The Influence of Folate and Multivitamin Use on the Familial Risk of Colon Cancer in Women[1]

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Abstract

Low intake of folate and methionine and heavy alcohol consumption have been associated with an increased overall risk of colon cancer, possibly related to their role in methylation pathways. We estimated the relative risk (RR) of colon cancer according to a history of colorectal cancer in a first-degree relative and categories of folate, methionine, and alcohol intake in a prospective cohort study of 88,758 women who completed family history and detailed food frequency questionnaires. During 16 years of follow-up, colon cancer was diagnosed in 535 women. The inverse association of folic acid with colon cancer risk was greater in women with a family history. Compared with women who consumed 200 μg or less of folic acid/day, the age-adjusted RR of colon cancer for those who consumed >400 μg/day was 0.81 (95% confidence interval, 0.62–1.07) in women without a family history of colorectal cancer and 0.48 (95% confidence interval, 0.28–0.83) in women with a family history (*P* for interaction = 0.02). The influence of family history was markedly diminished by use of multivitamins containing folic acid (*P* for interaction = 0.04). High levels of dietary methionine also reduced the effect of family history (*P* for interaction = 0.05), whereas moderate to heavy alcohol consumption increased the risk associated with family history (*P* for interaction = 0.004). Other risk factors for colorectal cancer did not significantly modify the influence of family history. Our results suggest that higher intake of folate and methionine, regular use of multivitamins containing folate, and avoidance of moderate to heavy alcohol consumption may diminish the excess risk of colon cancer associated with a family history of the disease.

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Introduction

Epidemiological studies indicate that a history of colorectal cancer in a first-degree relative (*e.g.*, parent or sibling) elevates an individual's lifetime risk of colorectal cancer ~2-fold (1–5). Although a specific gene associated with common (*i.e.*, sporadic) colorectal cancer has not yet been identified, kindred studies suggest that familial clustering of colorectal cancer probably occurs as a result of a partially penetrant inherited susceptibility that may explain a substantial proportion of common colorectal cancer (6, 7). In view of the known associations of colon cancer with environmental exposures, these pedigree analyses suggest that inheritance determines individual susceptibility for colon cancer, whereas environmental factors define which genetically susceptible individuals will ultimately develop the disease.

Several studies indicate that inherited polymorphisms of genes responsible for methyl-group metabolism are associated with colorectal cancer risk, and the effect of these genes is modified by consumption of folate, methionine, and alcohol (8–14). Other studies suggest that methionine intake may diminish the excess risk associated with a family history (15), whereas alcohol may enhance the effect of family history (4, 15). Independent of family history, high intake of alcohol and low intake of folate or methionine has been associated with an increased risk of colorectal neoplasia (16–30).

By studying interactions between exposures and family history, one can both define methods for targeted prevention in genetically susceptible individuals as well as generate hypotheses for the mechanisms by which these susceptibilities are conferred. We therefore used data from a large prospective cohort study to determine the influence of dietary methyl-group availability on the excess incidence of colon cancer associated with a family history of the disease.

Materials and Methods

Study Population. The Nurses' Health Study was established in 1976 when 121,700 female registered nurses residing in the United States, 30–55 years of age, completed a mailed questionnaire on known or suspected risk factors for cancer and coronary heart disease (31). Every two years, participants have been sent follow-up questionnaires to update information on potential risk factors and to identify newly diagnosed cases of cancer and other diseases.

Exposure Data. Participants provided information on smoking history, age, height, weight, physical activity, aspirin use, postmenopausal hormone use, and examination by colonoscopy or sigmoidoscopy as well as the indications for the procedure. A history of colorectal cancer in a father, mother, sister, or brother was elicited on the 1982 questionnaire and was updated in 1988 and 1992. No questions were asked about family size, and no attempt was made to validate reports of cancer in family members. In addition, we did not ask study participants who reported a family history of colorectal cancer to distinguish

whether their relative's diagnosis was colon cancer or rectal cancer.

The 1980 Nurses' Health Study questionnaire included a 61-item semiquantitative food frequency questionnaire to assess diet as well as supplemental vitamin use. The participants were asked to report the average frequency of consumption of each item during the previous year. In addition, for each item we asked whether consumption had greatly increased or greatly decreased during the previous 10 years. Current multivitamin use was assessed in each biennial questionnaire beginning in 1980. We also asked about the brands of multivitamin typically used as well as the brand and types of breakfast cereal used. Women who were current multivitamin users in 1980 were asked to state how many years they had been taking multivitamins. 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 the United States Department of Agriculture sources supplemented with other data (32), including data on specific brands and types of multivitamins and breakfast cereals. The reproducibility and validity of these questionnaires have been documented previously (16, 33–35).

To evaluate the validity of the reported nutrient consumption measured by the questionnaire, comprehensive diet records, plasma high-density lipoprotein levels, and erythrocyte folate levels were obtained from a subsample of participants (16, 33–35). Mean daily intakes of nutrients, including alcohol, as assessed by the questionnaire and by the diet records were very similar. The Spearman correlation coefficient for alcohol intake measured by the diet records and the questionnaire was 0.90 (16). In addition, alcohol intake as reported by the questionnaire was correlated with plasma high-density lipoprotein ($r = 0.40$, $P < 0.001$). Furthermore, within our relatively folate-replete patient population, the Pearson correlation between folate intake by the questionnaire (including supplements) and erythrocyte folate from the blood samples was 0.55 (16, 18). The mean erythrocyte folate levels (ng/ml) and SE of the mean by quintile of total folate intake from lowest to highest quintile were 301 ± 15, 341 ± 10, 355 ± 11, 355 ± 11, and 406 ± 21.

Population for Analysis. The dietary questionnaire was returned by 98,462 nurses in 1980. We excluded women with 10 or more food items left blank or implausibly high or low scores for total food intake, as well as those who reported previous cancer (other than nonmelanoma skin cancer), ulcerative colitis, or a familial polyposis syndrome. This left 88,758 women for the analysis.

Identification of Cases of Colon Cancer. On each questionnaire we inquired whether colon or rectal cancer had been diagnosed and, if so, the date of the diagnosis. For this analysis, the follow-up rate was 96% of the total possible person-years through June 1, 1996. Medical records were obtained from 92% of the cases and were reviewed by physicians who were blinded to questionnaire data. Although pathology reports and hospital records could not be obtained for 8% of cases, we based our analysis on all incident colorectal cancers because the accuracy of self-reporting was high (95%). We excluded the small number of cancers that were not adenocarcinomas as well as carcinomas *in situ*. This left 786 cases of invasive colorectal adenocarcinoma. Of these, 535 were in the colon, 164 were in the rectum, and 87 were at undetermined sites. In a previous analysis of this cohort, a family history of colorectal cancer was associated with a significant increase in the risk of colon cancer (RR,[3] 2.01; 95% CI, 1.44–2.14) but had no influence on the risk of rectal cancer (RR, 0.98; 95% CI, 0.44–2.18; Ref. 5); as a result, we did not consider incident rectal cancer among the study participants in this analysis.

Data Analysis. We examined the risk of colon cancer according to total and dietary folate intake in 1980 and family history of colorectal cancer. Data on family history of colorectal cancer was based on the response to the baseline questionnaire and updated, thereafter, in 1988 and 1992 (based on a previous analysis in this cohort; Ref. 5). We categorized women into four groups of folate intake (≤200, 201–300, 301–400, and >400 μg/day) based on a previous analysis of folate and colon cancer risk in this cohort (16). In addition, categories of methionine intake (≤1.4, 1.5–1.8, 1.9–2.2, and >2.2 g/day) and alcohol intake (0, 0.1–15, 15.1–30, and >30 g/day) were based on a previous analysis of colorectal neoplasia in this cohort (18).

To examine the influence of folate from food only, we categorized participants according to dietary folate intake in 1980 after excluding long-term (>5 years) multivitamin supplement users. For this analysis, multivitamin use was updated biennially. The exclusion of longer-term supplement users was based on a previous analysis of folate and colon cancer in this cohort (16). In that analysis, short-term users of multivitamins (≤5 years) experienced a RR of 1.02 (95% CI, 0.75–1.35) when compared with women who did not report multivitamin use, whereas multivitamins use for 6–10 years was associated with a nonsignificant reduction in risk (RR, 0.83; 95% CI, 0.64–1.09). Consequently, in that original publication and in the current analysis, short-term multivitamin users were included in the analysis of folate from food sources and long-term users were excluded.

To examine the influence of duration of folate supplement use, family history, and risk of colon cancer, we computed duration of use of multivitamins containing folate and updated this variable every 2 years on the basis of the brand and type of multivitamin use reported biennially from 1980 to 1996. Before 1973, 100 μg was the maximum dose allowed in supplements according to the United States Food and Drug Administration regulations, and many supplement formulations did not contain folic acid (36). Thus, we considered 1973 (when doses of 400 μg were first allowed) to be the earliest possible starting point.

We also computed duration in the highest tertile of total folate intake (dietary plus supplement use) and updated this variable based on the 1980, 1984, 1986, 1990, and 1994 dietary assessments. Within each follow-up period for this subanalysis, only women in either the lowest or highest tertiles were included; women in the lower tertile of folate intake were considered the reference category.

Because of the limited number of participants with higher levels of methionine intake (>2.2 g/day) or alcohol intake (>30 g/day), we had insufficient power to analyze duration of high intake of methionine or alcohol intake among participants with a family history. Nonetheless, methionine intake as measured by the 1980 questionnaire was correlated with subsequent measures over time ($r = 0.38$, comparing intake from the 1980 questionnaire with the 1986 questionnaire; $P < 0.001$). In addition, alcohol intake as measured by the 1980 questionnaire was correlated with alcohol intake measured by the 1986 questionnaire ($r = 0.63$; $P < 0.001$).

For the primary analysis, we used incidence rates of colon cancer with person-years of follow-up as the denominator. For

[3] The abbreviations used are: RR, relative risk; CI, confidence interval.

each participant, person-years of follow-up were counted from the date of return of the 1980 questionnaire to May 31, 1996. For the participants who received a diagnosis of colon cancer or who died from another cause, person-years of follow-up were calculated according to the most recently completed questionnaire, but the period of follow-up terminated with the diagnosis of colon cancer or death.

Age-adjusted RRs with 95% CIs were calculated after stratification according to 5-year age categories using the Mantel-Haenszel summary estimator. For multivariate analysis, we used pooled logistic regression, which accounts for varying times to the outcome event (37) and is asymptotically equivalent to a Cox regression model with time-dependent covariates, given short-time intervals and a low probability of outcome (38). A participant contributed up to eight observations based on eight 2-year periods from 1980 to 1996; 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.

The basic model included known or suspected risk factors for colon cancer, including age (5-year categories), pack-years of smoking before age 30 years (0, 1–5, 6–10, 11–15, or >15 pack-years, based on a previous analysis in this cohort; Ref. 39), body-mass index (in quintiles), regular vigorous exercise ≥1 day/week (yes or no), regular aspirin use (≥2 times per week; yes or no), screening endoscopy (yes or no), beef, pork, or lamb as a main dish (<1 per month, 1–3 per month, 1 per week, 2–4 per week, or ≥ 5 per week), alcohol consumption (0, <15, 15–30, or >30 g/day), and energy-adjusted intake folate (≤200, 201–300, 301–400, and >400 μg/day), and methionine (≤1.4, 1.5–1.8, 1.9–2.2, and >2.2 g/day). Intakes of folate, methionine, and other nutrients were adjusted for total energy intake by using residual analysis (40). We used all variables as assessed in 1980 with the exception of age and screening endoscopy use, which were updated biennially. In additional models, we considered intake of total fat, animal fat, calcium and dietary fiber; intake of vitamins A, C, D, and E; and postmenopausal estrogen use.

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*s are two-sided. Tests for interaction were performed by entering into the model the cross-product terms of family history (as an indicator variable) and the other risk factor for colon cancer (measured continuously for all ordinal variables; Ref. 41).

Results

Among 88,758 women eligible for analysis, 6,956 (7.8%) reported a history of colorectal cancer in one or more first-degree relatives in 1982. In 1988, when family history of colorectal cancer was updated, 9,561 (10.8%) reported an affected first-degree relative. By 1992, 11,808 (13.3%) reported such a history. During the 16 years of follow-up (1,375,165 person-years), we observed 535 confirmed colon cancers. Baseline characteristics of the study participants according to the baseline report of family history of colorectal cancer are shown in Table 1. Women with and without a positive family history demonstrated similar patterns of age, dietary intake, body mass index, physical activity, and smoking history. However, women with a family history of colorectal cancer underwent screening endoscopy during the study period more frequently than those without a family history.

Participants who reported a history of colorectal cancer in one or more first-degree relatives experienced an age-adjusted RR of colon cancer of 2.00 (95% CI, 1.63–2.46) compared with those without a family history. The RR associated with family history was not materially altered by multivariate adjustment for other known or suspected risk factors for the disease (RR, 1.91; 95% CI, 1.53–2.36).

Table 1 Characteristics of the study participants according to the presence or absence of a family history of colorectal cancer[a]

	No family history n = 81,802	Family history n = 6,956
Age (yr)	46.7	46.8
Body mass index (kg/m^2)	24.3	24.3
Alcohol intake (g/day)	6.6	6.8
Dietary intake[b]		
Folate (μg/day)[c]	365	365
Methionine (g/day)	1.9	1.9
Animal fat (g/day)	52.2	51.9
Dietary fiber (g/day)	16.8	16.9
Beef, pork or lamb as a main dish (servings/week)	2.6	2.6
Calcium (mg/day)[c]	732	732
Vitamin D (IU/day)[c]	292	291
Regular aspirin use (%)[d]	23.5	26.4
Multivitamin supplement use (%)	34.0	34.1
Mean duration of multivitamin use at baseline (yr)[e]	4.4	4.3
Mean duration of multivitamin use in 1994 (yr)[f]	8.0	8.1
Screening endoscopy use at baseline (%)	5.4	5.7
Screening endoscopy use through study follow-up (%)[g]	20.3	43.1
Regular vigorous exercise (%)[b]	44.7	46.0
Mean pack-years of smoking before age 30 yr	3.0	2.9

[a] Based on reports of a family history of colorectal cancer in one or more first-degree relatives obtained at study initiation. Values are means directly standardized according to the age distribution of the cohort.
[b] Dietary values represent the mean energy-adjusted intake.
[c] Includes the use of supplements.
[d] Defined as aspirin use on two or more days/week.
[e] Mean duration of use among multivitamin users at baseline.
[f] Mean duration of use among multivitamin users in 1994.
[g] Includes screening endoscopy use during study follow-up and at baseline.
[h] Regular vigorous exercise was defined as vigorous physical activity (enough to work up a sweat) on one or more days/week.

The inverse association of folic acid with colon cancer was greater in women with a family history of colorectal cancer (Fig. 1*a*). Incorporating the cross-product interaction term of family history and folate consumption into the multivariate model, we observed a significant difference in the relative effect of family history with different levels of folate intake (*P* for interaction = 0.02). Compared with women without a family history who consumed 200 μg or less of folate/day (1980–1990), women without a family history who consumed >400 μg/day experienced a multivariate RR of 0.91 (95% CI, 0.69–1.19), women with a family history who consumed 200 μg or less/day experienced a RR of 2.49 (95% CI, 1.66–3.75), and women with a family history who consumed >400 μg/day experienced a RR of 1.30 (95% CI, 0.82–2.08). Although total folate intake had only a minimal protective influence on the risk of colon cancer among women without a family history, greater folate intake was associated with substantially reduced risk among those with a family history (*P* for trend = 0.01). Among women without a family history, the age-adjusted RR of colon cancer for those who consumed >400 μg of folate/day was 0.81 (95% CI, 0.62–1.07) compared with women who consumed 200 μg or less per day. Among women with a family history, the RR for those who consumed >400 μg/day was 0.48 (95% CI, 0.28–0.83) when compared with women with a family history who consumed 200 μg or less per day.

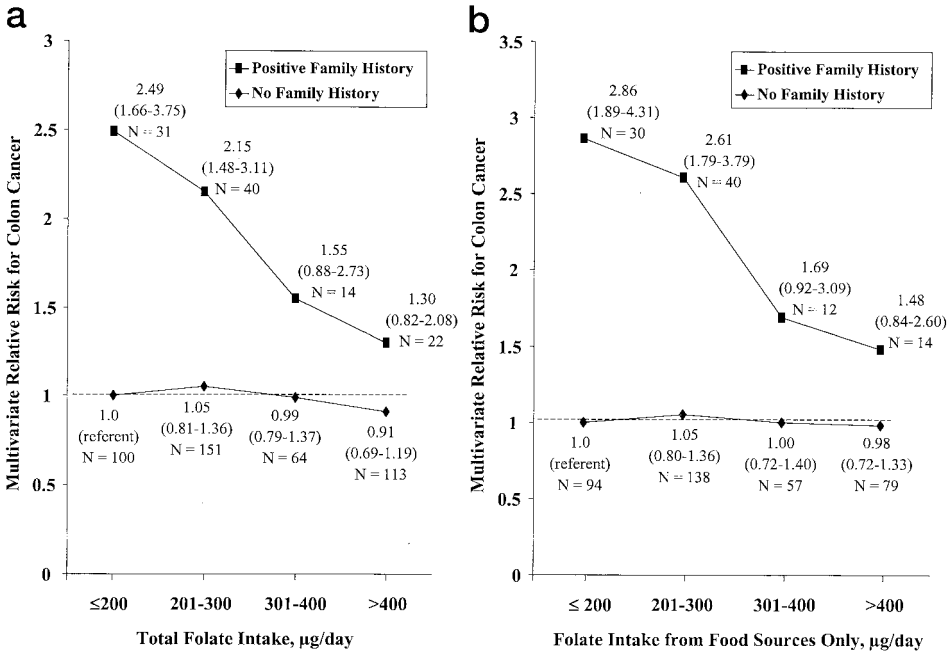

Fig. 1. *a*, multivariate relative risk of colon cancer according to total folate intake in 1980 (dietary and supplemental sources) and family history of colorectal cancer. *b*, multivariate relative risk of colon cancer according to folate intake in 1980 from food sources only and family history of colorectal cancer. In *b*, women who used multivitamin supplements for >5 years were excluded. *Numbers* in *parentheses*, 95% CIs; *N*, number of cases for each category.

Table 2 RR of colon cancer according to multivitamin supplement use in 1980 and family history of colorectal cancer[a,b]

Multivitamin use	No family history				Positive family history			
	No. of cases	Person-years	Age-adjusted RR (95% CI)	Multivariate RR (95% CI)	No. of	Person-years	Age-adjusted RR (95% CI)	Multivariate RR(95% CI)
No	298	820,189	1.0	1.0	85	90,041	2.30 (1.82–2.90)	2.18 (1.71–2.78)
Yes	130	420,485	0.84 (0.68–1.03)	0.89 (0.72–1.10)	22	47,320	1.13 (0.73–1.74)	1.13 (0.73–1.75)

[a] Multivariate RRs are adjusted for age (5-year categories), pack-years of smoking before age 30 years (0, 1–5, 6–10, 11–15, or >15 pack-years), body-mass index (in quintiles), regular vigorous exercise (≥1 day/week), regular aspirin use (≥2 times per week), screening endoscopy (yes or no), beef, pork, or lamb as a main dish (<1 per month, 1–3 per month, 1 per week, 2–4 per week, or ≥5 per week), alcohol consumption (abstinence, history of greatly reduced consumption, or <15, 15–30, or >30 g/day), and energy-adjusted levels of methionine (≤1.4, 1.5–1.8, 1.9–2.2, >2.3 g/day).
[b] *P* for interaction for the cross-product term of multivitamin supplement use in 1980 and family history of colorectal cancer = 0.04.

We further assessed the influence of dietary folate (as measured in 1980) after excluding long-term (>5 years) multivitamin supplement users (Fig. 1*b*). For this analysis, multivitamin use was updated biennially. After adjusting for other covariates, the cross-product interaction term of family history and dietary folate consumption was borderline significant in the multivariate model (*P* for interaction = 0.05). Although dietary folate had no appreciable influence on the risk among women without a family history of colorectal cancer, dietary folate was inversely associated with risk among women with a family history (*P* for trend = 0.04).

In 1980, 84% of all women whose folate intake exceeded 400 μg/day consumed folate supplements in the form of multivitamins. We therefore examined the risk of colon cancer according to both multivitamin use in 1980 and family history of colorectal cancer (Table 2). A multivariate test for statistical interaction between multivitamin use and family history was significant (*P* = 0.04). Among women without a family history, the age-adjusted RR of colon cancer for women who reported multivitamin use in 1980 was 0.84 (95% CI, 0.68–1.03) compared with those who did not use multivitamins. Among women with a family history, the RR for those who reported multivitamin use in 1980 was 0.48 (95% CI, 0.28–0.83) when compared with women with a family history who did not use multivitamins.

The influence of family history of colorectal cancer was markedly diminished by multivitamins containing folic acid. Among women who did not use multivitamins, participants with a family history experienced a RR of colon cancer of 2.16 (95% CI, 1.60–2.76) when compared with those without a family history. In contrast, among women who used multivitamins, the RR of colon cancer associated with a family history was 1.25 (95% CI, 0.79–1.96).

We examined the temporal influence of folate intake according to family history. Duration of multivitamin use was calculated using responses to the duration-of-use question in 1980 and the subsequent biennial follow-up. A multivariate test for statistical interaction between duration of multivitamin use and family history did not reach statistical significance (*P* = 0.12). As depicted in Fig. 2*a*, increasing duration of multivitamin use was not associated with a reduction in colon cancer risk among women without a family history of colorectal cancer, whereas increasing duration of multivitamin use was inversely associated with risk among women with a family history. Compared with women with a positive family history who did not use multivitamins, women with a positive family history who

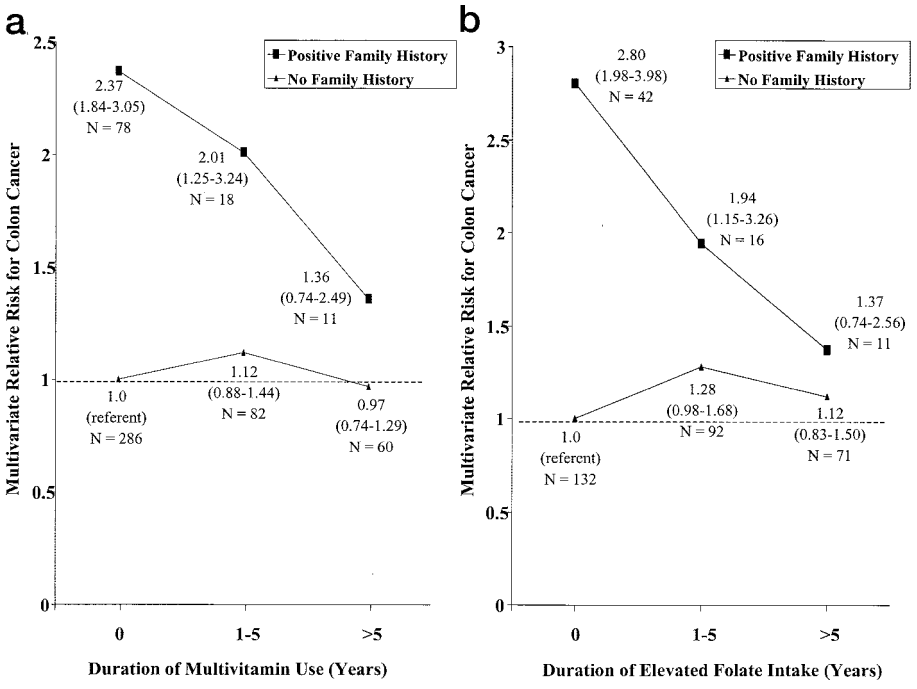

Fig. 2. *a*, multivariate relative risk of colon cancer according to duration of multivitamin use and family history of colorectal cancer. *b*, multivariate relative risk of colon cancer according to duration of high folate use (highest tertile) and family history of colorectal cancer. Only women in either the lowest or highest tertiles were included in *b*; women in the lower tertile of folate intake were considered the referents. *Numbers* in *parentheses*, 95% CIs; *N*, number of cases for each category.

reported 1–5 years of use experienced an age-adjusted RR of 0.80 (95% CI, 0.48–1.35), and those who reported >5 years experienced a RR of 0.54 (95% CI, 0.29–1.00; *P* for trend = 0.06).

We further examined this temporal relation by considering the duration that the participant remained in the highest tertile of total folate intake. These data were based on responses to the 1980 dietary questionnaire and updated in 1984, 1986, 1990, and 1994. A multivariate test for statistical interaction between duration of high folate use and family history was significant ($P = 0.04$). As shown in Fig. 2*b*, duration of high folate intake was not associated with colon cancer risk among women without a family history of the disease, whereas duration of high folate intake was inversely associated with risk among women with a family history. When compared with women with a positive family history in the lowest tertile of folate intake, women with a family history in the highest tertile of folate intake for 1–5 years experienced an age-adjusted RR of 0.67 (95% CI, 0.38–1.20), and those in the highest tertile for >5 years experienced a RR of 0.46 (95% CI, 0.24–0.89; *P* for trend = 0.04).

We examined dietary and nondietary colon cancer risk factors other than folate, including other vitamins present in multivitamin preparations. We conducted multivariate models that included the cross-product term of family history and folate intake as well as the cross-product term of family history and one other covariate. In each model that included the interaction with intake of vitamins A, C, D, E, calcium, dietary fiber, total fat, animal fat, and red meat, aspirin use, pack-years of smoking before age 35, postmenopausal hormone use, and screening endoscopy use, the cross-product term of family history and folate intake remained statistically significant ($P \leq 0.02$). In contrast, after adjustment for the interaction of family history and folate intake, none of the other covariates had a significant interaction with family history ($P \geq 0.30$).

We also observed a significant interaction between family history and methionine intake (Fig. 3*a*). A test for statistical interaction using the cross-product term of methionine intake and family history was significant in the multivariate model ($P = 0.05$). Although methionine intake had no relation to colon cancer risk in women without a family history, methionine intake was associated with a significant reduction in risk among those with a family history (P for trend = 0.04). When the cross-product terms representing the interactions of family history with folate and methionine intake were simultaneously included in the model, the interaction term of folate intake and family history remained significant, whereas the interaction term of methionine intake and family history became slightly attenuated (*P* for interaction = 0.02 and 0.07 for folate and methionine, respectively).

We also examined the influence of alcohol intake on the risk of colon cancer according to family history (Fig. 3*b*). A multivariate test for statistical interaction using the cross-product term between alcohol intake (categorized dichotomously as ≥30 g/day *versus* <30 g/day) and family history was significant ($P = 0.004$). Compared with women without a family history who abstained from alcohol, women without a family history who consumed ≥30 g of alcohol/day experienced a multivariate RR of 0.88 (95% CI, 0.56–1.39), women with a family history who abstained experienced a RR of 1.91 (95% CI, 1.32–2.78), and women with a family history who consumed ≥30 g/day experienced a RR of 3.80 (95% CI, 2.13–6.76). When the cross-product terms representing the interaction between alcohol intake and family history were added to a model that also included the interaction between folate intake and family history, both remained significant (*P* for interaction = 0.004 and 0.02 for alcohol and folate, respectively).

We had previously observed the interaction between family history of colorectal cancer and folate intake using follow-up from 1980 through 1990.[4] Compared with women with-

[4] Unpublished data.

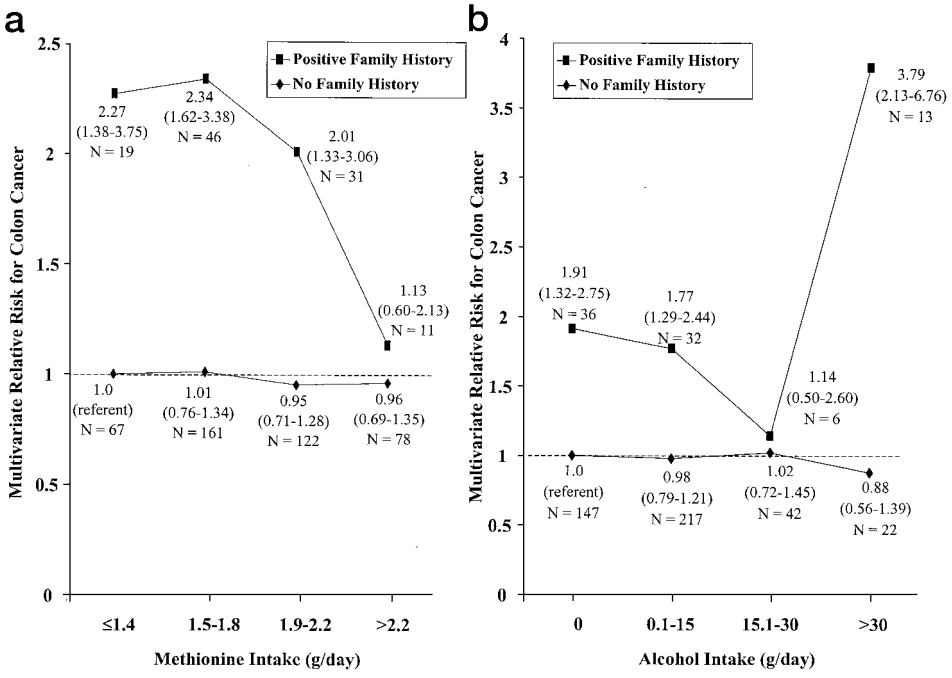

Fig. 3. *a*, multivariate relative risk of colon cancer according to methionine intake in 1980 and family history of colorectal cancer. *b*, multivariate relative risk of colon cancer according to alcohol intake in 1980 and family history of colorectal cancer. *Numbers* in *parentheses*, 95% CIs; *N*, number of cases for each category.

out a family history who consumed ≤200 μg of folate/day (1980–1990), women without a family history who consumed >400 μg/day experienced a multivariate RR of 0.94 (95% CI, 0.65–1.34), women with a family history who consumed ≤200 μg/day experienced a RR of 2.71 (95% CI, 1.55–4.73), and women with a family history who consumed >400 μg/day experienced a RR of 0.85 (95% CI, 0.39–1.87; *P* for interaction = 0.01). To further explore the consistency of this relationship, we assessed the interaction between family history and folate intake (reported in 1980) using follow-up from 1990 through 1996. Compared with women without a family history who consumed ≤200 μg/day (1990–1996), women without a family history who consumed >400 μg/day experienced a multivariate RR of 0.87 (95% CI, 0.56–1.36), women with a family history who consumed ≤200 μg/day experienced a RR of 2.31 (95% CI, 1.25–4.27), and women with a family history who consumed >400 μg/day experienced a RR of 1.37 (95% CI, 0.70–2.71; *P* for interaction = 0.04).

Discussion

In this large prospective study, we found that quantities of folate that are considerably greater than those needed to prevent clinical folate deficiency substantially attenuated the excess risk of colon cancer among women with a family history. Concordant with that finding, we observed a marked reduction in the effect of family history among multivitamin users. Our results indicate that individuals with a family history who use multivitamin supplements for >5 years may decrease their risk of colon cancer by almost 50%. In addition, high levels of dietary methionine appeared to reduce the effect of family history, whereas moderate to heavy alcohol consumption appeared to increase the risk associated with family history.

The relative risk of colon cancer associated with a history of one or more affected first-degree relatives observed in this study is consistent with the findings of previous studies (1–4), including an earlier report from this cohort (5). Other studies have demonstrated an inverse association between folate intake (17–30) and the risk of colorectal neoplasia, including a previous report from this cohort (16). However, in the current analysis, the inverse association with folate intake was far greater among people with a family history of colorectal cancer than among those without a family history.

Few studies have addressed whether specific dietary factors and micronutrients influence the risk of colon cancer associated with a family history (4, 15, 42–44). Two studies reported that alcohol consumption markedly increased the familial risk of colorectal cancer (4, 15), and one found that methionine diminished the effect of family history (15). Le Marchand *et al.* observed that family history was not associated with colorectal cancer among men in the "low risk" or "healthy" tertile of all colorectal cancer life-style risk factors, whereas men with a family history in the highest or "unhealthy" tertile of lifestyle variables experienced a RR of 11.7 (15). Similarly, Slattery *et al.* (43) noted that the influence of family history on colon cancer risk was largely abrogated among individuals in the lowest level of a "Western" pattern diet. In contrast, individuals in the highest level of a Western diet, characterized by heavy meat, egg, potato, high-fat dairy, refined grain, and sugar consumption, experienced the greatest risk associated with a family history. In the latter two analyses, it is uncertain which specific components of a "healthy" or "non-Western" pattern lifestyle were responsible for the attenuation of familial risk.

The mechanisms by which folate may modify the effect of family history are unclear. Folate and methionine are important factors in DNA methylation, whereas alcohol antagonizes methylation pathways (45). Genomic and proto-oncogene-specific DNA hypomethylation seems to be an early and consistent event in colon carcinogenesis (46–50). In addition, folate is required to convert deoxyuridylate into thymidylate. Blount *et al.* (51) demonstrated that folate deficiency was related to massive misincorporation of uracil into human DNA

and increased chromosomal breaks, and these changes were reversible with folate supplementation.

Defective DNA mismatch repair appears to account for the genetic susceptibility associated with hereditary nonpolyposis colorectal cancer (52). In the present analysis of sporadic colorectal cancer, the interactions of folate, methionine, and alcohol with family history suggest that individuals with a family history of colorectal cancer are more susceptible to dietary methyl deficiency, possibly as a result of low-penetrance aberrations in DNA methylation or DNA repair. However, our data do not exclude the possibility that folate, methionine, and alcohol intake may each modify the effect of family history through alternative or independent mechanisms.

The strengths of our study include its prospective design; repeated assessments of family history, diet, and multivitamin use; validation of folate intake with a biochemical marker; detailed data on many potential confounders; and high follow-up response rate. Data on family history were obtained only from the study participants; we did not ask relatives to verify these reports, and we did not determine family size. Because the participants were all nurses, the accuracy of the reports is likely to be high. Moreover, because the data on family history were collected before the diagnosis of any cases of colorectal cancer, any errors in recall would have attenuated rather than exaggerated a true association. Familial clustering of colorectal cancer is heterogeneous in nature, and our data on family history may include cases of colorectal cancer that occurred as a result of chance, a common environmental exposure, or a genetic predisposition. Nonetheless, these errors of misclassification would result in an underestimation of the true interaction between dietary exposures and the effect of family history. In addition, because our study was exclusively among women, it is uncertain whether these results are generalizable to men.

We cannot exclude the possibility that our findings are attributable to chance. However, several of our findings suggest that the effect of family history on the risk of colon cancer may be modified by folic acid intake. These findings include: (*a*) the fact that both dietary and supplementary folate significantly attenuated the effect of family history; (*b*) the lack of confounding from considered variables; (*c*) the biologically predicted interaction with methionine and alcohol intake; (*d*) the strength of the interaction, particularly with long-term multivitamin use and long-term high folate consumption; (*e*) the dose-responses observed for both amount and duration of folate intake; and (*f*) the consistency of the interaction in two separate time periods. Residual confounding from imperfect measurement of the considered variables is plausible but unlikely, given the similar results of the age-adjusted and multivariate models.

The influence of multivitamin supplements on the familial risk of colorectal cancer appears to principally reflect the folic acid content of multivitamins. However, one plausible alternative explanation remains uncontrolled confounding from other nutrients in multivitamin supplements. Nevertheless, the strong evidence of benefit from multivitamins, whether or not it is ultimately attributable to folic acid, is of interest in itself.

In conclusion, we observed that the excess risk associated with a family history of colorectal cancer was substantially reduced by higher intake of folic acid. These findings may provide potential avenues for prevention in a relatively high-risk population and generate new hypotheses for mechanisms of familial clustering of colorectal cancer.

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