continue participating were randomized in a 1:1 ratio to 1 mg per day of folic acid or placebo within strata defined by study center, sex and age (60 years or younger vs. older

than 60 years). The period of treatment and follow-up was originally planned to be 3 years (first follow-up interval); however, because of concern that longer exposure to folic acid might be required to observe an anti-neoplastic effect, participants were asked to continue protocol folate treatment for a second colonoscopic surveillance cycle (usually 3 or 5 years) (second follow-up interval). Thus, two surveillance intervals were included in this analysis. When a surveillance colonoscopy was not performed at the end of the first follow-up interval, we used the last examination at least one 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 lifestyle 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 non-fasting participants into 7-ml EDTA Vacutainer brand tubes. Plasma levels of folate were determined by a microbiological assay using a colistin sulphate 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). Red blood cell (RBC) folate was determined by the ACS:180® folate assay, a competitive immunoassay using direct chemiluminescent technology (Bayer Corporation, Tarrytown NY). Plasma folate was conducted at BEVITAL AS, Bergen, Norway.

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 2 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 centimeter 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. Over-dispersed 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, 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 lifestyle 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 (years), but since there was no significant change in the estimates of risk we did not include this variable in final models. Models for the 2nd 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 prior to and after December 31, 1996 (mid point between years folic acid was introduced in U.S. 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, 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 less then 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 (rho values range: 0.16-0.64, p values<0.0001). Plasma folate levels increased significantly from baseline to first follow-up in the placebo group (mean ± SD = 23.7±17.0 vs. 29.9±14.3 nmol/L, p<0.0001) and much more markedly with folic acid treatment (mean ± SD=24.1±18.2 vs. 74.5 ± 35.8 nmol/L, p<0.0001).

Association of folic acid treatment and risk of colorectal adenomas stratified by baseline dietary, total intake, 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, non-significant 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 RR at the lowest (RR=0.85, 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 for interaction = 0.01). For advanced lesions, the RR's for study folic acid treatment were statistically similar across tertiles of folate status. During the second follow-up interval, the folic acid RR's did not differ by baseline folate status. When we stratified by baseline multivitamin use, results were broadly similar among multivitamin users and non-users (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 for trend = 0.01) and for plasma folate levels (RR for third tertile versus first = 0.72, 95% CI, 0.54-0.97, p for 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 three years of the study, there was observational evidence of an inverse association of folate 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 endpoint.

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 prior to the late 1990's, when fortification of cereal grains with folic acid in the U.S. 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 non-multivitamin 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 non-significant 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 while folate at moderate doses may be protective compared to 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 appeared 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 appeared 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 up-regulation of folate receptors (29) compared to 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 (DHFR). But while small oral doses of folic acid are efficiently reduced, intakes above 400 μg can saturate DHFR's ability 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 non-specific 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 to those with lower dietary folate intake or circulating levels. Possible explanations include the lack of statistical power or a threshold effect. Powers et al. 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 (33). Their data suggest than 1,200 μg of folic acid/day for 45 days does not elicit any additional significant increase in colon folate over 400 μg of folic acid/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 (FPGS) 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 clinically important endpoint. We used a validated semi-quantitative 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 pre-fortification and one post-fortification, our mean baseline levels are more similar to post-fortification levels (serum: 26.9 nmol/L and RBC: 590 nmol/L) (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, since 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 endpoints 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.

Acknowledgements

This project has been funded in part with federal funds (N01-CO-12400, R01-CA-059005, U54-CA-100971) from the National Cancer Institute, National Institutes of Health. J.C.F. is supported in part by a post-PhD Research Fellowship from the National Cancer Institute of Canada (#017602). We thank all the individuals who participated in this clinical trial.

This work was supported in part by funding (R01-CA-059005, U54-CA-100971) from the National Cancer Institute, National Institutes of Health.

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Table 1

Selected characteristics of the study participants[§]

Characteristic	1st Follow-up	2nd 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%)

[§] counts do not necessarily add to the total sum due to missing data.

[¥] using standard definitions by Polyp Prevention Study Group (3, 10);

Table 2

Folic acid treatment at 1mg/day and risk of any adenomas and advanced lesions by baseline dietary, total intake, plasma and RBC folate levels[§]

	1st Follow-up				2nd Follow-up			
	Placebo (n=486)	Folic Acid (n= 501)	RR (95% CI)	p-value[¥]	Placebo (n=373)	Folic Acid (n=403)	RR (95% CI)	p-value[¥]
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: 353.1-1285.6 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: 353.1-1285.6 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: 552.0-1807.8 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: 552.0-1807.8 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 adenoma								
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: 450.0-1133.0 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: 450.0-1133.0 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)	

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

[‡] Adjusted for age, sex, center, duration of follow-up, multivitamin use, aspirin treatment group;

[*] Ranges are based on 2000 calories/day;

[¥] p-value for heterogeneity;

§ counts do not necessarily add to the total sum due to missing data.

Table 3

Association of baseline dietary, total intake, plasma and RBC folate levels and risk of any colorectal adenomas by folic acid treatment group[§]

	1st Follow-up				2nd 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: 353.1-1285.6 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-value for trend		0.26		0.60		0.51		0.05
p-value for 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: 552.0-1807.8 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-value for trend		0.01		0.41		0.60		0.25
p-value for 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-value for trend		0.03		0.54		0.84		0.52
p-value for 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: 450.0-1133.0 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-value for trend		0.21		0.96		0.02		0.20
p-value for heterogeneity		0.60				0.50		

[¥] 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;

[‡] Adjusted for age, sex, center, duration of follow-up, aspirin treatment group, multivitamin use;

[*] Ranges are based on 2000 calories/day;

[§] counts do not necessarily add to the total sum due to missing data.