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Multivitamin Use, Folate, and Colon Cancer in Women in the Nurses' Health Study

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Background: High intake of folate may reduce risk for colon cancer, but the dosage and duration relations and the impact of dietary compared with supplementary sources are not well understood.

Objective: To evaluate the relation between folate intake and incidence of colon cancer.

Design: Prospective cohort study.

Setting: 88 756 women from the Nurses' Health Study who were free of cancer in 1980 and provided updated assessments of diet, including multivitamin supplement use, from 1980 to 1994.

Patients: 442 women with new cases of colon cancer.

Measurements: Multivariate relative risk (RR) and 95% CIs for colon cancer in relation to energy-adjusted folate intake.

Results: Higher energy-adjusted folate intake in 1980 was related to a lower risk for colon cancer (RR, 0.69 [95% CI, 0.52 to 0.93] for intake >400 μg/d compared with intake ≤200 μg/d) after controlling for age; family history of colorectal cancer; aspirin use; smoking; body mass; physical activity; and intakes of red meat, alcohol, methionine, and fiber. When intake of vitamins A, C, D, and E and intake of calcium were also controlled for, results were similar. Women who used multivitamins containing folic acid had no benefit with respect to colon cancer after 4 years of use (RR, 1.02) and had only nonsignificant risk reductions after 5 to 9 (RR, 0.83) or 10 to 14 years of use (RR, 0.80). After 15 years of use, however, risk was markedly lower (RR, 0.25 [CI, 0.13 to 0.51]), representing 15 instead of 68 new cases of colon cancer per 10 000 women 55 to 69 years of age. Folate from dietary sources alone was related to a modest reduction in risk for colon cancer, and the benefit of long-term multivitamin use was present across all levels of dietary intakes.

Conclusions: Long-term use of multivitamins may substantially reduce risk for colon cancer. This effect may be related to the folic acid contained in multivitamins.

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Folate is essential for regenerating methionine, the methyl donor for DNA methylation, and for producing the purines and pyrimidines required for DNA synthesis. Inadequate availability of folate may contribute to aberrations in DNA methylation and may lead to abnormalities in DNA synthesis or repair, either of which may influence colon carcinogenesis. Epidemiologic evidence, including that from two prospective studies done in men (1, 2), suggests that inadequate intake of folate may increase risk for colon cancer (3). Prospective data on women are limited, but one case–control study found a lower risk for colon cancer among women who used supplements that contained folic acid (4). Whether intake from dietary sources higher than that considered adequate to avoid deficiency confers additional benefits is unknown. In addition, the temporal relation between folate status and incidence of colon cancer is unclear. In this report, we examine the temporal and dosage relations between intake of folate, both from supplements and from foods, and risk for colon cancer in women in the Nurses' Health Study. We pay particular attention to the problem of possible confounding by multivitamin use.

Methods

Study Sample

The Nurses' Health Study began in 1976 when 121 700 U.S. female registered nurses 30 to 55 years of age completed a mailed questionnaire on risk factors for cancer and coronary heart disease (5). Every 2 years, we update information and ask women to report newly diagnosed cases of cancer. In 1980, we used a semiquantitative food-frequency questionnaire to establish a "dietary cohort." For this analysis, we excluded women with implausibly high or low scores for total energy intake; those

who left 10 or more items blank on the food-frequency questionnaire; and those who reported previous cancer (other than nonmelanoma skin cancer), ulcerative colitis, or a familial polyposis syndrome. We also excluded women who provided incomplete information on aspirin and multivitamin use in 1980. After exclusions, 88 756 women formed the analytic cohort.

Dietary Assessment

The 1980 semiquantitative food-frequency questionnaire (6, 7) included items on 61 foods and beverages plus vitamin and mineral supplements. Similar but expanded questionnaires were administered in 1984, 1986, and 1990. Current multivitamin use was assessed in each biennial questionnaire from 1980 to 1992. We also asked about the brands and types of breakfast cereal and multivitamins typically used, and we asked women who were current multivitamin users in 1980 to state how many years they had been taking multivitamin supplements. For each food listed, a commonly used unit or portion size was specified. Each woman was asked how often over the past year, on average, she had consumed that amount of each food; she could choose from nine possible responses. We computed nutrient intakes by multiplying the consumption frequency of each unit of every food by the nutrient content of the specified portions by using composition values from U.S. Department of Agriculture sources (8) supplemented with other data, including data on specific brands and types of multivitamins and breakfast cereal.

In addition to giving information on diet, participants provided information on age, weight, height, smoking history, physical activity, aspirin use, colonoscopy or sigmoidoscopy, and parental history of colorectal cancer.

Identification of Cases of Colon Cancer

When a woman (or next of kin for decedents) reported a diagnosis of colon or rectal cancer, we asked for permission to obtain hospital records and pathology reports. The responses to the follow-up questionnaires accounted for 96% of potential person-years through the end of the follow-up period (June 1994). Most deaths were reported by family members or the postal system in response to the follow-up questionnaires or were identified through the National Death Index (9). A study physician who was blinded to exposure information reviewed medical records and extracted pertinent data. We confirmed a total of 655 new cases of colorectal adenocarcinoma (excluding carcinoma in situ). Of these, 442 were in the colon (218 in the proximal colon [cecum to splenic flexure] and 224 in the distal colon), 143 were in the rectum, and 70 were at undetermined sites.

Data Analysis

We analyzed total, supplementary, and dietary intake of folate in relation to risk for colon cancer. Because risk factors for rectal cancers may differ, we did not consider them in the major analyses but report results for them separately. We conducted additional analyses for total colon cancer, including cases of colorectal cancer for which subsite information was unavailable (these cases may have included some cases of rectal cancer), colorectal cancer, proximal colon cancer, and distal colon cancer.

We first examined total and dietary intake of folate in 1980 in relation to risk for colon cancer in the period from 1980 to 1994. Then, to examine the potential time lag between folate intake and risk for colon cancer, we computed the time elapsed since the start of use of multivitamin supplements containing folic acid and updated this variable every 2 years on the basis of the brand and type of multivitamins used and the frequency of multivitamin use reported biennially from 1980 to 1992. For example, if a woman began using multivitamin supplements in 1976, she was considered a user of 4 years in 1980 and a user of 6 years in 1982. Before 1973, 100 μg of folic acid was the maximum dose allowed in supplements according to U.S. Food and Drug Administration (FDA) regulations, and many supplement formulations at that time did not contain folic acid. Thus, we considered 1973 (when doses of 400 μg were first allowed [10]) to be the earliest possible starting point. Although our analysis of duration of use did not require that users report multivitamin consumption on each questionnaire, multivitamin use tended to be consistent. For example, 70% of women who had 15 or more years of use reported multivitamin use on most questionnaires, and 75% of women using multivitamins in 1980 also took multivitamins in 1992.

Our basic model included variables suspected to be related to risk for colon cancer, including cigarette smoking before age 30 years; family history of colorectal cancer; physical activity level; body mass index (kg/m^2); aspirin use; and intakes of red meat (beef, pork, or lamb as a main dish), alcohol, and fiber. Intakes of folate, methionine, and other nutrients were adjusted for total energy intake by using residual analysis (11). We used all variables as assessed in 1980 with the exception of age, which was updated biennially. In additional models, we considered intake of total and saturated fat; intake of calcium; intake of vitamins A, C, D, and E; postmenopausal estrogen use; and history of endoscopic screening.

Women were followed from the month in 1980 in

Table 1. Age-Standardized Characteristics According to Folate Intake in 1980 in Participants in the Nurses' Health Study*

Characteristic	Folate Intake in 1980			
	≤200 μg/d	201–300 μg/d	301–400 μg/d	>400 μg/d
Participants, *n*	21 272	28 773	12 857	25 854
Mean age, *y*	51.4	52.7	53.4	52.9
Total folate, *μg*†	161	253	342	676
Dietary folate, *μg*	161	253	329	334
Multivitamin supplement use, %	2.0	1.6	9.1	86.3
Aspirin use, %	29.5	24.5	40.4	34.7
Family history of colon cancer, %	9.2	8.2	12.4	10.4
Body mass index, kg/m^2	24.3	24.1	23.1	23.8
Mean pack-years of smoking before age 30 years	4.5	4.6	3.1	4.3
Participants in top quartile of physical activity, %	25.5	25.6	25.0	24.8
Daily intake				
Saturated fat, *g*	31.3	26.8	26.7	25.7
Cholesterol, *mg*	316	315	340	349
Dietary fiber, *g*	11.3	17.3	22.0	18.3
Alcohol, *g*	9.5	9.4	7.9	7.0
Methionine, *g*	1.78	1.82	2.00	1.97
Vitamin A, *IU*	7092	9410	17 126	17 214
Vitamin C, *mg*	160	380	397	446
Vitamin D, *IU*	198	160	194	547
Calcium, *mg*	683	694	755	767
Beef, pork, or lamb as a main dish, *servings/wk*	2.9	2.1	2.4	1.9

* All factors except age were standardized to the age distribution of the entire cohort.
† Folate and all nutrients were adjusted for total energy intake by using residual analysis.
‡ Percentage.

which they responded, and they accumulated person-time until the month of diagnosis, the month of death from other causes, or June 1994. We used the Mantel–Haenszel summary estimator to adjust for age (across 5-year categories). For multivariate analyses, we used pooled logistic regression, which accounts for varying time to the outcome event (12) and is asymptotically equivalent to a Cox regression model with time-dependent covariates, given short time intervals and a low probability of outcome (13). A participant contributed up to seven observations based on seven 2-year periods from 1980 to 1994; if a woman received a diagnosis of colon cancer or died of any cause, the subsequent 2-year periods were censored. Each 2-year set of observations contributed by each participant was pooled in the logistic regression analysis.

We tested for trends, controlling for multiple covariates by modeling the specific exposure as a continuous variable in a logistic model that included the covariates. All reported *P* values are two-sided.

Results

We examined, by level of folate intake in 1980, the distribution of various factors possibly related to colon cancer (**Table 1**). The first three categories of folate intake reflected primarily dietary sources, whereas 86.3% of women with intake exceeding 400 μg/d used multivitamin supplements. The group with the second-highest intake, of which 9.1% took supplements, had the highest frequency of aspirin use, the lowest average body mass index, the lowest frequency of cigarette use in young adulthood, and the highest intake of dietary fiber. Alcohol use decreased with increasing folate intake. In multivariate models, we adjusted for these factors in addition to strong predictors of colon cancer (physical activity, red meat, and methionine) in this population.

In 1986, we assessed erythrocyte folate level in a subsample of 188 women. When we used the 1980 questionnaire to categorize folate intake, the mean erythrocyte folate level was 302 ng/mL for folate intake of 200 μg/d or less ($n = 37$), 348 ng/mL for intake of 201 to 300 μg/d ($n = 67$); 346 ng/mL for intake of 301 to 400 μg/d ($n = 41$); and 387 ng/mL for intake of more than 400 μg/d ($n = 43$). The lowest erythrocyte levels in these four intake categories were 168, 197, 209, and 217 ng/mL, respectively; all were within the normal range (normal range > 150 ng/mL). The first three categories primarily represented dietary sources of folate; intake of more than 400 μg usually reflected supplement use. Among multivitamin users, the daily supplementary dose of folic acid was 100 μg or more for 97% of users, 200 μg or more for 87% of users, and 400 μg or more for 69% of users.

Higher total intake of folate in 1980 was related to a lower risk for colon cancer (**Table 2**). The age-adjusted and multivariate analyses (including age; aspirin use; physical activity; body mass index; pack-years of smoking before age 30 years; family history of colorectal cancer; intake of beef, pork, or lamb as a main dish; and intake of alcohol, fiber, and methionine) yielded similar results. Results were almost identical when we included 70 cases of large-bowel cancer of unknown site (relative risk

Table 2. Relative Risk for Colon Cancer According to Folate Intake from Dietary and Supplementary Sources in the Nurses' Health Study (1980–1994)

Variable	Folate Intake in 1980				*P* for Trend*
	≤200 μg/d	201–300 μg/d	301–400 μg/d	>400 μg/d	
Person-years	291 318	394 161	176 373	353 540	
Cases, *n*	114	158	64	106	
Relative risk†	1.0	0.92	0.79	0.67	
Relative risk (95% CI)‡	1.0	0.92 (0.70–1.19)	0.79 (0.56–1.12)	0.69 (0.52–0.93)	0.01

* Test for trend based on folate as a continuous variable in the multivariate model.
† Age-adjusted relative risk for energy-adjusted folate intake.
‡ After controlling for age (5-year categories); aspirin use (yes/no); physical activity (quintiles); body mass index (five categories); pack-years of smoking before age 30 years (four categories); family history of colorectal cancer (yes/no); intake of beef, pork, or lamb as a main dish (6 categories); and intake of alcohol (continuous), fiber (quintiles), and methionine (quintiles).

[RR], 0.69 [95% CI, 0.52 to 0.91] for intake of more than 400 μg/d compared with intake of 200 μg/d or less; *P* for trend = 0.005). Further adjustment for total and saturated fat intake, postmenopausal estrogen use, and history of endoscopic screening did not change our results appreciably (data not shown).

We next examined nutrients other than folate that are present in multivitamin supplements. In five models that included age; folate intake; intake of vitamins A, E, C, and D; and intake of calcium, total folate intake remained inversely associated with risk for colon cancer (*P* for trend for folate = 0.0006, 0.02, 0.02, 0.06, and 0.003, modeling for vitamins A, E, C, and D and calcium, respectively). In contrast, after adjustment for folate intake, none of these nutrients was significantly related to risk for colon cancer ($P = 0.15$ for vitamin A [positive trend], $P > 0.2$ for vitamin E, $P > 0.2$ for vitamin C, $P > 0.2$ for vitamin D, and $P > 0.2$ for calcium).

The relation between folate intake in 1980 and subsequent risk for colon cancer varied by time; an inverse association was limited primarily to the latter part of the follow-up period. For June 1980 to May 1988 (214 cases), the multivariate relative risk was 0.85 (CI, 0.56 to 1.30) for intake greater than 400 μg/d compared with intake of 200 μg/d or less (*P* for trend > 0.2); for June 1988 to May 1994 (228 cases), the multivariate relative risk was 0.56 (CI, 0.37 to 0.84) (*P* for trend = 0.006). We further examined temporal relations by considering onset of use of multivitamin supplements containing substantial amounts of folate; these data were based on responses to the duration-of-use question in 1980 and was updated biennially. As the **Figure** shows, women who used multivitamins had no benefit with respect to colon cancer after 4 years of use (RR, 1.02 [CI, 0.75 to 1.35]) and had only nonsignificant risk reductions after 5 to 9 years (RR, 0.83 [CI, 0.64 to 1.09]) and 10 to 14 years of use (RR, 0.80 [CI, 0.61 to 1.05]). After 15 years of use, however, risk for colon cancer was markedly lower (RR, 0.25 [CI, 0.13 to 0.51]). Among supplement users only, the *P* value for the trend of decreasing risk over time with multivitamin use was 0.0003. Directly on the basis of our data, we computed 68 new cases of colon cancer per 10 000 women 55 to 69 years of age over a 10-year period compared with 15 cases per 10 000 women 55 to 69 years of age who had used multivitamins for at least 15 years.

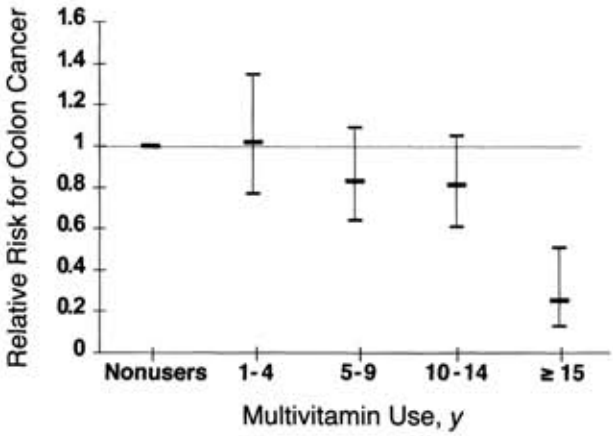

Figure. Relative risk for colon cancer according to years since the start of use of multivitamins containing folic acid in the Nurses' Health Study (1980 to 1994). Age; aspirin use; physical activity; body mass index; pack-years of smoking before age 30 years; family history of colorectal cancer; intake of beef, pork, or lamb as a main dish; and intake of alcohol, fiber, and methionine were controlled for. Vertical bars represent 95% CIs.

Because FDA regulations limited the amount of folic acid contained in multivitamin supplements to 100 μg before 1973, the longest possible duration of use of supplements containing 400 μg of folic acid by 1988 was 15 years. Thus, the category of 15 years of use or more was limited to follow-up time after 1988. We therefore analyzed years since the start of multivitamin use in the follow-up period 1988 to 1994 and found results nearly identical to those found when we used the person-time of the entire follow-up period (multivariate RR, 0.29 [CI, 0.15 to 0.56]; $P < 0.001$; *P* value for trend < 0.001).

The lower risk for colon cancer seen after 15 years of multivitamin use was observed for proximal (multivariate RR, 0.16 [CI, 0.06 to 0.52]) and distal (RR, 0.37 [CI, 0.15 to 0.90]) colon cancer. However, long-term multivitamin use did not influence risk

for rectal cancer (RR, 1.27 [CI, 0.67 to 2.46]). Because of the null association with rectal cancer, the inverse association of total colorectal cancer with at least 15 years of multivitamin use was attenuated compared with the association of colon cancer alone with at least 15 years of use statistically, but it remained significant (RR, 0.53 [CI, 0.35 to 0.80]; $P = 0.003$).

For folate obtained from food only, a nonsignificant inverse association from 1988 to 1994 was noted when long-term (≥15 years) supplement users were excluded (**Table 3**). However, long-term multivitamin use was associated with a substantially lower risk for colon cancer across all levels of dietary folate. Women with low dietary folate intake (≤200 μg/d) seemed to benefit most from supplementary folate (RR, 0.15). We used only the follow-up period 1988 to 1994 in this analysis because supplement use of 15 years or more (as defined here) could only begin in 1988, and our goal was to keep the six categories of dietary folate by supplement use comparable (**Table 3**).

Because low methionine intake may increase folate requirements, we examined whether an interaction between methionine and folate was evident with regard to risk for colon cancer. On the basis of folate intake in 1980, the apparent benefit of folate was limited to women in the lower half of methionine intake (**Table 4**). The P value for statistical interaction between folate and methionine (assessed by including the product of folate and methionine in a model with these individual nutrients) was 0.005; a similar interaction was noted between methionine intake and dietary folate, excluding supplement users ($P = 0.02$). Among women with low methionine intake, a significant inverse association between colon cancer and dietary folate was noted (P for trend = 0.04).

Discussion

Our results indicate that using multivitamin supplements for 15 or more years may decrease risk for colon cancer by about 75%; the data are consistent with the hypothesis that folate intake is the principal nutritional factor associated with risk reduction. The CIs are also consistent with a substantially weaker benefit. A reduction of 20% was suggested after 5 years, but a larger study is needed to confirm this finding. Controlling for various known and suspected risk factors for colon cancer did not alter this relation. An unconsidered correlate of folate intake could have accounted for our findings, but most of the covariates associated with "health-seeking behavior" that we examined did not have a simple linear correlation with folate intake. Although the prevalence of presumably health-seeking behaviors (lower body mass index, lower prevalence of smoking, lower intake of saturated fat, and higher intakes of fiber and aspirin) corresponded more closely to dietary folate intake (up to 400 μg/d, mostly from fruits and vegetables), the lowest risk for colon cancer was seen among women taking multivitamin supplements (**Table 1**).

Because the highest folate intakes were due primarily to multivitamin supplement use, a component of multivitamin use other than folate may have accounted for our findings. However, because other nutrients in multivitamins that may be protective tended to come from diverse dietary sources, these could be distinguished from folate by using multivariate models. For example, vitamin D and folic acid are both present in most brands of multivitamin supplements, but dietary sources of these nutrients differ. When folate and vitamin D were modeled together, an inverse association with colon cancer existed for folate (RR, 0.75 [CI, 0.53 to 1.05]; P for trend = 0.06) but not for vitamin D (RR, 0.86 [CI, 0.60 to 1.28]; P for trend > 0.2). Moreover, the trend toward protection was seen over the entire range of folate intake (**Table 2**), whereas supplement users were mostly in the top category; this finding suggests that both dietary and supplemental intakes were beneficial.

Our findings support recent studies that have examined folate in relation to risk for colon cancer. Four case–control studies have found a higher risk

Table 3. Relative Risk for Colon Cancer According to Duration of Use of Multivitamin Supplements Containing Folic Acid and Energy-Adjusted Dietary Folate Intake in the Nurses' Health Study (1988–1994)

Variable	Dietary Folate Intake in 1980		
	≤200 μg/d	201–300 μg/d	>300 μg/d
Nonusers or women using supplements for < 15 years			
Cases/person-years	67/143 155	99/203 410	53/119 952
Relative risk (95% CI)*	1.0	0.96 (0.70–1.32)	0.82 (0.56–1.20)
Women using supplements for ≥ 15 years			
Cases/person-years	1/13 502	6/25 860	2/17 510
Relative risk (95% CI)*	0.15 (0.02–1.02)	0.44 (0.19–1.02)	0.21 (0.05–0.84)

* After controlling for age; aspirin use; physical activity; body mass index; pack-years of smoking before age 30 years; family history of colorectal cancer; intake of beef, pork, or lamb as a main dish; and intake of alcohol and methionine.

Table 4. Relative Risk for Colon Cancer According to Intakes of Total Folate and Methionine in 1980 in the Nurses' Health Study

Variable	Folate Intake in 1980				*P* for Trend*
	≤200 μg/d	201–300 μg/d	301–400 μg/d	>400 μg/d	
Methionine intake <1.8 g/d					
Cases/person-years	84/184 496	89/214 526	26/84 503	42/174 528	
Relative risk (95% CI)†	1.0	0.81 (0.59–1.11)	0.57 (0.36–0.90)	0.48 (0.33–0.71)	<0.001
Methionine intake ≥1.8 g/d					
Cases/person-years	30/102 027	68/181 415	39/93 874	64/180 023	
Relative risk (95% CI)†	0.57 (0.37–0.87)	0.66 (0.47–0.92)	0.70 (0.47–1.06)	0.62 (0.44–0.88)	>0.2

* Test for trend based on separate models stratified by methionine level.
† After controlling for age; aspirin use; physical activity; body mass index; pack years of smoking before age 30 years; family history of colorectal cancer; intake of beef, pork, or lamb as a main dish; and intake of alcohol and fiber.

for colon cancer among persons with low folate intake (14–17). Evidence from four prospective studies provides relevant information: Three of these studies (1, 2), including our current study, support an inverse association between higher folate intake and lower risk for cancer, and the other (18), which did not have comprehensive dietary data, showed an inverse association between plasma folate levels and risk for colon cancer. One case–control study found that folic acid from multivitamin supplements was associated with half the risk for colon cancer (4). In the Health Professionals Follow-up Study, men who used supplements for more than 10 years had a moderately reduced risk for colon cancer (multivariate RR, 0.74 [CI, 0.47 to 1.17]), particularly distal colon cancer (RR, 0.62 [CI, 0.32 to 1.31]). Low dietary or erythrocyte folate levels have also been associated with an increased risk for colon adenomas (19–24).

Approximately one quarter of Americans began consuming an additional 400 μg of folic acid daily from vitamin supplements or fortified breakfast cereals in 1973 (when use of this amount of folic acid was first allowed) or soon thereafter (10). Of note, an unexplained sharp reduction in colorectal cancer incidence occurred in white persons in the United States in the late 1980s (25). In our cohort, risk for colon cancer decreased in the latter half of the 1980s among women who had been using supplements with folic acid since 1973. Some have speculated that increased use of sigmoidoscopy and fecal occult blood tests (triggering colonoscopy and leading to removal of precancerous lesions) played a role in this decline in colon cancer incidence (25). In our population, however, the percentage of women who had had endoscopy by 1988 was identical (18.9%) for both long-term (≥15 years) supplement users and other women. Moreover, the reduction in risk was, if anything, stronger for proximal cancers, whereas a benefit from sigmoidoscopic screening should be largely limited to the distal colon. The potential role of folic acid status in altering population rates of colon cancer warrants further study. In addition, the effect of this secular reduction in colon cancer rates, which may be related to multivitamin supplement use, needs to be considered when results from studies of other etiologic factors (for example, aspirin use [26]) conducted in the United States in the 1980s are interpreted.

At the time of this study, the recommended daily allowance of folate for women was 180 μg (27); recently, this was increased to 400 μg. Folate supplementation was beneficial for women with relatively high dietary intake of folate (>300 μg/d). Few women were likely to have frank folate deficiency; none of the 188 women in our sample was clinically deficient. As seems to be the case for prevention of neural tube defects (28), folate levels higher than those needed to prevent anemia may be required to minimize risk for colon cancer. Of concern, the prevalence of folate deficiency is higher in the general population (29–31); 88% of the population have folate intake less than 400 μg/d (27), although folate status should improve because of the current fortification of cereal-grain products (32).

The inverse association between colon cancer and folate was stronger with supplemental folate than with dietary folate. This may reflect the high level of folic acid in a typical supplement (400 μg) and the high bioavailability of supplementary folate (folic acid or pteroyl-monoglutamic acid). In contrast, polyglutamyl folate from foods must be deconjugated to the monoglutamyl form in the intestine before absorption and thus has substantially lower bioavailability (33). In a recent small intervention study in women, erythrocyte folate levels increased significantly over 3 months in response to 400 μg of folic acid from supplements or fortified foods but not from an additional 400 μg of dietary folate (34). Thus, although foods naturally high in folate may provide other beneficial micronutrients, consumption of these foods is probably less effective than use of supplements and fortified foods in enhancing folate status.

The mechanisms whereby folate may reduce car-

cinogenesis are unclear. Different endogenous forms of folate, 5-methyl tetrahydrofolate and 5,10-methylene tetrahydrofolate, are essential for DNA methylation and DNA synthesis, respectively. When levels of 5,10-methylene tetrahydrofolate are low, misincorporation of uracil for thymidine may occur during DNA synthesis (35), possibly increasing spontaneous mutation rates (36), sensitivity to DNA-damaging agents (37), frequency of chromosomal aberrations (38, 39), and errors in DNA replication (39–41). In a recent study (42), folate deficiency was related to the massive incorporation of uracil into human DNA and to increased chromosomal breaks, and these processes returned to normal when participants received folic acid supplementation. When methionine intake is low, levels of S-adenosylmethionine decrease; this stimulates the enzyme methylene tetrahydrofolate reductase (MTHFR) to convert 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, which is required for methionine synthesis. If compensatory production of methionine is hindered by an insufficient 5-methyl tetrahydrofolate level, the methyl supply for DNA methylation may be inadequate. Hypomethylation of DNA is one of the earliest events in colon carcinogenesis (43–48), although whether DNA methylation directly influences the process remains unproven. Recent findings in two cohorts (18, 49) show that homozygotes bearing a polymorphism of the *MTHFR* gene (677C→T; alanine-to-valine), which correlates with reduced enzyme activity (50, 51), have a lower risk for colon cancer, possibly because low activity of this enzyme maintains high cellular levels of 5,10-methylene tetrahydrofolate. This relation between a functional polymorphism of this folate-metabolizing gene and risk for colon cancer provides independent evidence of a role of folate that cannot be attributed to confounding by a correlate of folate intake.

Strengths of our study include its prospective design, repeated assessments of diet and multivitamin use, validation of folate intake with a biochemical marker, detailed data on many potential confounders, and high follow-up response rate. Nevertheless, because this was an observational rather than a randomized study, we cannot definitively attribute our results to folate. However, several of our findings suggest that folate is the relevant nutrient; these findings include 1) the fact that that dietary and supplementary folate combined to produce an inverse linear dose–response relation with colon cancer; 2) the lack of confounding from considered variables; 3) the biologically predicted interaction with methionine intake; and 4) the strength of the association, particularly with long-term multivitamin use. Residual confounding from imperfect measurement of the considered variables is plausible but unlikely, given the similar results of the age-adjusted and the multivariate models (**Table 2**). The most plausible alternative explanation of our findings (other than causality) is uncontrolled confounding from a nutrient or nutrients in multivitamin supplements. The strong evidence of benefit from multivitamins, whether or not it is ultimately attributable to folic acid, is of interest in itself. Definitive proof of a benefit of folic acid may require a randomized, double-blind clinical trial.

Another limitation of our study is that folate intake is assessed with error. Although folate intake based on questionnaire responses predicts erythrocyte folate levels, some degree of measurement error is inevitable. When reporting errors are independent of outcome, as they are likely to be in our study because of its prospective design, their tendency is to bias results toward the null. Thus, measurement error is unlikely to account for the inverse association but may artifactually narrow the observed CI (52).

A further limitation is our inability to assess folic acid obtained from multivitamins before 1973; this inability compelled us to assume that exposure to folic acid from multivitamins was initiated in 1973 even among women who were using multivitamins before this year. This assumption seems reasonable because the allowable limit of folic acid was only 100 μg and numerous multivitamin formulations before 1973 did not include folic acid. The major impact of any inaccuracies induced by this assumption would be that we may have underestimated the induction period between onset of multivitamin use and reduced risk for colon cancer. For example, a woman who began using multivitamins in 1968 may have derived some benefit from that point on, but we would have started counting her multivitamin exposure at 1973 rather than 1968, essentially ignoring the 5 additional years of exposure.

Folate has been shown to protect against neural tube defects and possibly cardiovascular disease by decreasing homocysteine levels (53). A previous report from the Nurses' Health Study (54) indicated that folate and multivitamin use were related to lower risk for coronary heart disease. Further study and resolution of the relation between folic acid intake and risk for colon cancer would contribute to the ongoing debate over the value of increasing folate intake in the general population.

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