Folic Acid for the Prevention of Colorectal Adenomas
A Randomized Clinical Trial

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Context Laboratory and epidemiological data suggest that folic acid may have an antineoplastic effect in the large intestine.

Objective To assess the safety and efficacy of folic acid supplementation for preventing colorectal adenomas.

Design, Setting, and Participants A double-blind, placebo-controlled, 2-factor, phase 3, randomized clinical trial conducted at 9 clinical centers between July 6, 1994, and October 1, 2004. Participants included 1021 men and women with a recent history of colorectal adenomas and no previous invasive large intestine carcinoma.

Intervention Participants were randomly assigned in a 1:1 ratio to receive 1 mg/d of folic acid (n=516) or placebo (n=505), and were separately randomized to receive aspirin (81 or 325 mg/d) or placebo. Follow-up consisted of 2 colonoscopic surveillance cycles (the first interval was at 3 years and the second at 3 or 5 years later).

Main Outcome Measures The primary outcome measure was occurrence of at least 1 colorectal adenoma. Secondary outcomes were the occurrence of advanced lesions ($\geq$25% villous features, high-grade dysplasia, size $\geq$1 cm, or invasive cancer) and adenoma multiplicity (0, 1-2, or $\geq$3 adenomas).

Results During the first 3 years, 987 participants (96.7%) underwent colonoscopic follow-up, and the incidence of at least 1 colorectal adenoma was 44.1% for folic acid (n=221) and 42.4% for placebo (n=206) (unadjusted risk ratio [RR], 1.04; 95% confidence interval [CI], 0.90-1.20; P=.58). Incidence of at least 1 advanced lesion was 11.4% for folic acid (n=57) and 8.6% for placebo (n=42) (unadjusted RR, 1.32; 95% CI, 0.90-1.92; P=.15). A total of 607 participants (59.5%) underwent a second follow-up, and the incidence of at least 1 colorectal adenoma was 41.9% for folic acid (n=127) and 37.2% for placebo (n=113) (unadjusted RR, 1.13; 95% CI, 0.93-1.37; P=.23); and incidence of at least 1 advanced lesion was 11.6% for folic acid (n=35) and 6.9% for placebo (n=21) (unadjusted RR, 1.67; 95% CI, 1.00-2.80; P=.05). Folic acid was associated with higher risks of having 3 or more adenomas and of noncolorectal cancers. There was no significant effect modification by sex, age, smoking, alcohol use, body mass index, baseline plasma folate, or aspirin allocation.

Conclusions Folic acid at 1 mg/d does not reduce colorectal adenoma risk. Further research is needed to investigate the possibility that folic acid supplementation might increase the risk of colorectal neoplasia.

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FOLIC ACID AND ITS DERIVATIVES (folate) are essential nutrients in humans and play an important role in nucleotide synthesis and methylation reactions.[1] Folate deficiency leads to macrocytic anemia, and abundant evidence indicates that even in well-nourished western populations, folate supplementation reduces the risk of neural tube defects.[2]

Considerable epidemiological evidence suggests that a low-folate diet is associated with an increased risk of colorectal neoplasia,[3-5] particularly in concert with alcohol, which can antagonize the metabolism of folate.[6,7] Much animal data support an antineoplastic effect of folate. However, in some animal studies, folate deficiency protects against, and supplementation increases, experimental carcinogenesis.[3]

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Nevertheless, on the whole, the biological and epidemiological evidence supports the potential for folate supplementation to prevent colorectal neoplasia in humans.

Adenomas are precursors of most colorectal cancers[8,9] and are an appropriate end point for assessing efficacy of chemopreventive agents against the development of large intestine cancer. To evaluate the chemopreventive effect of folate in humans, we conducted a randomized trial of folic acid supplementation, with and without aspirin, for the prevention of new colorectal adenomas in persons with a recent history of these lesions.

METHODS
Study Design
The study design has been previously described.[10] In brief, the Aspirin/Folate Polyp Prevention Study was a double-blind, placebo-controlled, randomized clinical trial of the efficacy of oral aspirin, folic acid, or both to prevent colorectal adenomas in persons with a history of adenomas. Using a 3×2 factorial design, our study compared 81 mg/d and 325 mg/d of aspirin with placebo and 1 mg/d of 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 findings regarding aspirin have been previously reported.[10] In brief, we reported that low-dose aspirin (81 mg/d) had a moderate, statistically significant chemopreventive effect, reducing the risk of colorectal adenomas by 19%, while high-dose aspirin (325 mg/d) provided no significant benefit.[10] This article focuses on folic acid.

The folic acid investigation was initially designed to parallel the investigation for aspirin, evaluating a 3-year treatment period. However, because longer exposure to folic acid might be required to observe an antineoplastic effect,[11] participants who underwent a colonoscopy during the first follow-up interval were invited to continue their blinded randomized treatment (folic acid or placebo) for an additional 3 or 5 years. Because of the expected rapid effect of aspirin, and the anticipated difficulty in maintaining adherence, we did not attempt to prolong the aspirin investigation. Our analysis of folic acid includes both follow-up intervals.

The trial involved 9 clinical centers (Cleveland Clinic Foundation, Cleveland, Ohio; University of Colorado Health Sciences Center, Denver; Dartmouth-Hitchcock Medical Center, Lebanon, NH; Henry Ford Health Sciences Center, Detroit, Mich; University of Iowa College of Medicine, Iowa City; University of Minnesota, Minneapolis; University of North Carolina School of Medicine, Chapel Hill; University of Southern California, Los Angeles; and University of Toronto, Toronto, Ontario). An independent data and safety monitoring committee reviewed the study semiannually. Human subjects committees at the clinical centers approved the study protocol and materials distributed to the participants. All participants provided written informed consent.

Study Population, Randomization, and Interventions
Recruitment occurred between July 6, 1994, and March 20, 1998. Potential participants were identified by clinical center staff using colonoscopy and pathology reports. Those participants eligible were aged 21 to 80 years and had at least 1 of the following criteria: at least 1 histologically confirmed adenoma removed within 3 months before recruitment, at least 1 histologically confirmed adenoma removed within 16 months before recruitment and a lifetime history of 2 or more confirmed adenomas, or a histologically confirmed adenoma of at least 1 cm in diameter removed within 16 months before recruitment. We required that each participant had a complete colonoscopy, with removal of all known polyps, within 3 months of enrollment. Exclusion criteria included a history of familial polyposis syndromes, invasive large intestine cancer, malabsorption syndromes, any condition that could be worsened by supplemental aspirin or folic acid, and any condition commonly treated with aspirin, nonsteroidal anti-inflammatory drugs, or folate (eg, recurrent arthritis, atherosclerotic vascular disease, and folic acid deficiency).[10] To avoid the potential for folate supplementation to mask vitamin B_{12} deficiency,[12] we measured plasma vitamin B_{12} levels in all participants before randomization and excluded those with evidence of deficiency (<162 pg/mL). We also assayed methylmalonic acid in those participants whose vitamin B_{12} levels were marginal (162-366 pg/mL). Participants having increased methylmalonic acid (>396 nmol/L) were not randomized. Additionally, participants who required baseline methylmalonic acid testing, and who were in the highest quintile of acceptable methylmalonic acid at baseline, were retested before treatment continuation in the second follow-up interval. Women of childbearing potential had to provide agreement to use effective birth control for the duration of the study.

Participants completed a questionnaire regarding personal characteristics, medical history, and lifestyle habits. To describe the population of participants, the questionnaire inquired about race/ethnicity using the following categories: non-Hispanic white, non-Hispanic black, Hispanic, American Indian or Alaskan Native, Asian or Pacific Islander, other (participants specified using a text field), and uncertain (no participants selected this category).

After recruitment, participants began a 3-month, single-blind, run-in period to assess tolerance of aspirin and adherence with pill taking. During this run-in period, participants received 325 mg/d of aspirin and a placebo tablet identical in appearance to the folic acid tablets. Run-in participants who reported taking at least 80% of the tablets and who wished to continue participating underwent randomization. We used a computer-generated randomization in blocks of 6 to allocate participants in a 1:1 ratio to 1 mg/d of folic acid or placebo within strata de-

fined by study center, sex, and age ($\leq$60 years vs >60 years). The study was double-blinded. Treatment assignments were concealed from participants and study staff except for the pharmacist technician and the statistical analyst (L.A.M.). Study tablets (provided by Wyeth Consumer Health Care, Madison, NJ) were distributed in calendar packs or bottles. Placebo tablets were identical in appearance to folic acid tablets. On October 1, 2004, funding for the folic acid treatment was discontinued, and participants were asked to stop taking all study tablets by October 1, 2004. As originally planned, extended treatment and follow-up would have ended December 31, 2006.

Follow-up

Adenoma occurrence was determined by colonoscopy and pathology review. Follow-up was conducted in 2 intervals. The first interval corresponded to the initial 3-year protocol, in which participants were to undergo a complete colonoscopy 34 to 40 months after the qualifying examination. The planned length of the second follow-up interval was at the discretion of the participant's physician and was generally 3 or 5 years. Whenever possible, participants who discontinued taking study tablets were followed up (in both intervals) for study end points.

Participants were regularly counseled regarding avoidance of folic acid–containing supplements (as well as avoidance of aspirin and other nonsteroidal anti-inflammatory drugs during the first study interval). During the first follow-up interval, participants received questionnaires every 4 months regarding adherence to study treatment; use of medications, over-the-counter drugs, and nutritional supplements; intestine procedures (in particular endoscopy and surgery); and the occurrence of symptoms, illnesses, and hospitalizations. During the second follow-up interval, questionnaires were administered every 4 months to participants who continued taking study tablets and annually to all other participants.

Important medical events reported by participants were verified with medical record review. The follow-up period for such events was from randomization until study withdrawal or October 1, 2004, whichever occurred first. Records for all large intestine procedures (endoscopy or surgery) were obtained. Slides for all tissue removed from the intestine were retrieved and sent to a single study pathologist (D.C.S.) for uniform review who classified lesions as neoplastic (adenomatous, including sessile serrated adenomas[13]) or nonneoplastic.

The primary outcome measure was the occurrence of at least 1 colorectal adenoma. Prespecified secondary outcomes were advanced lesions (tubulovillous adenomas [25%-75% villous features], villous adenomas [$\geq$75% villous features], large adenomas [$\geq$1 cm in diameter], adenomas with high-grade dysplasia, or invasive cancer), adenoma multiplicity (0, 1-2, or $\geq$3 adenomas), and adverse events.

Plasma folate was evaluated at the end of the first follow-up interval to ascertain adherence with randomized treatment. Plasma levels of vitamin B_{12} and folate were determined by microbiological assays using a chloramphenicol-resistant strain of *Lactobacillus casei* and colistin-sulfate resistant strain of *Lactobacillus leichmannii*, respectively.[14,15] Methylmalonic acid[16] and total plasma homocysteine[17] were assayed by gas chromatography/mass spectroscopy.

Statistical Analysis and Sample Size

Fisher exact and *t* tests were used for comparisons between groups in categorical and continuous variables, respectively. Analyses of adenoma occurrence were performed for each of the 2 follow-up intervals and for the 2 intervals combined. The period of risk for the first follow-up interval was from 1 year after randomization through the 3-year examination (including findings from interim examinations, if any). If a 3-year follow-up colonoscopy was not performed, the last examination at least 1 year after randomization was used to

mark the end of the first follow-up interval. The second follow-up interval pertained to participants who underwent a 3-year examination and was defined as the time from the end of the first interval through the first subsequent surveillance colonoscopy or until October 1, 2004, whichever was earlier.

The predefined primary statistical analysis was a χ^2 test comparing the risk of 1 or more adenomas in the 2 treatment groups. Unadjusted risk ratios (RRs) and 95% confidence intervals (CIs) were also used to compare folic acid with placebo. Adjusted RRs were obtained from generalized linear models in which age, sex, clinical center, number of lifetime adenomas, duration of follow-up, and aspirin treatment group were covariates. These models used a natural logarithm link function and Poisson distributed errors and were adjusted for overdispersion and underdispersion. The possibility that baseline factors modified the folic acid effect was assessed using interaction terms and Wald tests. For example, to evaluate whether randomized aspirin allocation modified the effect of folic acid, we fit the regression model, including indicator variables for folic acid and aspirin dose as well as appropriate interaction terms. We then used a 2-*df* Wald test to test for interaction. The following potential effect modifiers were considered: sex, alcohol consumption (users vs nonusers), smoking status (current and former users vs nonusers), presence of advanced lesions (none vs $\geq$1) at baseline examinations, and the following covariates disaggregated at the median: age (<57 vs $\geq$57 years), plasma folate level ($\leq$8.4 vs >8.4 ng/mL [$\leq$19.0 vs >19.0 nmol/L]), and body mass index (calculated as weight in kilograms divided by height in meters squared, $\leq$26.7 vs >26.7). Adenoma multiplicity was assessed by grouping participants as having 0, 1 to 2, or 3 or more adenomas and using the χ^2 test to compare the treatment groups.

We performed analyses on the intention-to-treat population consisting of all randomized participants who un-

derwent a follow-up examination, including those who discontinued randomized supplementation. In addition, we used multiple imputation[18] to estimate the folic acid effect after imputing missing examination data. The imputation used logistic regression to model adenoma occurrence with the following baseline covariates: age, sex, clinical center, number of lifetime adenomas, aspirin treatment, and folic acid treatment. We imputed a sufficient number of complete data sets to achieve more than 99% relative efficiency and combined the results using established methods[18] to provide summary RRs, 95% CIs, and *P* values. Finally, we considered the subset of participants who continued their randomized treatment into the second follow-up interval. Two-sided *P*<.05 was considered statistically significant. All statistical analyses were performed by using SAS version 9.1 (SAS Institute, Cary, NC) and Stata version 9 (StataCorp LP, College Station, Tex).

During final data review, we discovered that 3 participants did not satisfy all initial eligibility criteria. Two participants had low vitamin B_{12} levels (<162 pg/mL) but were tested for methylmalonic acid, and their methylmalonic acid levels were in the acceptable range. One participant was enrolled on the basis of having had an adenoma of at least 1 cm removed within 16 months of recruitment, but the actual size of the adenoma was less than 1 cm. Six participants received treatment in the second follow-up interval despite either increased methylmalonic acid (n=2) or no methylmalonic acid testing (n=4). We analyzed our primary outcome measure both including and excluding these participants and found no appreciable difference in the results. Therefore, we included these participants in all analyses reported herein.

A sample size of 1000 participants was selected to provide power of at least 80% to detect a risk reduction with as-pirin (25% reduction with low-dose and 55% reduction with high-dose) or folic acid (40% reduction) using a 2-sided statistical significance level of *P*<.05. This assumed a 35% adenoma occurrence rate in the placebo group and a follow-up rate of 80%. The power to detect a 40% decrease in risk with folic acid supplementation was 94%.

RESULTS
Participants, Follow-up, and Adherence

Of the 1409 participants who began the run-in period, 1021 underwent randomization in both the folic acid and aspirin components of the study (**FIGURE 1**). An additional 100 participants were randomized only to the aspirin component and are thus excluded from this analysis. Of the 288 participants who were not randomized, 73 (25.3%) had bleeding or another possible adverse event, 62 (21.5%) were unable to avoid taking medication or supplements prohibited by the study, 34 (11.8%) were nonadherent, and the remaining 119 (41.3%) were excluded for other reasons (1 participant died, 64 were found to be ineligible, 28 had an intercurrent illness, and 26 declined to continue the study). A total of 505 participants were randomized to the placebo group and 516 participants were randomized to 1 mg/d of folic acid (with or without aspirin). A total of 987 participants (96.7%) underwent a follow-up colonoscopy at least 1 year following randomization during the first follow-up interval. The remaining 34 participants either died (n=9) or were lost to follow-up (n=25). The mean (SD) time from randomization to completion of the first follow-up interval was 32.7 (3.6) months. A total of 926 participants (90.7%) participated in the second follow-up interval (729 [71.4%] continued randomized folic acid/placebo treatment and 197 [19.3%] discontinued study tablets but agreed to be followed up for study end points). One participant was discontinued from randomized treatment due to a high methylmalonic acid level and was followed up observationally. During the

Figure 1. Design of the Trial and Flow of Participants
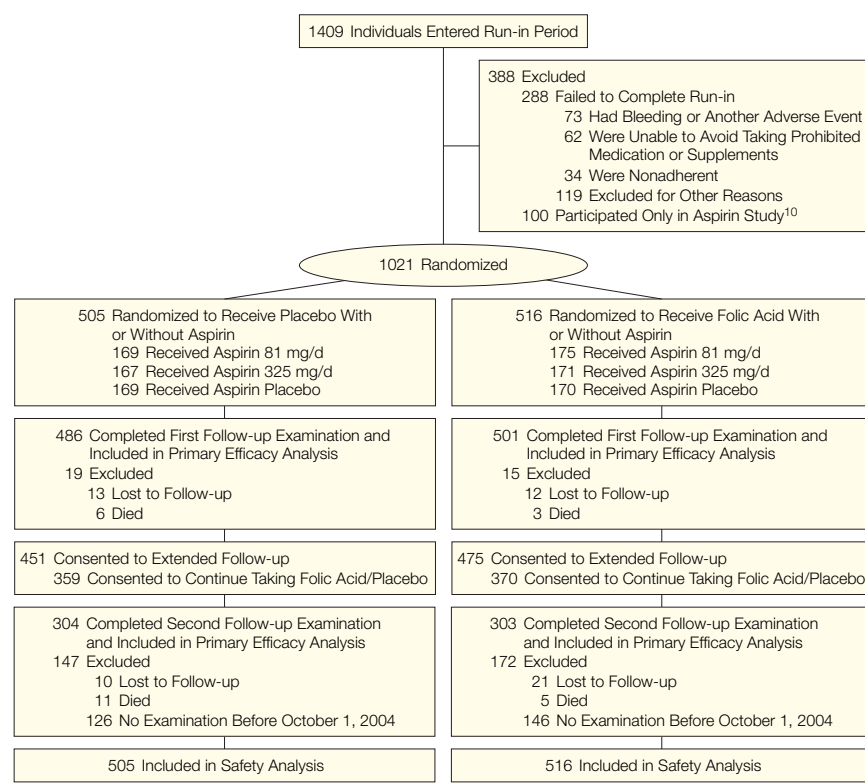
second follow-up interval, 16 participants died, 31 were lost to follow-up, and 272 did not have an examination before treatment was discontinued on October 1, 2004. The remaining 607 participants (59.5%) completed the second follow-up interval a mean (SD) of 41.8 (11.8) months after the 3-year follow-up. Approximately, 50% of participants in the second follow-up interval had planned to undergo colonoscopic follow-up 4 or less years after the 3-year examination.

There were no important differences in the baseline characteristics between the folic acid and placebo groups (TABLE 1). Reported adherence with the study protocol was excellent (TABLE 2). Overall, 87% of participants took their allocated study pills at least 6 days per week during the first follow-up interval. Pill taking adherence decreased during the second follow-up interval, with 71% of participants taking study pills at least 6 days per week. A significant portion of this decrease was due to lack of consent to extended protocol treatment. Avoidance of nonstudy folic acid supplements was also excellent, with 87% of participants avoiding them completely during the first follow-up interval. The use of folic acid supplements was not restricted among those participants who did not consent to extended protocol treatment. Thus, reported avoidance of folic acid decreased to 73% during the second follow-up interval.

Allocation to the folic acid group resulted in a pronounced increase in plasma folate and a modest decrease in total plasma homocysteine. Follow-up measurements (at the 3-year examination) for both folate and homocysteine were available from 419 participants in the placebo group and 430 participants in the folic acid group. Mean (SD) plasma folate increased from 10.4 (7.5) ng/mL at baseline to 13.2 (6.3) ng/mL at follow-up in the placebo group and from 10.5 (7.9) ng/mL to 32.8 (15.8) ng/mL in the folic acid group ($P<.001$). Mean (SD) plasma homocysteine decreased from 1.32 (0.39) mg/L to 1.24 (0.34) mg/L in the pla-

cebo group and from 1.34 (0.40) mg/L to 1.21 (0.30) mg/L in the folic acid group ($P=.02$). Among participants in the folic acid group, mean plasma folate decreased as self-reported adherence decreased. At the 3-year examination, the mean (SD) plasma folate was 33.3 (15.4) ng/mL among the 383 participants in the folic acid group who reported taking study tablets 6 to 7 days per week and 25.7 (18.5) ng/mL among the 31 participants in the folic acid group who reported taking study tablets less than 6 days per week ($P=.009$).

Primary Outcome Measure

In the first follow-up interval, adenomas occurred in 206 participants (42.4%) in the placebo group and 221 participants (44.1%) in the folic acid group (unadjusted RR, 1.04; 95% CI, 0.90-1.20; $P=.58$) (TABLE 3). In the second follow-up interval, adenomas occurred in 113 participants (37.2%) in the placebo group and 127 participants (41.9%) in the folic acid group (unadjusted RR, 1.13; 95% CI, 0.93-1.37; $P=.23$). Among the 607 participants with end point information in

Table 1. Baseline Characteristics of the Participants*

Characteristic	Placebo (n = 505)	Folic Acid (n = 516)	P Value
Age, mean (SD), y	57 (9.5)	57 (9.6)	.97
Male sex	321 (63.6)	330 (64.0)	.95
Race/ethnicity†			
Non-Hispanic white	431 (85.3)	443 (85.9)	
Non-Hispanic black	35 (6.9)	28 (5.4)	.54
Hispanic	22 (4.4)	30 (5.8)	
Asian, Pacific Islander, or other	17 (3.4)	15 (2.9)	
BMI, mean (SD)	27.4 (4.5)	27.5 (4.6)	.81
Current cigarette smoker‡	68 (13.6)	79 (15.4)	.42
No. of previous lifetime adenomas			
1-2	347 (68.9)	363 (70.8)	.54
≥3	157 (31.2)	150 (29.2)	
No. of adenomas on examinations qualifying for study entry			
1-2	439 (86.9)	450 (87.2)	.93
≥3	66 (13.1)	66 (12.8)	
Advanced lesion on examinations qualifying for study entry	139 (27.5)	151 (29.3)	.58
Colorectal cancer in first-degree relative	154 (37.9)	159 (38.5)	.89
Using multivitamins	191 (37.8)	176 (34.1)	.24
Dietary intake, mean (SD), kcal/d	1632 (654)	1637 (674)	.91
Dietary folate intake, mean (SD), µg/d	325 (163)	320 (147)	.62
Alcohol intake, mean (SD), drinks per d	0.6 (1.1)	0.6 (1.1)	.92
Plasma folate, mean (SD), ng/mL	10.4 (7.5)	10.5 (7.9)	.89
Total plasma homocysteine, mean (SD), mg/L	1.32 (0.39)	1.34 (0.40)	.62
Specific criteria for study entry§			
Adenoma removed ≤3 mo before recruitment	496 (98.2)	506 (98.1)	>.99
Adenoma removed ≤16 mo before recruitment and ≥2 lifetime adenomas	265 (52.5)	270 (52.3)	>.99
Adenoma ≥1 cm in diameter removed ≤16 mo before recruitment	99 (19.6)	127 (24.6)	.06

Abbreviation: BMI, body mass index, calculated as weight in kilograms divided by height in meters squared.
SI conversions: To convert plasma folate to nmol/L, multiply by 2.266; and plasma homocysteine to µmol/L, multiply by 7.397.
*Data are presented as No. (%) unless otherwise specified. Percentages do not always sum to 100 due to rounding. Data were missing for BMI for 2 participants, smoking status for 4 participants, number of previous lifetime adenomas for 4 participants, family history of colorectal cancer for 202 participants, dietary intake for 46 participants, alcohol intake for 48 participants, plasma folate level for 118 participants, and total plasma homocysteine for 116 participants.
†Self-reported on the participant questionnaire. "Other" was specified by the participant and consisted of "American Indian or Alaska native" (2 in placebo and 2 in folic acid groups), "South African" (1 in placebo group), "white/American Indian" (1 in placebo group), "Chinese/Italian" (1 in folic acid group), "Middle Eastern" (1 in folic acid group), or "Asian/black" (1 in folic acid group).
‡Defined as someone who currently smokes at least 1 cigarette per day and has a history of smoking at least 1 cigarette per day for at least 1 year.
§Categories are not mutually exclusive; many participants qualified under more than 1 criterion.

both follow-up intervals, the adenoma rate (any adenoma in either interval) was 65.5% in the placebo group and 71.3% in the folic acid group (RR, 1.09; 95% CI, 0.98-1.21; $P=.12$). Adjustment for age, sex, clinical center, length of follow-up, number of lifetime adenomas at baseline, and randomized aspirin treatment did not substantially affect these findings. Results using multiple imputation to account for missing follow-up examinations were also similar.

We evaluated the folic acid effect in subgroups defined by the following baseline factors: sex, age, alcohol intake, smoking status, plasma folate level, body mass index, and presence/absence of advanced lesions. There

was no significant effect modification in either follow-up interval. We also found no significant interaction between folic acid and randomized aspirin treatment ($P=.24$) (FIGURE 2). However, the suggestion of an increased risk with folic acid was confined to participants not allocated to aspirin.

The effect of folic acid supplementation among the 501 participants who agreed to continue pill taking (folic acid or placebo) in the second follow-up interval (254 allocated to the placebo group and 247 allocated to the folic acid group) was similar to that in the intention-to-treat analysis. Within this subgroup, adenomas occurred in 92 participants (36.2%) in the placebo group

and 106 participants (42.9%) in the folic acid group (RR, 1.18; 95% CI, 0.95-1.47; $P=.13$).

Secondary Outcome Measures

In both follow-up intervals, participants in the folic acid group tended to have higher rates of advanced adenomas and multiple adenomas (Table 3). In the first follow-up interval, advanced lesions occurred in 42 participants (8.6%) in the placebo group and 57 participants (11.4%) in the folic acid group (unadjusted RR, 1.32; 95% CI, 0.90-1.92; $P=.15$). The respective numbers in the second follow-up interval were 21 (6.9%) and 35 (11.6%) for both groups (unadjusted RR, 1.67; 95% CI, 1.00-2.80; $P=.05$). Among participants with follow-up information in both intervals, the overall rate of advanced lesions (any advanced lesion in either interval) was 17.1% in the placebo group and 23.1% in the folic acid group (RR, 1.35; 95% CI, 0.98-1.86; $P=.07$). Randomized aspirin treatment did not significantly modify the effect of folic acid on advanced adenomas in the first follow-up interval ($P=.34$) (Figure 2).

Three or more adenomas occurred in 38 participants (7.8%) in the placebo group and 47 participants (9.4%) in the folic acid group in the first follow-up interval (unadjusted RR, 1.20; 95% CI, 0.80-1.81; $P=.38$). The respective rates in the second follow-up interval were 13 (4.3%) and 30 (9.9%) for both groups (unadjusted RR, 2.32; 95% CI, 1.23-4.35; $P=.007$).

Table 2. Percentage Self-reported Adherence With Study Treatment and Avoidance of Folate Supplements, According to Treatment Assignment and Study Follow-up Period*

| | Reported Frequency of Tablet Taking, % | | | |
| | First Follow-up Interval | | Second Follow-up Interval | |
	Placebo (n = 486)	Folic Acid (n = 501)	Placebo (n = 304)	Folic Acid (n = 303)
Adherence with study treatment, d per wk				
6-7	87.4	87.2	72.4	69.0
3-5	6.2	5.6	4.9	5.0
<3	3.9	3.2	18.4	19.5
Unknown	2.5	4.0	4.3	6.6
Use of (nonstudy) folic acid supplementation, d per wk				
None	86.0	87.2	71.7	74.3
1-4	6.2	4.8	9.9	11.2
>4	7.4	7.0	18.1	13.5
Unknown	0.4	1.0	0.3	1.0

*Only patients who underwent a follow-up colonoscopy are included. First follow-up interval included the initial 3-year protocol, and the second follow-up interval was 3 or 5 years later. The percentage adherence with study treatment for less than 3 days per week in the second follow-up interval includes 50 patients in the placebo group and 56 patients in the folic acid group who did not consent to extended treatment.

Table 3. Risk of Adenoma After Randomization in the Intention-to-Treat Population*

| | First Follow-up Interval | | | | Second Follow-up Interval | | | |
| | No. (%) of Participants | | | | No. (%) of Participants | | | |
End Point	Placebo (n = 486)	Folic Acid (n = 501)	Unadjusted RR (95% CI)	P Value	Placebo (n = 304)	Folic Acid (n = 303)	Unadjusted RR (95% CI)	P Value
Any adenoma	206 (42.4)	221 (44.1)	1.04 (0.90-1.20)	.58	113 (37.2)	127 (41.9)	1.13 (0.93-1.37)	.23
Advanced lesion	42 (8.6)	57 (11.4)	1.32 (0.90-1.92)	.15	21 (6.9)	35 (11.6)	1.67 (1.00-2.80)	.05
No. of adenomas								
1-2	168 (34.6)	174 (34.7)	1.00 (0.85-1.19)	.66	100 (32.9)	97 (32.0)	0.97 (0.77-1.22)	.02
≥3	38 (7.8)	47 (9.4)	1.20 (0.80-1.81)		13 (4.3)	30 (9.9)	2.32 (1.23-4.35)	

Abbreviations: CI, confidence interval; RR, risk ratio.
*The intention-to-treat population consisted of all randomized participants with a follow-up examination, including those participants who discontinued randomized supplementation. First follow-up interval included the initial 3-year protocol, and the second follow-up interval was 3 or 5 years later. P values are based on χ² tests. For number of adenomas, P values are global for the 3 categories (0, 1-2, and ≥3 adenomas), and separate RRs are shown to summarize the effect of folic acid on each adenoma-multiplicity category.

Results were similar when the analysis was restricted to the 501 participants who agreed to extended treatment with folic acid or placebo in the second follow-up interval. In this subgroup, advanced lesions occurred in 18 participants (7.1%) in the placebo group and 29 participants (11.7%) in the folic acid group (RR, 1.66; 95% CI, 0.95-2.90; P=.08); and 3 or more adenomas occurred in 11 participants (4.3%) in the placebo group and 27 participants (10.9%) in the folic acid group (RR, 2.52; 95% CI, 1.28-4.98; P=.008).

We evaluated the folic acid impact on sessile serrated adenomas and found no significant effect in either follow-up interval. In the first follow-up interval, sessile serrated adenomas occurred in 43 participants (8.9%) in the placebo group and 54 participants (10.8%) in the folic acid group (RR, 1.22; 95% CI, 0.83-1.78; P=.31). In the second follow-up interval, sessile serrated adenomas occurred in 11 participants (3.6%) in the placebo group and 17 participants (5.6%) in the folic acid group (RR, 1.55; 95% CI, 0.74-3.26; P=.25).

No significant association was found between allocation to folic acid and risks of death, colorectal cancer, myocardial infarction, coronary revascularization, or stroke (TABLE 4). A higher rate of noncolorectal cancers was observed among participants allocated to the folic acid group (54 [10.5%] vs 32 [6.3%], respectively; P=.02). This difference was due to an excess of prostate cancer, with 24 cases (7.3%) in the folic acid group and 9 cases (2.8%) in the placebo group (P=.01).

COMMENT

In this double-blind, placebo-controlled, randomized clinical trial, we found that folic acid supplementation did not decrease the risk of adenoma occurrence among participants with a recent history of adenomas. There was no evidence of benefit even among subgroups that might be considered sensitive to the chemopreventive effects of folate, such as those participants with low baseline folate stat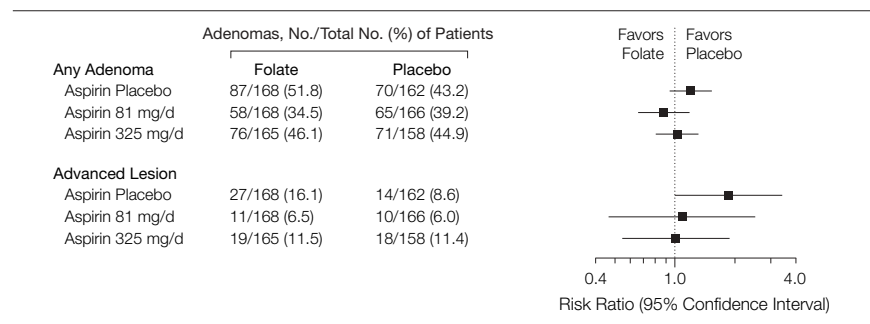us, those participants who drank alcohol, and cigarette smokers. Indeed, there was a suggestion of an increase in risk for advanced lesions and in adenoma multiplicity among those participants randomized to the folic acid group.

Our results may have been affected by fortification of the food supply with folate, which began in 1996, shortly after enrollment in this study was initiated, and became mandatory in 1998, largely to reduce the risks of neural tube defects by increasing maternal folate levels during early pregnancy.[19] The fortification levels (an average of 140 µg per 100 g of grain product) were expected to increase dietary folate intake by 70 to 120 µg/d in middle-aged and older adults.[20] Although this is only a fraction of the amount in our supplement, it is possible that by increasing the floor folate status of our participants, as well as increasing the peak folate levels in those receiving active treatment, fortification may have altered the impact of our supplement. This is consistent with a recent observational study that found that adenoma risk is inversely associated with plasma folate levels only among individuals not taking multivitamins, a group with lower folate status than multivitamin users.[21] The increase in plasma folate and the decrease in total plasma homocysteine over time in our placebo group was likely due to folate fortification and probably underlies the modest change in homocysteine in the folic acid group. These data clearly indicate that we conducted our study in a folate-replete population.

Previous research regarding the effect of folate on carcinogenesis is difficult to integrate into one coherent picture. Animal studies have provided conflicting evidence, suggesting that folate may have a dual effect on carcinogenesis, protecting normal mucosa, but enhancing progression of early lesions.[3] Although some animal studies reported that folate deficiency enhances experimental carcinogenesis,[22,23] other evidence suggests that deficiency may reduce the development of colorectal cancer.[24,25] Similarly, an animal study reported that supplementation above nutritional requirements may limit intestine carcinogenesis,[23] although other findings reported an increase in risk.[25,26] Evidence also exists suggesting that aggressive supple-

Figure 2. Unadjusted Risk Ratios Comparing Folic Acid vs Placebo by Randomized Aspirin Treatment in the Intention-to-Treat Population in the First Follow-up Interval

	Adenomas, No./Total No. (%) of Patients		
Any Adenoma	Folate	Placebo	
Aspirin Placebo	87/168 (51.8)	70/162 (43.2)	
Aspirin 81 mg/d	58/168 (34.5)	65/166 (39.2)	
Aspirin 325 mg/d	76/165 (46.1)	71/158 (44.9)	
Advanced Lesion			
Aspirin Placebo	27/168 (16.1)	14/162 (8.6)	
Aspirin 81 mg/d	11/168 (6.5)	10/166 (6.0)	
Aspirin 325 mg/d	19/165 (11.5)	18/158 (11.4)	

Favors Folate   Favors Placebo

Risk Ratio (95% Confidence Interval) 0.4 1.0 4.0

Table 4. Incidence of Serious Adverse Events After Randomization

	No. (%) of Participants		
Adverse Event	Placebo (n = 505)	Folic Acid (n = 516)	P Value
Death	19 (3.8)	10 (1.9)	.09
Noncolorectal cancer	32 (6.3)	54 (10.5)	.02
Colorectal cancer	4 (0.8)	3 (0.6)	.72
Myocardial infarction	8 (1.6)	14 (2.7)	.28
Coronary revascularization	16 (3.2)	16 (3.1)	>.99
Stroke	5 (1.0)	9 (1.7)	.42

mentation may enhance the growth of established, microscopic lesions.[23] Overall, epidemiological data from cohort and case-control studies have tended to find folate intake to be inversely associated with risks of colorectal cancer[4] and adenomas.[4,27-32] Analyses of red blood cell or serum folate levels also largely suggest a protective effect of folate on the risk of colorectal cancer or adenomas,[31,33-35] although data have not been entirely consistent.[36,37]

One hypothesis to explain the purported chemopreventive properties of folate pertains to DNA methylation, which can have strong effects on gene expression[38] and implications for the susceptibility of DNA to damage.[39] Overall, genomic hypomethylation is one of the earliest molecular abnormalities in human colorectal neoplasia,[40,41] observed even in small adenomas.[40-43] Because folate is an important factor for the transfer of methyl groups, it is critical to DNA methylation. Low folate status may also contribute to imbalances in nucleotide pools, DNA strand breaks, and mutations.[44] The pathway that may be most relevant for the development of colorectal cancer in the setting of methylation disturbances is the serrated polyp, which is associated with a higher prevalence of CpG island methylator phenotype[45]; however, we found no folic acid effect on sessile serrated adenoma occurrence.

We found no clear evidence that folic acid supplementation provided any health benefits. Although a significant excess of prostate cancers was observed in the folate group, this might be spurious given the number of adverse events evaluated. A recent randomized trial of folic acid in combination with B vitamins for vascular disease also suggested that treatment with these agents may increase the risk of colon cancer (RR, 1.36; 95% CI, 0.89-2.08; $P=.16$) and prostate cancer (RR, 1.21; 95% CI, 0.86-1.72; $P=.28$), although these results were not statistically significant.[46] Observational studies regarding prostate cancer have been mixed. Some studies reported an increased risk of prostate cancer in association with high folate in-

take or blood levels, but these results were not statistically significant or were limited to early-stage cancers.[47-49] A case-control study reported a statistically significant, favorable role of dietary folate on prostate cancer risk.[50]

In conclusion, our study indicates that folate, when administered as folic acid for up to 6 years, does not decrease the risk of adenoma formation in the large intestine among individuals with previously removed adenomas. The evidence for an increased risk of adenomas is equivocal and requires further research. In view of the fortification of the US food supply with folate, and some suggestions that folate could conceivably increase the risk of neoplasia even outside the colorectum,[51-55] this line of investigation should have a high priority.

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REFERENCES

1. Herbert V. Folic Acid. In: Shils M, Olson J, Shike M, Ross AC, eds. *Nutrition in Health and Disease.* Baltimore, Md: Williams & Wilkins; 1999.
2. Shaw GM, Schaffer D, Velie EM, Morland K, Harris JA. Periconceptional vitamin use, dietary folate, and the occurrence of neural tube defects. *Epidemiology.* 1995;6:219-226.
3. Kim Y-I. Folate, colorectal carcinogenesis and DNA methylation: lessons from animal studies. *Environ Mol Mutagen.* 2004;44:10-25.
4. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. *J Nutr.* 2002; 132(suppl):2350S-2355S.
5. Harnack L, Jacobs DR, Nicodemus K, Lazovitch D, Anderson K, Folsom AR. Relationship of folate vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. *Nutr Cancer.* 2002;43:152-158.
6. Giovannucci E, Stampfer MJ, Colditz GA, et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. *J Natl Cancer Inst.* 1993;85:875-884.
7. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst.* 1995;87:265-273.
8. Peipins LA, Sandler RS. Epidemiology of colorectal adenomas. *Epidemiol Rev.* 1994;16:273-297.
9. Neugut AI, Jacobson JS, DeVivo I. Epidemiology of colorectal adenomatous polyps. *Cancer Epidemiol Biomarkers Prev.* 1993;2:159-176.
10. Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med.* 2003;348:891-899.
11. Giovannucci E, Stampfer MJ, Colditz GA, et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med.* 1998;129:517-524.
12. Mills JL. Fortification of foods with folic acid: how much is enough? *N Engl J Med.* 2000;342:1442-1445.
13. Snover DC. Serrated polyps of the large intestine. *Semin Diagn Pathol.* 2005;22:301-308.
14. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol.* 1997;281:43-53.
15. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. *J Clin Pathol.* 1991;44:592-595.
16. Rasmussen K. Solid-phase sample extraction for rapid determination of methylmalonic acid in serum and urine by a stable-isotope-dilution method. *Clin Chem.* 1989;35:260-264.

17. Windelberg A, Arseth O, Kvalheim G, Ueland PM. Automated assay for the determination of methylmalonic acid, total homocysteine, and related amino acids in human serum or plasma by means of methylchloroformate derivatization and gas chromatography-mass spectrometry. *Clin Chem.* 2005;51:2103-2109.
18. Little RJA, Rubin DB. *Statistical Analysis With Missing Data.* New York, NY: John Wiley & Sons; 1987.
19. Final Rule on Food Labeling, Health Claims and Label Statements, Folic Acid and Neural Tube Defects, 58 *Federal Register* 2606 (1993).
20. Food Standards: Amendment of the Standards of Identity for Enriched Grain Products to Require Addition of Folic Acid, 58 *Federal Register* 53305-53312 (1993).
21. Martínez ME, Giovannucci E, Jiang R, et al. Folate fortification, plasma folate, homocysteine and colorectal adenoma recurrence. *Int J Cancer.* 2006;119:1440-1446.
22. Cravo ML, Mason JB, Dayal Y, et al. Folate deficiency enhances the development of colonic neoplasia in dimethylhydrazine-treated rats. *Cancer Res.* 1992;52:5002-5006.
23. Kim Y-I, Salomon RN, Graeme-Cook F, et al. Dietary folate protects against the development of macroscopic colonic neoplasia in a dose responsive manner in rats. *Gut.* 1996;39:732-740.
24. Le Leu RK, Young GP, McIntosh GH. Folate deficiency reduces the development of colorectal cancer in rats. *Carcinogenesis.* 2000;21:2261-2265.
25. Song J, Medline A, Mason JB, Gallinger S, Kim Y-I. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. *Cancer Res.* 2000;60:5434-5440.
26. Wargovich MJ, Chen CD, Jimenez A, et al. Aberrant crypts as a biomarker for colon cancer: evaluation of potential chemopreventive agents in the rat. *Cancer Epidemiol Biomarkers Prev.* 1996;5:355-360.
27. Benito E, Cabeza E, Moreno V, Obrador A, Bosch FX. Diet and colorectal adenomas: a case-control study in Majorca. *Int J Cancer.* 1993;55:213-219.
28. Tseng M, Murray SC, Kupper LL, Sandler RS. Micronutrients and the risk of colorectal adenomas. *Am J Epidemiol.* 1996;144:1005-1014.
29. Baron JA, Sandler RS, Haile RW, Mandel JS, Mott LA, Greenberg ER. Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. *J Natl Cancer Inst.* 1998;90:57-62.
30. Breuer-Katschinski B, Nemes K, Marr A, et al; Colorectal Adenoma Study Group. Colorectal adenomas and diet: a case-control study. *Dig Dis Sci.* 2001;46:86-95.
31. Bird CL, Swendseid ME, Witte JS, et al. Red cell and plasma folate, folate consumption, and the risk of colorectal adenomatous polyps. *Cancer Epidemiol Biomarkers Prev.* 1995;4:709-714.
32. Whelan RL, Horvath KD, Gleason NR, et al. Vitamin and calcium supplement use is associated with decreased adenoma recurrence in patients with a previous history of neoplasia. *Dis Colon Rectum.* 1999;42:212-217.
33. Paspatis GA, Kalafatis E, Oros L, Xourgias V, Koutsioumpa P, Karamanolis DG. Folate status and adenomatous colonic polyps: a colonoscopically controlled study. *Dis Colon Rectum.* 1995;38:64-67.
34. Kato I, Dnistrian AM, Schwartz M, et al. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. *Br J Cancer.* 1999;79:1917-1921.
35. Martínez ME, Henning SM, Alberts DS. Folate and colorectal neoplasia: relation between plasma and dietary markers of folate and adenoma recurrence. *Am J Clin Nutr.* 2004;79:691-697.
36. Glynn SA, Albanes D, Pietinen P, et al. Colorectal cancer and folate status: a nested case-control study

among male smokers. *Cancer Epidemiol Biomarkers Prev.* 1996;5:487-494.
37. Van Guelpen B, Hultdin J, Johansson I, et al. Low folate levels may protect against colorectal cancer. *Gut.* 2006;55:1461-1466.
38. Razin A, Cedar H. DNA methylation and gene expression. *Microbiol Rev.* 1991;55:451-458.
39. Pogribny IP, Basnakian AG, Miller BJ, Lopatina NG, Poirier LA, James SJ. Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. *Cancer Res.* 1995;55:1894-1901.
40. Bedford MT, van Helden PD. Hypomethylation of DNA in pathological conditions of the human prostate. *Cancer Res.* 1987;47:5274-5276.
41. Gama-Sosa MA, Slagel VA, Trewyn RW, et al. The 5-methylcytosine content of DNA from human tumors. *Nucleic Acids Res.* 1983;11:6883-6894.
42. Goelz SE, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science.* 1985;228:187-190.
43. Feinberg AP, Gehrke CW, Kuo KC, Ehrlich M. Reduced genomic 5-methylcytosine content in human colonic neoplasia. *Cancer Res.* 1988;48:1159-1161.
44. Blount BC, Ames BN. DNA damage in folate deficiency. *Baillieres Clin Haematol.* 1995;8:461-478.
45. Kambara T, Simms LA, Whitehall VL, et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut.* 2004;53:1137-1144.
46. Lonn E, Yusuf S, Arnold MJ, et al; Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med.* 2006;354:1567-1577.
47. Stevens VL, Rodriguez C, Pavluck AL, McCullough ML, Thun MJ, Calle EE. Folate nutrition and prostate cancer incidence in a large cohort of US men [published online ahead of print March 22, 2006]. *Am J Epidemiol.* 2006;163:989-996.
48. Hultdin J, Van Guelpen B, Bergh A, Hallmans G, Stattin P. Plasma folate, vitamin B12 and homocysteine and prostate cancer risk: a prospective study. *Int J Cancer.* 2005;113:819-824.
49. Weinstein SJ, Hartman TJ, Stolzenberg-Solomon R, et al. Null association between prostate cancer and serum folate, vitamin B6, vitamin B12 and homocysteine. *Cancer Epidemiol Biomarkers Prev.* 2003;12:1271-1272.
50. Pelucchi C, Galeone C, Talamini R, et al. Dietary folate and risk of prostate cancer in Italy. *Cancer Epidemiol Biomarkers Prev.* 2005;14:944-948.
51. Bills ND, Hinrichs SH, Morgan R, Clifford AJ. Delayed tumor onset in transgenic mice fed a low-folate diet. *J Natl Cancer Inst.* 1992;84:332-337.
52. Baggott JE, Vaughn WH, Juliana MM, Eto I, Krumdieck CL, Grubbs CJ. Effects of folate deficiency and supplementation on methylnitrosourea-induced rat mammary tumors. *J Natl Cancer Inst.* 1992;84:1740-1744.
53. Kotsopoulos J, Medline A, Renlund R, et al. Effects of dietary folate on the development and progression of mammary tumors in rats. *Carcinogenesis.* 2005;26:1603-1612.
54. Charles D, Ness AR, Campbell D, Smith GD, Hall MH. Research pointers: taking folate in pregnancy and risk of maternal breast cancer. *BMJ.* 2004;329:1375-1376.
55. Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF, et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr.* 2006;83:895-904.