Folate intake and risk of colorectal cancer and adenoma: modification by time[1–4]

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ABSTRACT

Background: Experimental and observational studies have suggested that folate may play dual roles in colorectal cancer risk depending on the timing and dose.

Objective: We examined the latency between folate intake and the incidence of colorectal cancer.

Design: We prospectively examined associations between folate intake assessed every 2 to 4 y by using validated food-frequency questionnaires and risk of colorectal cancer and adenoma in the Nurses' Health Study and Health Professionals Follow-Up Study, which included 2299 incident colorectal cancers and 5655 colorectal adenomas from 1980 to 2004.

Results: There was an association between total folate intake 12–16 y before diagnosis and lower risk of colorectal cancer (relative risk: 0.69; 95% CI: 0.51, 0.94; $\geq$800 compared with $<$250 μg folate/d), but there was no association between intake in the recent past and colorectal cancer risk. Long- and short-term intakes of total folate were associated with a lower risk of colorectal adenoma, with a strong association with intake 4–8 y before diagnosis (odds ratio: 0.68; 95% CI: 0.60, 0.78; $\geq$800 compared with $<$250 μg folate/d). The current use of multivitamins for $>$15 y, but not a shorter duration of use, was associated with lower risk of colorectal cancer; and a shorter duration of use was related to lower risk of adenoma. We did not observe an adverse effect of total folate or synthetic folic acid on risk of colorectal cancer or adenoma even during the folic acid fortification era.

Conclusion: Folate intake is inversely associated with risk of colorectal cancer only during early preadenoma stages. *Am J Clin Nutr* 2011;93:817–25.

INTRODUCTION

Folate plays an essential role in one-carbon metabolism as a carrier of single-carbon units, including participation in DNA methylation and DNA biosynthesis (1–3). Some findings have suggested that, although folate intake may decrease risk of early lesions of colorectal neoplasia, high intakes of folate after the neoplastic lesion develops may increase risk of colorectal cancer (4, 5). This has raised concern that fortification of flour and uncooked cereal grains with synthetic folic acid may have increased the incidence of colorectal cancer (6–8). However, detailed epidemiologic analyses of the temporal relation of folate intake to the diagnosis of colorectal adenoma and carcinoma are lacking. Also, to our knowledge, no epidemiologic studies have separately examined natural folate and synthetic folic acid.

We previously examined the associations of baseline intakes of total (from foods and supplements) and dietary (from foods) folate to risks of colorectal cancer and adenoma in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) during 6–20 y of follow-up (9–12). Inverse associations were seen, but our previous analyses only included a single measurement of baseline folate intake. Because we have obtained repeated assessments of folate intake every 2 to 4 y and have extended the follow-up $\leq$24 y, we were able to examine the latency between total folate intake, dietary folate (from foods), natural folate (from foods only and not including folic acid from fortified foods or supplements), and folic acid (from fortified foods and supplements) and diagnosis and higher intakes because of fortification.

SUBJECTS AND METHODS

Study population

The NHS was established in 1976 when 121,700 female registered nurses who were 30–55 y of age returned a mailed questionnaire. The HPFS was initiated in 1986 when 51,529 male health professionals aged 40–75 y returned a mailed questionnaire. Participants provided detailed information about their medical histories, lifestyles, and risk factors for chronic diseases on biennial follow-up questionnaires. For the analyses presented in the current study, follow-up started in 1980 in the NHS and in 1986 in the HPFS when the collection of dietary information

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began. The follow-up rates for both study populations were >90% of potential person-time.

For colorectal cancer and adenoma analyses, we excluded participants who did not return the baseline food-frequency questionnaire (FFQ), had been previously diagnosed with cancer (except nonmelanoma skin cancer), left an extensive number of items blank on the baseline FFQs, reported an implausible energy intake at baseline (<500 or >3500 kcal/d for women and <800 or >4200 kcal/d for men), or had ulcerative colitis. The analyses of colorectal adenomas were further limited to participants who reported having undergone a colonoscopy or sigmoidoscopy because adenomas are mostly asymptomatic and, therefore, diagnosed during routine screening or for symptoms not related to adenomas. As a result, 87,861 women in the NHS and 47,290 men in the HPFS were included in the colorectal cancer analyses; and 50,637 women and 29,015 men were included in the adenoma analyses. The NHS was approved by the Institutional Review Board of the Brigham and Women's Hospital (Boston, MA); the HPFS was approved by the Institutional Review Board of the Harvard School of Public Health (Boston, MA).

Dietary and nondietary assessment

Dietary information was collected from NHS participants by using validated FFQs in 1980, 1984, and 1986 and every 4 y thereafter and from HPFS participants every 4 y since 1986. Participants were asked how frequently, on average, during the past year they consumed one standard serving of a specific food item in 9 categories. Responses on frequencies of a specified serving size for each food item were converted to average daily intakes. We calculated folate intake from foods and supplements (total intake), dietary intake from unfortified and fortified foods, and synthetic folic acid intake from supplements and folic acid–fortified foods, taking into account mandatory fortification since 1998. Natural folate intake from foods only was also calculated by subtracting the synthetic folic acid intake from the total folate intake. To calculate the folic acid intake, we took into account the brand and type of multivitamins and the brand of breakfast cereal over the entire follow-up period, folic acid supplements assessed from 1984, and other fortified grains assessed from 1998. Quantities of folate and other nutrients from foods were calculated by multiplying the reported frequency of each food by the nutrient content of one serving of that food primarily on the basis of the US Department of Agriculture Nutrient Database (13) that corresponded to each time that FFQs were administered, taking into account the fortification of folic acid, and then energy adjusted by using the nutrient residual method (14). The duration of multivitamin use was calculated on the basis of the reported duration at baseline and updated by using subsequent responses to current multivitamin use.

The folate intake from the FFQ completed before mandatory fortification has been shown to predict erythrocyte folate concentrations before fortification in these cohorts (Pearson's correlation coefficient: 0.55 in the NHS and 0.56 in the HPFS) (9).

Information on weight, physical activity, aspirin use, endoscopy, postmenopausal hormone use (in the NHS), and menopausal status (in the NHS) was updated almost every 2 y. Family histories of colorectal cancer in parents and siblings were elicited in the 1982 questionnaire and were updated in 1988, 1992, 1996, and 2000 in the NHS and in 1986, 1990, 1992, and 1996 in the HPFS. The average number of cigarettes smoked per day in the age range of <15, 15–19, and 20–29 y (in the HPFS), the age when subjects started to smoke, the average number of cigarettes smoked per day in the first 5 y, the age when subjects last smoked in the past (in the NHS), and height were assessed at baseline.

Ascertainment of colorectal cancer and adenomas

Self-reported information on new diagnoses of colorectal cancer or polyps was obtained on each questionnaire; participants (or next of kin for participants who died) who reported a diagnosis of colorectal cancer or polyps were asked for permission to access medical records related to the diagnosis. The National Death Index (15) was also used to identify fatalities. Investigators blinded to the risk factor status of participants reviewed medical records of colorectal cancers or polyps. For the adenoma analyses, by using pathologic records, polyps were classified as adenomatous, hyperplastic, or other nonadenomatous. Only adenomatous polyps were included as cases. We considered only adenomas of the distal colon and rectum as cases because we assumed that a substantial proportion of the procedures performed early in the study period may have been sigmoidoscopies, which only reached the descending colon and the sigmoid colon. We included the first adenomas in this analysis. A total of 2299 (1312 in the NHS and 987 in the HPFS) cases were included in the colorectal cancer analyses, and 5655 (3101 in the NHS and 2554 in the HPFS) cases were included in the colorectal adenoma analyses. We performed a sensitivity analysis by including all adenomatous polyps (4419 adenomatous polyps in the NHS and 3729 adenomatous polyps in the HPFS) for total folate intake and showed that the results were similar to those in the analysis that included only distal and rectal adenomas (data not shown).

Statistical analyses

We calculated the study-specific, age- and energy-adjusted least-square means and SEs of intakes of total, dietary, and natural folate and synthetic folic acid with the SAS PROC GLM procedure (SAS 9.1; SAS Institute Inc, Cary, NC). For colorectal cancer analyses, we calculated the relative risks (RRs) and 95% CIs for each study by using the Cox proportional hazards model (16) with SAS PROC PHREG (SAS 9.1; SAS Institute Inc) (17). Person-years of follow-up were calculated from the date that the baseline questionnaire was returned to the date of colorectal cancer diagnosis, date of death, or end of follow-up (31 May 2004 for women and 31 January 2004 for men), whichever came first. We stratified the data by age in months at the start of follow-up and calendar year of the current questionnaire cycle. We evaluated whether the proportional hazards assumption was satisfied by adding interaction terms between age and cumulative average total folate intake and showed that the terms were not statistically significant; thus, the assumption was satisfied. In the multivariate models for both the colorectal cancer and adenoma analyses, we also adjusted for possible risk factors listed in footnotes to the tables. For the confounding variables, we used cumulative average dietary intakes; nondietary covariates were updated every 2 y. We presented only multivariate results here. The magnitude of the inverse association in the age-adjusted analysis was generally slightly stronger than the multivariate results (data not shown).

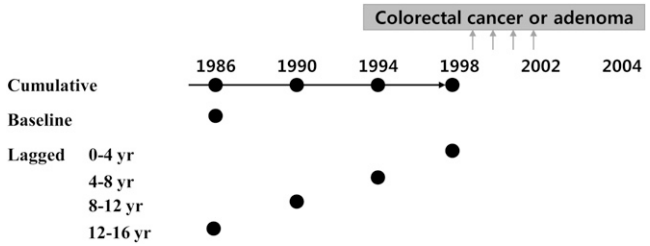

FIGURE 1. Analysis of folate intake in relation to colorectal cancer and adenoma (eg, the assessment of folate to predict colorectal cancer or adenoma diagnosed between 1998 and 2002). ●, food-frequency questionnaires used for folate intake.

When we further adjusted for intakes of vitamin B-6, vitamin B-12, and methionine in the baseline analysis of colorectal cancer and the simple update analysis of colorectal adenoma, the results did not appreciably change (data not shown).

For the colorectal adenoma analyses, we calculated odds ratios (ORs) and 95% CIs of colorectal adenoma by using the multiple logistic regression model (SAS PROC LOGISTIC; (SAS 9.1; SAS Institute Inc) (17) to account for the presence or absence of prevalent adenoma at the time of endoscopy.

For both the colorectal cancer and adenoma analyses, we combined study-specific $\log_e$ RRs (ORs) by using a random-effects model (18–20). The heterogeneity between the 2 studies was assessed by the Q statistic (20, 21). To calculate the P value for the test for trend, participants were assigned the median value of intake category, and this variable was treated as a continuous term.

Participants were categorized by using absolute cutoffs or quantiles on the basis of the distribution of folate intake. We analyzed baseline folate intakes, varying lag-time folate intakes, and cumulative average folate intakes (**Figure 1**). For baseline analyses in the NHS, we used the average folate intake of the 1980 and 1984 FFQs in the NHS, in which we used 1984 as the starting year to use the increased comprehensiveness of the 1984 FFQ. To evaluate the latency between folate intake and colorectal cancer, we performed analyses by using varying lag times. For example, in the NHS, for a latency of 0–4 y before diagnosis (simple update), we used folate intake in 1980 for follow-up from 1980 to 1984, intake in 1984 for follow-up from 1984 to 1986, intake in 1986 for follow-up from 1986 to 1990, and so forth. For a latency of 4–8 y, we used folate intake in 1980 for cases diagnosed in 1984–1988, intake in 1984 for cases diagnosed in 1988–1990, and so forth. In the adenoma analysis, the same method was applied to cases and noncases on the basis of the most recent endoscopy year. Cumulative average folate intakes were calculated from 1980 in the NHS and from 1986 in the HPFS, when the first FFQs were administered. For example, in the NHS, folate intake in 1980 was used for cases diagnosed in 1980–1984, the average intake in 1980–1984 was used for

TABLE 1
Folate intake and supplement use in the Nurses' Health Study (NHS) in 1980–2002 and in the Health Professionals Follow-Up Study (HPFS) in 1986–2002

	Year						
	1980	1984	1986	1990	1994	1998	2002
Intake (μg/d)[1]							
NHS							
Total folate intake	368 (0.9)	384 (1.0)	403 (1.0)	430 (1.1)	457 (1.1)	638 (1.2)	764 (1.2)
Dietary folate intake	260 (0.4)	274 (0.4)	287 (0.4)	310 (0.5)	306 (0.5)	392 (0.5)	443 (0.5)
Natural folate intake	241 (0.3)	245 (0.3)	250 (0.3)	265 (0.4)	261 (0.4)	278 (0.4)	293 (0.4)
Synthetic folic acid intake	158 (1.0)	146 (1.1)	157 (1.1)	169 (1.2)	197 (1.3)	358 (1.3)	469 (1.4)
HPFS							
Total folate intake	—	—	479 (1.4)	506 (1.6)	528 (1.7)	718 (1.9)	858 (2.0)
Dietary folate intake	—	—	356 (0.6)	373 (0.7)	353 (0.8)	463 (0.8)	522 (0.9)
Natural folate intake	—	—	309 (0.5)	317 (0.5)	302 (0.6)	333 (0.6)	354 (0.7)
Synthetic folic acid intake	—	—	193 (1.3)	202 (1.5)	230 (1.7)	386 (1.8)	503 (2.0)
Supplement use[2]							
NHS							
Multivitamins	34	37	42	38	47	61	69
Folic acid supplements	—	1.6	1.2	0.8	1.4	7.0	9.8
HPFS							
Multivitamins	—	—	42	39	49	60	67
Folic acid supplements	—	—	2.8	1.1	1.8	7.5	13
Supplement sources[3]							
NHS							
Multivitamins	29	26	26	26	30	32	35
Folic acid supplements	—	1.7	1.2	0.7	1.2	4.1	5.0
HPFS							
Multivitamins	—	—	23	25	31	31	32
Folic acid supplements	—	—	2.4	0.9	1.5	4.4	6.1

[1] Values are study-specific, age- and energy-adjusted least-square means; SEMs in parentheses. Total folate intake was from foods and supplements. Dietary folate intake was from fortified and unfortified foods. Natural folate intake was derived from unfortified foods alone. Synthetic folic acid was derived from folic acid–fortified foods and supplements.

[2] Values are study-specific, age-standardized percentages of population who took supplements.

[3] Values are percentage contributions of multivitamins and folic acid supplements toward total folate intake.

TABLE 2
Risk of colorectal cancer according to folate intake in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS)[1]

	Baseline	Simple update (0–4-y lag)	4–8-y lag	8–12-y lag	12–16-y lag	Cumulative average
No. of cases (NHS, HPFS)	1069, 987	1120, 813	1010, 684	829, 477	645, 277	1312, 987
Total folate[2]						
<250 μg/d	1.00	1.00	1.00	1.00	1.00	1.00
250 to <400 μg/d	0.91 (0.76, 1.08)	0.96 (0.82, 1.12)	0.94 (0.70, 1.27)	0.91 (0.78, 1.06)	0.83 (0.70, 0.99)	0.95 (0.81, 1.12)
400 to <600 μg/d	0.86 (0.69, 1.06)	1.00 (0.84, 1.19)	0.83 (0.70, 0.99)	0.88 (0.73, 1.06)	0.82 (0.50, 1.36)	0.90 (0.78, 1.04)
600 to <800 μg/d	0.84 (0.69, 1.03)	0.92 (0.77, 1.10)	0.85 (0.71, 1.02)	0.91 (0.75, 1.11)	0.73 (0.57, 0.94)	0.84 (0.70, 1.00)
≥800 μg/d	0.74 (0.60, 0.92)	0.88 (0.72, 1.07)	0.92 (0.75, 1.13)	0.88 (0.68, 1.13)	0.69 (0.51, 0.94)	0.85 (0.68, 1.08)
P for trend[3]	0.02	0.19	0.67	0.45	0.04	0.07
P for heterogeneity[4]	0.91	0.57	0.98	0.94	0.36	0.50
Dietary folate[2,5]						
<250 μg/d	1.00	1.00	1.00	1.00	1.00	1.00
250 to <300 μg/d	1.02 (0.90, 1.16)	0.95 (0.82, 1.12)	0.97 (0.82, 1.16)	1.08 (0.93, 1.27)	0.91 (0.76, 1.09)	1.07 (0.94, 1.22)
300 to <400 μg/d	1.04 (0.90, 1.19)	0.99 (0.81, 1.21)	0.96 (0.83, 1.10)	0.88 (0.66, 1.18)	0.84 (0.70, 1.01)	1.02 (0.89, 1.15)
400 to <500 μg/d	0.89 (0.74, 1.08)	1.13 (0.85, 1.50)	0.92 (0.76, 1.11)	0.95 (0.67, 1.33)	0.92 (0.68, 1.24)	1.00 (0.83, 1.21)
≥500 μg/d	0.86 (0.67, 1.10)	1.03 (0.81, 1.31)	0.91 (0.72, 1.16)	0.82 (0.58, 1.18)	0.60 (0.39, 0.91)	1.07 (0.82, 1.38)
P for trend[3]	0.32	0.51	0.49	0.43	0.01	0.92
P for heterogeneity[4]	0.91	0.26	0.98	0.23	0.83	0.99
Natural folate[2,5]						
<200 μg/d	1.00	1.00	1.00	1.00	1.00	1.00
200 to <250 μg/d	0.93 (0.81, 1.07)	1.24 (0.94, 1.63)	1.00 (0.84, 1.19)	0.99 (0.84, 1.18)	0.93 (0.77, 1.12)	1.09 (0.79, 1.51)
250 to <300 μg/d	0.96 (0.83, 1.11)	1.10 (0.92, 1.31)	0.92 (0.78, 1.08)	0.96 (0.80, 1.15)	0.91 (0.74, 1.13)	1.00 (0.86, 1.16)
300 to <400 μg/d	1.02 (0.87, 1.20)	1.18 (1.00, 1.40)	0.92 (0.78, 1.09)	0.98 (0.81, 1.19)	0.89 (0.71, 1.11)	1.08 (0.92, 1.27)
≥400 μg/d	0.94 (0.74, 1.18)	1.23 (0.81, 1.89)	1.05 (0.83, 1.32)	0.96 (0.72, 1.27)	0.85 (0.60, 1.19)	1.13 (0.87, 1.46)
P for trend[3]	0.83	0.16	0.92	0.53	0.08	0.66
P for heterogeneity[4]	0.98	0.08	0.33	0.34	0.47	0.52
Synthetic folic acid[2]						
<50 μg/d	1.00	1.00	1.00	1.00	1.00	1.00
50 to <100 μg/d	0.87 (0.70, 1.09)	1.03 (0.88, 1.19)	1.04 (0.89, 1.21)	0.81 (0.60, 1.10)	0.77 (0.57, 1.05)	0.89 (0.77, 1.02)
100 to <200 μg/d	0.81 (0.70, 0.95)	0.94 (0.81, 1.10)	0.89 (0.75, 1.05)	0.83 (0.68, 1.02)	0.92 (0.73, 1.17)	0.97 (0.86, 1.10)
200 to <400 μg/d	0.91 (0.79, 1.04)	0.93 (0.80, 1.08)	0.90 (0.77, 1.05)	0.92 (0.77, 1.10)	0.81 (0.52, 1.27)	0.89 (0.78, 1.00)
≥400 μg/d	0.85 (0.75, 0.98)	0.91 (0.77, 1.07)	0.96 (0.76, 1.21)	0.91 (0.77, 1.08)	0.78 (0.64, 0.97)	0.93 (0.81, 1.06)
P for trend[3]	0.03	0.11	0.66	0.40	0.06	0.26
P for heterogeneity[4]	0.36	0.22	0.11	0.39	0.36	0.75

[1] All values are pooled multivariate relative risks; 95% CIs in parentheses. Total folate was from foods and supplements. Follow-up years were 1980–2004 in the NHS and 1986–2004 in the HPFS for the 0–4-y lag, 1984–2004 in the NHS and 1990–2004 in the HPFS for the 4–8-y lag, 1988–2004 in the NHS and 1994–2004 in the HPFS for the 8–12-y lag, and 1992–2004 in the NHS and 1998–2004 in the HPFS for the 12–16-y lag. Natural folate was derived from foods alone. Synthetic folic acid was derived from supplements and folic acid–fortified foods.

[2] A Cox proportional hazards model was used and adjusted for age, calendar year, pack-years of smoking before age 30 y (never smoker, 1–4 pack-years, 5–10 pack-years, or ≥11 pack-years of smoking), physical activity (quintiles of metabolic equivalent tasks/wk), aspirin dose (never, past, or current use of 1–2, 3–5, 6–14, or ≥15 tablets/wk), height (continuous), BMI (in kg/m^2; <23, 23 to <25, 25 to <30, 30 to <35, or ≥35), family history of colorectal cancer in parents and siblings (yes or no), menopausal status and hormone therapy use (only in women; premenopausal women and never use, past use, or current use in postmenopausal women), history of endoscopy (yes or no), red meat intake (quintiles), alcohol intake (never and 0.1–9.9, 10–14.9, 15–29.9, or ≥30 g alcohol/d), calcium intake from foods (continuous), and total energy intake (continuous).

[3] Two-sided; calculated by using Wald's test statistic.

[4] Two-sided; calculated by using the *Q* test statistic.

[5] Additionally adjusted for multivitamin use (never; past; current: 1–5, 6–9, 10–15, 16–19, or ≥20 y).

cases diagnosed in 1984–1986, and so forth. To examine whether the association for total folate intake varied by alcohol intake (ie, nondrinkers and 0.1–14.9 and ≥15 g alcohol/d) and family history of colorectal cancer and whether the association for natural folate intake varied by multivitamin use (ie, never, past, or current use), we used a mixed-effects metaregression model (22); a 2-sided Wald's test statistic was used to test the null hypothesis that there was no modification of the folate-colorectal cancer association by levels of the potential effect modifiers.

All statistical tests were 2-sided, and $P < 0.05$ was considered significant. We used SAS 9.1 (SAS Institute Inc) for analyses.

RESULTS

Folate intake over time

Overall, total folate intake from foods and supplements have increased over the past 2 decades, particularly between intake assessed by using 1994 FFQs and intake assessed by using 1998 FFQs because of mandatory folic acid fortification since January 1998 (**Table 1**). Percentage increases in the age- and energy-adjusted mean of intake assessed in 1994 to that in 1998 were 40% in the NHS and 36% in the HPFS for total folate intake, 28% in the NHS and 31% in the HPFS for dietary folate intake, and 82% in the NHS and 68% in the HPFS for

TABLE 3
Risk of colorectal adenoma according to folate intake in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS)[1]

	Baseline	Simple update (0–4-y lag)	4–8-y lag	8–12-y lag	12–16-y lag	Cumulative average
No. of cases (NHS, HPFS)	2740, 2553	2813, 2293	2731, 1925	2561, 1470	2138, 1005	3101, 2554
Total folate[2]						
<250 μg/d	1.00	1.00	1.00	1.00	1.00	1.00
250 to <400 μg/d	0.99 (0.80, 1.21)	1.00 (0.89, 1.13)	1.05 (0.95, 1.16)	1.05 (0.95, 1.16)	0.99 (0.89, 1.09)	0.97 (0.87, 1.08)
400 to <600 μg/d	1.00 (0.85, 1.17)	1.03 (0.91, 1.17)	0.91 (0.81, 1.02)	0.99 (0.88, 1.11)	1.01 (0.89, 1.14)	0.93 (0.70, 1.22)
600 to <800 μg/d	0.87 (0.64, 1.19)	0.85 (0.71, 1.03)	0.82 (0.67, 1.00)	0.93 (0.82, 1.05)	0.82 (0.71, 0.94)	0.75 (0.55, 1.03)
≥800 μg/d	0.85 (0.65, 1.11)	0.75 (0.59, 0.95)	0.68 (0.60, 0.78)	0.89 (0.76, 1.03)	0.89 (0.74, 1.06)	0.73 (0.57, 0.94)
P for trend[3]	0.05	0.003	<0.001	0.009	0.008	0.005
P for heterogeneity[4]	0.08	0.10	0.39	0.88	0.37	0.15
Dietary folate[2,5]						
<250 μg/d	1.00	1.00	1.00	1.00	1.00	
250 to <300 μg/d	1.01 (0.77, 1.32)	1.07 (0.81, 1.43)	1.03 (0.93, 1.13)	1.07 (0.97, 1.18)	0.99 (0.88, 1.13)	1.03 (0.85, 1.25)
300 to <400 μg/d	0.97 (0.87, 1.08)	0.97 (0.74, 1.27)	0.91 (0.83, 1.00)	1.01 (0.92, 1.11)	0.96 (0.87, 1.07)	0.94 (0.73, 1.21)
400 to <500 μg/d	0.99 (0.88, 1.12)	0.91 (0.65, 1.29)	0.75 (0.65, 0.87)	0.91 (0.80, 1.04)	0.91 (0.78, 1.05)	0.92 (0.74, 1.14)
≥500 μg/d	1.03 (0.87, 1.20)	0.75 (0.51, 1.11)	0.78 (0.68, 0.90)	1.20 (0.94, 1.52)	1.02 (0.84, 1.24)	0.71 (0.59, 0.85)
P for trend[3]	0.27	0.005	<0.001	0.69	0.41	0.008
P for heterogeneity[4]	0.54	0.004	0.63	0.14	0.44	0.49
Natural folate[2,5]						
<200 μg/d	1.00	1.00	1.00	1.00	1.00	1.00
200 to <250 μg/d	0.89 (0.73, 1.07)	1.00 (0.85, 1.17)	1.00 (0.89, 1.14)	1.01 (0.84, 1.22)	1.04 (0.93, 1.16)	0.98 (0.88, 1.08)
250 to <300 μg/d	0.94 (0.80, 1.12)	0.95 (0.83, 1.07)	0.99 (0.89, 1.10)	1.07 (0.96, 1.19)	0.94 (0.83, 1.06)	1.01 (0.84, 1.22)
300 to <400 μg/d	0.92 (0.83, 1.02)	0.82 (0.73, 0.93)	0.80 (0.71, 0.90)	0.96 (0.85, 1.07)	0.93 (0.82, 1.06)	0.90 (0.75, 1.07)
≥400 μg/d	0.99 (0.85, 1.15)	0.73 (0.55, 0.98)	0.69 (0.43, 1.09)	1.09 (0.92, 1.29)	0.99 (0.81, 1.19)	0.73 (0.61, 0.89)
P for trend[3]	0.75	0.07	0.11	0.91	0.29	<0.001
P for heterogeneity[4]	0.94	0.04	0.001	0.49	0.74	0.56
Synthetic folic acid[2]						
<50 μg/d	1.00	1.00	1.00	1.00	1.00	1.00
50 to <100 μg/d	0.98 (0.89, 1.09)	0.95 (0.85, 1.06)	0.91 (0.79, 1.05)	0.90 (0.74, 1.08)	1.01 (0.85, 1.20)	1.02 (0.93, 1.12)
100 to <200 μg/d	1.02 (0.93, 1.12)	1.01 (0.88, 1.17)	0.86 (0.77, 0.95)	1.06 (0.95, 1.18)	0.96 (0.84, 1.09)	0.97 (0.89, 1.06)
200 to <400 μg/d	0.94 (0.86, 1.03)	0.96 (0.86, 1.06)	0.84 (0.77, 0.93)	0.91 (0.82, 1.00)	0.96 (0.86, 1.08)	0.85 (0.77, 0.94)
≥400 μg/d	0.89 (0.81, 0.98)	0.76 (0.61, 0.95)	0.70 (0.61, 0.80)	0.87 (0.79, 0.97)	0.91 (0.81, 1.03)	0.75 (0.65, 0.87)
P for trend[3]	0.009	0.006	<0.001	0.007	0.10	<0.001
P for heterogeneity[4]	0.67	0.02	0.14	0.62	0.36	0.13

[1] All values are pooled multivariate odds ratios; 95% CIs in parentheses. Follow-up years were 1980–2004 in the NHS and 1986–2004 in the HPFS for the 0–4-y lag, 1984–2004 in the NHS and 1990–2004 in the HPFS for the 4–8-y lag, 1988–2004 in the NHS and 1994–2004 in the HPFS for the 8–12-y lag, and 1992–2004 in the NHS and 1998–2004 in the HPFS for the 12–16-y lag. Total folate was derived from foods and supplements. Natural folate was derived from foods alone. Synthetic folic acid was derived from supplements and folic acid–fortified foods.

[2] A logistic regression model was used and adjusted for age (<50, 50–54, 55–59, 60–64, 65–69, or ≥70 y), endoscopy before baseline (yes or no), recent endoscopy year (continuous), pack-years of smoking before age 30 y (never smoker and 1–4, 5–10, or ≥11 pack-years of smoking), physical activity (quintiles of metabolic equivalent tasks/wk), aspirin dose (never, past, or current use of 1–2, 3–5, 6–14, or ≥15 tablets/wk), height (continuous), BMI (in kg/m^2; <23, 23 to <25, 25 to <30, 30 to <35, or ≥35), family history of colorectal cancer in parents and siblings (yes or no), menopausal status and hormone therapy use (only in women; premenopausal women and never use, past use, or current use in postmenopausal women), red meat intake (quintiles), alcohol intake (never and 0.1–9.9, 10–14.9, 15–29.9, or ≥30 g/d), calcium intake from foods (continuous), total energy intake (continuous), and indication for endoscopy (routine screening compared with other indications)

[3] Two-sided; calculated by using the Wald's test statistic.

[4] Two-sided; calculated by using the *Q* test statistic.

[5] Additionally adjusted for multivitamin use (never; past; current: 1–5, 6–9, 10–15, 16–19, or ≥20 y).

synthetic folic acid intake, whereas natural folate intake increased by <11%. Multivitamins contributed to 23–35% of total folate intake over the entire follow-up period. There was also an overall upward trend in the proportion of participants who took multivitamins or folic acid supplements over the past 2 decades.

Baseline characteristics of participants in the NHS and HPFS according to total folate intake have been presented elsewhere (23). Participants who consumed higher total folate were more likely to be physically active and to take multivitamins and less likely to smoke or eat red meat.

Folate associated with colorectal cancer

In men and women combined, we showed a significant inverse association between baseline total folate intake from foods and supplements and risk of colorectal cancer during the overall follow-up (**Table 2**). We did not observe a significant association for baseline dietary folate (from foods including fortified foods) or natural folate intake (from foods only and not including folic acid from fortified foods). When we examined varying lag-time periods, we showed no associations for lag of 0–4, 4–8, or 8–12 y. However, for the 12–16-y lag, we showed a significant inverse

TABLE 4
Risk of colorectal cancer or adenoma according to multivitamin use in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS)

	Colorectal cancer[1]		Colorectal adenoma[2]	
Duration of multivitamin use[3]	No. of cases (NHS, HPFS)	Values[4]	No. of cases (NHS, HPFS)	Values[5]
Never	430, 303	1.00	785, 670	1.00
Past	279, 199	0.99 (0.82, 1.21)	686, 535	0.96 (0.88, 1.04)
Current				
1–5 y	334, 172	0.96 (0.81, 1.13)	763, 570	0.91 (0.80, 1.03)
6–9 y	86, 88	0.89 (0.75, 1.06)	303, 215	0.73 (0.66, 0.81)
10–15 y	84, 121	0.89 (0.76, 1.05)	233, 293	0.80 (0.72, 0.90)
16–19 y	26, 48	0.71 (0.53, 0.96)	110, 121	0.90 (0.71, 1.14)
≥20 y	73, 52	0.77 (0.64, 0.94)	221, 144	0.76 (0.65, 0.89)

[1] A Cox proportional hazards model was used and adjusted for age, calendar year, pack-years of smoking before age 30 y, physical activity, aspirin dose, height, BMI, family history of colorectal cancer in parents and siblings, menopausal status and hormone therapy use, history of endoscopy, and intakes of red meat, alcohol, calcium from foods, and total energy.

[2] A logistic regression model was used and adjusted for age, endoscopy before baseline, recent endoscopy year, pack-years of smoking before age 30 y, physical activity, aspirin dose, height, BMI, family history of colorectal cancer in parents and siblings, menopausal status and hormone therapy use, red meat intake, alcohol intake, calcium intake from foods, total energy intake, and indication for endoscopy.

[3] Calculated on the basis of the reported duration at baseline and updated by subsequent responses to current multivitamin use.

[4] Values are pooled multivariate relative risks; 95% CIs in parentheses.

[5] Values are pooled multivariate odds ratios; 95% CIs in parentheses.

association for total folate intake and dietary folate intake but no significant association for natural folate intake. Likewise, we showed the strongest inverse association for the 12–16-y lag when we analyzed total folate intake in the quintiles (data not shown). When we included simple updated total folate intake (lag of 0–4 y) and baseline total folate intake in the same model, we still showed an inverse association for baseline total folate intake (P for trend = 0.02), but simple updated total folate intake had no association with colorectal cancer risk as we observed when simple updated intake was analyzed in a separate model. We did not find an inverse association for the cumulative average total, dietary, or natural folate intake with colorectal cancer risk. To examine any adverse effect of high total folate intake, we analyzed study-specific deciles (cutoff points were 646 μg folate/d in the NHS and 817 μg folate/d in the HPFS) by using the cumulative average intake; RRs (95% CI) that compared the top with bottom deciles were 0.91 (0.74, 1.11; P for trend = 0.13) for total folate intake, and 1.06 (0.87, 1.29; P for trend = 0.73) for natural folate intake.

When we examined the association for folic acid from fortified foods and supplements (synthetic folic acid), we also showed that baseline intake and intake assessed 12–16 y before diagnosis were inversely associated with colorectal cancer risk (P for trend = 0.03 and 0.06, respectively; Table 2). When we simultaneously included natural folate intake and synthetic folic acid intake in the same model in the 12–16-y lag-time analysis, we observed similar associations for natural folate and synthetic folic acid intake compared with those that we observed when analyzed in separate models (data not shown).

We also examined the associations for cumulative average total folate intake by limiting the follow-up period to the postfortification era (1998–2004; n = 612 cases). We did not find any increased risk associated with higher folate intake in the postfortification era (RR: 0.85 for ≥800 compared with <400 μg folate/d; 95% CI: 0.64, 1.12; P for trend = 0.61).

Folate associated with colorectal adenomas

We showed a strong inverse association for cumulative average total, dietary, or natural folate intake, but a weaker association for baseline intake, with colorectal adenoma (**Table 3**). When we examined the associations for total folate intake by using varying lag-time periods, we showed the strongest inverse association for total folate intake assessed 0–4 or 4–8 y before adenoma diagnosis, which was similar to that observed in the analysis by using quintiles of total folate (data not shown). We also showed a significant inverse trend of total folate and colorectal adenoma association for the 8–12- and 12–16-y lags. For dietary and natural folate, we showed an inverse association for intake assessed 0–4 or 4–8 y before diagnosis and no association for intake assessed 8–12 or 12–16 y before diagnosis.

When we examined the association for folic acid intake from fortified foods and supplements (synthetic folic acid), we showed stronger inverse associations for cumulative average intake or intake assessed 0–4 or 4–8 y before adenoma diagnosis compared with baseline or intake in the remote past (Table 3). When we simultaneously included natural folate intake and synthetic folic acid intake in the same model in the 0–4- and 4–8-y lag-time analyses, we showed similar associations for both natural folate and synthetic folic acid intake compared with those that we observed when analyzed in separate models (data not shown).

When we limited the follow-up period to the postfortification era (1998–2004; n = 2193 cases), the OR for ≥800 compared with <400 μg folate/d of the cumulative average total folate intake was 0.87 (95% CI: 0.72, 1.04; P for trend = 0.009).

Multivitamin use associated with colorectal cancer and adenomas

Multivitamin use for 16–19 and ≥20 y was associated with a 23–29% lower risk of colorectal cancer (**Table 4**). For colorectal adenomas, a 27% lower risk was noted for 6–9 y of use, and a similar reduction was seen with longer time periods of use.

TABLE 5
Colorectal cancer and adenoma according to folate intake by other factors[1]

Variables	Folate intake categories (μg/d)				*P* for trend[2]	*P* for heterogeneity[3]
	1	2	3	4		
Colorectal cancer[4]						
Baseline total folate (μg/d)	<250	250 to <400	400 to <600	≥600	—	—
Alcohol						
Nondrinkers (*n* = 373)	1.00	0.80 (0.60, 1.06)	0.70 (0.29, 1.67)	0.88 (0.62, 1.26)	0.99	0.94
<15 g/d, 1 drink (*n* = 1223)	1.00	0.91 (0.73, 1.13)	0.89 (0.69, 1.15)	0.78 (0.64, 0.96)	0.05	—
≥15 g/d (*n* = 457)	1.00	1.02 (0.77, 1.34)	0.88 (0.51, 1.52)	0.87 (0.61, 1.23)	0.23	—
Family history of colorectal cancer						
No family history (*n* = 1581)	1.00	0.94 (0.77, 1.14)	0.90 (0.76, 1.05)	0.82 (0.69, 0.99)	0.07	0.77
Family history (*n* = 472)	1.00	0.82 (0.63, 1.07)	0.75 (0.42, 1.33)	0.78 (0.55, 1.10)	0.24	—
Baseline natural folate (μg/d)	<200	200 to <300	300 to <400	≥400	—	—
Multivitamin use						
Never (*n* = 601)	1.00	0.94 (0.71, 1.24)	0.85 (0.59, 1.22)	0.97 (0.44, 2.14)	0.70	0.97
Past (*n* = 432)	1.00	0.83 (0.63, 1.09)	0.97 (0.69, 1.36)	0.81 (0.47, 1.41)	0.62	—
Current (*n* = 962)	1.00	0.95 (0.79, 1.14)	1.09 (0.87, 1.36)	0.96 (0.69, 1.34)	0.76	—
Colorectal adenoma[5]						
Simple updated total folate (μg/d)	<250	250 to <400	400 to <600	≥600	—	—
Alcohol						
Nondrinkers (*n* = 781)	1.00	1.27 (0.95, 1.69)	1.24 (0.91, 1.69)	0.93 (0.69, 1.26)	0.01	0.70
<15 g/d (*n* = 3234)	1.00	0.87 (0.74, 1.01)	0.90 (0.77, 1.06)	0.72 (0.62, 0.85)	<0.001	—
≥15 g/d (*n* = 1092)	1.00	0.99 (0.71, 1.39)	1.07 (0.80, 1.41)	0.86 (0.65, 1.14)	0.03	—
Family history of colorectal cancer						
No family history (*n* = 3815)	1.00	0.98 (0.85, 1.13)	0.99 (0.85, 1.15)	0.81 (0.70, 0.94)	<0.001	0.39
Family history (*n* = 1292)	1.00	0.90 (0.72, 1.14)	1.03 (0.81, 1.32)	0.72 (0.57, 0.91)	<0.001	—
Simple updated natural folate (μg/d)	<200	200 to <300	300 to <400	≥400	—	—
Multivitamin use						
Never (*n* = 1455)	1.00	0.94 (0.80, 1.12)	0.78 (0.62, 0.98)	0.76 (0.53, 1.09)	0.04	0.86
Past (*n* = 1220)	1.00	0.99 (0.80, 1.21)	1.01 (0.79, 1.30)	0.81 (0.53, 1.22)	0.07	—
Current (*n* = 2961)	1.00	1.03 (0.76, 1.39)	0.95 (0.66, 1.36)	0.73 (0.56, 0.95)	<0.001	—

[1] *n*, number of cases. Unless otherwise indicated, all values are pooled multivariate relative risks; 95% CIs in parentheses. We used the baseline folate intake for colorectal cancer and simple updated intake for colorectal adenoma because we considered those as the relevant timing of intakes on the basis of the main results in Tables 2 and 3.

[2] Two-sided; calculated by using Wald's test statistic.

[3] For the highest category. Two-sided and calculated by using Wald's test statistic.

[4] A Cox proportional hazards model was used and adjusted for age, calendar year, pack-years of smoking before age 30 y, physical activity, aspirin dose, height, BMI, family history of colorectal cancer in parents and siblings, menopausal status and hormone therapy use, history of endoscopy, red-meat intake, alcohol intake, calcium intake from foods, and total energy intake.

[5] A logistic regression model was used and adjusted for age, endoscopy before baseline, recent endoscopy year, pack-years of smoking before age 30 y, physical activity, aspirin dose, height, BMI, family history of colorectal cancer in parents and siblings, menopausal status and hormone therapy use, red-meat intake, alcohol intake, calcium intake from foods, total energy intake, and indication for endoscopy.

Folate associated with colorectal cancer and adenomas by other factors

The associations between total folate intake and colorectal cancer or adenomas did not vary significantly by cumulative average alcohol intake (*P* for interaction ≥ 0.70), which interfered with folate metabolism (24), although there was a slightly stronger inverse association for colorectal cancer or adenomas in those who consumed <1 drink/d (**Table 5**). The associations for total folate intake did not vary by family history of colorectal cancer. The associations for natural folate intake (Table 5) or dietary folate intake (data not shown) and risk of colorectal cancer or adenoma did not vary by multivitamin use (*P* for interaction > 0.37).

DISCUSSION

Total folate intake in the remote past was associated with lower risk of colorectal cancer. In contrast, total folate intake close to diagnosis was most strongly associated with lower risk of colorectal adenoma. Because the adenoma-carcinoma sequence is the process underlying the development of most colorectal cancer (25), our findings suggested that higher folate intake may reduce early development of colorectal carcinogenesis.

A pooled analysis of 13 prospective cohort studies showed an RR of 0.92 (95% CI: 0.84, 1.00) for dietary folate from foods and an RR of 0.85 (95% CI: 0.77, 0.95) for total folate intake from foods and supplements in relation to colorectal cancer risk (26). However, the pooled analysis did not address high folate intake (≥800 μg folate/d) because the cutoffs in the highest categories in the studies were not >600 μg folate/d. In our current analysis, we observed lower risk of colorectal cancer for baseline total folate intake but not for the cumulative average intake or intake close to diagnosis of colorectal cancer. For colorectal adenoma, most epidemiologic studies that examined baseline folate intake, including our study with a shorter follow-up (9), observed an inverse association (9, 27–29).

The Aspirin/Folate Polyp Prevention Study reported that treatment with 1 mg folic acid/d did not decrease colorectal adenoma recurrence but increased the number of advanced lesions (30). However, in other trials in the United Kingdom and United States, folic acid supplementation did not increase risk of adenoma recurrence (31, 32). In some animal studies, folate deficiency before the establishment of neoplastic foci promoted the development of colorectal cancer but inhibited the progression of existing neoplastic foci (4, 5). Synthetic folic acid (pteroylmonoglutamate) is the oxidized form of folate used in supplements and fortified food products, which has a higher bioavailability than does naturally occurring folate (pteroylpolyglutamate) (33). Increased folic acid intake resulted in a steady increase in circulating unmetabolized folic acid concentrations (34), which may have some unanticipated effects. For example, recent evidence showed that the activity of natural killer cells was reduced in women with higher plasma concentrations of unmetabolized folic acid (35). However, in our data, we did not observe any evidence of an adverse effect of folate intake on risk of colorectal cancer or first adenoma; instead, our findings suggested that a benefit of folate or synthetic folic acid may be limited to the initiation or early progression of colorectal cancer. Although possibly the inverse association for folate intake in the remote past diluted an adverse effect on colorectal cancer risk of folate intake close to the diagnosis because cumulative average intake was not associated with colorectal cancer risk, correlations between folate intakes years apart were not high. Spearman's correlation coefficients between folate intake in 1986 and in 2002 were 0.27 in the NHS and 0.30 in the HPFS. Also, when we adjusted for baseline folate intake, we saw no increase in risk with recent folate intake. However, because our study had 6 y of follow-up since mandatory folic acid fortification with 2 assessments of folic acid intake, the effect of increased folic acid intake on colorectal neoplasia needs to be further examined in a longer follow-up of postfortification period. As suggested by the Aspirin/Folate Polyp Prevention Study, supplemental folic acid of 1 mg folic acid/d added to high intake in the folic acid fortification era may potentially be harmful (30).

A clear inverse association between total folate intake 12–16 y before diagnosis and colorectal cancer risk suggested that the latency for colorectal cancer associated with folate intake is $\geq$10 y, which is similar to the progression time of adenomas that develop into cancers (36). The strongest inverse association between folate intake and adenoma risk beginning 0–8 y of use was more consistent with a shorter-term influence of folate on adenomas.

We also showed that >15 y of multivitamin use was associated with lower risk of colorectal cancer, as reported previously for colon cancer in the NHS (10), whereas >5 y of use was associated with lower risk of adenoma. The Cancer Prevention Study II Nutrition Cohort also observed lower risk of colorectal cancer in regular multivitamin users 10 y before enrollment (37). However, a recent study showed no association between >10 y of multivitamin use and colorectal cancer (38).

Because our study was an observational study, we cannot rule out the possibility of residual confounding, including components of multiple vitamins other than folic acid. However, we observed a similar pattern for natural folate intake with adjustment for multivitamin use. Also, the comprehensive assessment of known risk factors for colorectal cancer by using multiple questionnaires allowed adjustment for confounding factors. The strengths of our study include repeated measurements, assessment of colorectal cancer and adenoma cases and established risk factors, a large number of cases, and the inclusion of 2 independent cohorts. Also, because of the prospective design and high rates of follow-up, the recall bias and selection bias were unlikely to account for our findings. To our knowledge, because of multiple dietary assessments every 2–4 y beginning in the 1980s, our study is the only prospective cohort study that allowed analysis of the timing of folate intake in relation to progression of colorectal cancer over periods >10 y. Because folate intake in the postfortification era (from 1998) was assessed, we were able to examine total folate intake $\geq$800 μg folate/d. Although folate intake, multivitamin use, and other covariates may be measured with error, the misclassification was relatively low because we biennially collected updated information on multivitamin use and folic acid supplemental use.

In conclusion, we did not find any clear evidence that intake of folate, including folic acid added to foods or used as supplements, increased risk of colorectal cancer. Instead, our study supported the hypothesis that folate intake decreases risk of initiation or early development of colorectal cancer.

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