Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk[1–3]

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ABSTRACT

Background: Few epidemiologic studies have examined very high intakes of folate and whether consumption of nutrients involved in one-carbon metabolism is associated with breast cancer risk.

Objective: We prospectively examined whether the consumption of folate and nutrients involved in one-carbon metabolism (methionine, riboflavin, and vitamins B-6 and B-12) from self-reported intakes of diet (in year before baseline) and supplements (averaged over 10 y before baseline) were associated with the incidence of breast cancer and breast cancer tumor characteristics.

Design: Participants were 35,023 postmenopausal women aged 50–76 y in the VITamins And Lifestyle (VITAL) cohort study; breast cancer was diagnosed in 743 of these women between baseline (2000–2002) and 2006. Cox proportional hazards models were used to estimate multivariable-adjusted relative risks (RRs) and 95% CIs.

Results: Women consuming $\geq$1272 dietary folate equivalents (DFE)/d of total folate (10-y average) had a 22% decrease in breast cancer risk compared with women consuming $\leq$345 DFE/d (RR: 0.78; 95% CI: 0.61, 0.99; P for trend = 0.05). A greater benefit was observed for estrogen-receptor (ER) negative than for ER+ breast cancers (RR: 0.38; 95% CI: 0.18, 0.80; P for trend = 0.02; P = 0.02 for the difference between ER− and ER+). Neither current intakes of folate nor current or long-term intakes of other one-carbon nutrients were significantly associated with breast cancer risk. Multivitamin use attenuated the increased risk of breast cancer associated with alcohol drinking (P for interaction = 0.02).

Conclusions: Our study of predominantly supplement users suggests that high intakes of folate averaged over 10 y do not increase breast cancer risk, but may be protective, particularly against ER− breast cancers. *Am J Clin Nutr* 2009;89:624–33.

INTRODUCTION

Folate, a water-soluble B vitamin, is a carbon donor in one-carbon metabolism (1), where it participates in nucleotide synthesis and methylation reactions. Whereas folate supplementation has been thought of as chemoprotective (2), much evidence suggests that it may increase cancer risk (3–5). Cancer cells frequently up-regulate folate receptors, most likely to meet their accelerated need for nucleotides to support DNA synthesis and cell growth (6–8). Moreover, animal data show that folate administered later in colorectal carcinogenesis can promote tumors (9). In humans, a large chemoprevention trial (10, 11) reported an unexpected 67% increase in advanced colorectal adenomas with folic acid supplementation (1 mg/d) among patients with a history of adenoma. Additionally, a large observational cohort study (12) observed a statistically significant 32% increase in breast cancer risk among postmenopausal women consuming >853 μg folate/d, a level higher than evaluated in previously reported studies. Results from other epidemiologic investigations of folate intake and breast cancer risk have varied, reporting protective effects, increased risk, or no associations (reviewed in 13, 14).

High doses of folate from diet, fortified diet, and supplements are of concern because folate from synthetic sources (folic acid) is more bioavailable than from natural sources and is thus potentially more potent in fostering cancer growth (4). In 1998, the United States and Canada mandated the folic acid fortification of foods. Moreover, supplements are a major source of folic acid, and $\approx$50% of US adults take multivitamins (15–17). In one small study, 78% of postmenopausal women had unmetabolized synthetic folate in their blood (18).

Methionine, riboflavin, and vitamins B-6 and B-12 also have a role in one-carbon metabolism (19), but have been less studied in relation to breast cancer (13). Mechanistically, methionine is a one-carbon donor and is the precursor of *S*-adenosylmethionine—the universal donor of methylation reactions, including DNA methylation. Riboflavin and vitamins B-6 and B-12 are cofactors for methionine synthesis. Additionally, vitamin B-6, a coenzyme of serine hydroxymethyltransferase, is involved in nucleotide synthesis.

In this study of the VITAL (VITamins And Lifestyle) cohort, we investigated whether folate intake was associated with postmenopausal breast cancer risk. We additionally examined breast cancer risk by intakes of methionine, alcohol, riboflavin, and vitamins B-6 and B-12. Because supplement intake was the

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principal exposure in the VITAL Study, we were able to examine very high intakes of folate.

SUBJECTS AND METHODS

Study population

Participants were women in the VITAL cohort—a study principally designed to investigate supplement use and cancer risk. VITAL was initiated between 2000 and 2002, when 37,382 men and 40,337 women aged 50–76 y answered a self-administered questionnaire about their dietary intakes, supplement use, lifestyle factors, cancer risk factors, and health history. Participants lived in the 13-county area of western Washington State covered by the Surveillance, Epidemiology, and End Results (SEER) cancer registry. Additional study details, including demographic information, were published by White et al (20). This study was approved by the Fred Hutchinson Cancer Research Center Institutional Review Board, Seattle, WA.

For this analysis, women were excluded if they reported a history of breast cancer at baseline ($n = 3160$), were premenopausal, or had missing menopausal status ($n = 1921$). Women were considered postmenopausal if they had a natural menopause with no periods in the year before baseline, had ever used postmenopausal hormones (PMHs; defined below), had bilateral oophorectomy, or were 60 y or older at baseline. Because women with a hysterectomy without bilateral oophorectomy cannot report on menopause, they were considered to be postmenopausal if they had ever received hormone therapy or were 55 y or older at baseline. We further excluded participants if they reported gastrointestinal procedures that could seriously affect nutrient absorption ($n = 25$) (eg, intestinal bypass, gastroplasty, gastrostomy tube, and small bowel/ileum resection) or if they had received a diagnosis of in situ breast cancer ($n = 200$), had no date of diagnosis ($n = 3$), or had sarcoma, phyllodes, or lymphoma as breast cancer histologies ($n = 5$). After these exclusions, 35,023 postmenopausal women remained.

Baseline questionnaire

Dietary intakes were assessed by using a semiquantitative food-frequency questionnaire (FFQ), adapted from instruments developed for the Women's Health Initiative and other studies (21–23). On the FFQ, participants reported their usual frequency and portion size (small, medium, or large in reference to a given medium portion size) of 120 foods and beverages consumed during the year before baseline and also answered 12 adjustment questions. The average daily intake of dietary nutrients was calculated by multiplying the adjusted serving frequency (calculated as frequency times portion size) of each food or beverage item by its nutrient content and summing the nutrient contributions of all foods or beverages. We used the nutrient database, Minnesota Nutrient Data System for Research (University of Minnesota's Nutrition Coordinating Center, Minneapolis, MN), as the FFQ analytic program (24). This database took into account the US mandated folic acid fortification of grain products. Total alcohol intake was calculated from all reported past-year intakes of red wine, white wine, beer, and liquor/mixed drinks. Fruit and vegetable intakes were obtained by adding servings of all fruit and vegetable items, including fruit and vegetable juices and excluding potatoes (25).

Participants reported intakes of multivitamins and vitamin supplements, taken singly or as mixtures (eg, ''stress''/B-complex and antioxidant mixtures). For all supplement questions, a closed-ended format was used to inquire about current and past use, frequency, duration over the previous 10 y, and usual dose per day.

To obtain the nutrient composition of multivitamins, we asked participants to select from a list of 16 multivitamin brand names. If their brand was unlisted, we asked them to provide specific doses of the vitamins and minerals in their multivitamin. Participants were instructed to refer to the labels on their supplement bottles. Individuals who changed brands in the past 10 y selected from an additional list of brand names chosen to reflect market availability during that time period. For analyses, the nutrient content of specific brands was obtained from the Physician's Desk Reference for Nonprescription Drugs and Dietary Supplements (26) and information provided by the manufacturers. When the multivitamin brand or multivitamin contents were not provided by a participant, we defaulted to Centrum Silver (current use) and Centrum (past use), both from Wyeth (Madison, NJ), because they were the market leaders in 2001 and 1996, respectively, and the most common responses among VITAL participants. These supplement questions were based on our earlier methodologic work on supplement assessment (21, 27, 28). The validity and reliability of supplement reporting in this cohort was previously examined (29). For the supplements included in this article, the range of the test-retest intraclass correlation coefficients for 10-y supplemental intake was 0.80–0.84, and the range of the Pearson correlation coefficients between self-reported current intake and a home inventory of pill bottles was 0.69–0.82.

We examined dietary, supplemental, and total (diet plus supplemental) sources of folate, riboflavin, and vitamins B-6 and B-12 intakes; methionine was assessed from dietary sources only. We needed a conversion factor (30) for the calculation of total folate because synthetic folate (folic acid) is more bioavailable than is naturally occurring folate (polyglutamates). Thus, total folate, expressed in dietary folate equivalents (DFE), was obtained by first multiplying synthetic folate (supplements and fortified in foods) by a conversion factor of 1.7 and then adding intakes of natural food folate (μg). To examine the effect of timing of folate intake on breast cancer risk, we examined both ''current'' (ie, short-term) and ''10-year'' (ie, long-term) intakes of folate. We also examined current and 10-y intakes of the other B nutrients. Current daily supplemental intakes were calculated by summing intakes from current use of multivitamin preparations and individual vitamins: $\sum$(dose per day) $\times$ (days per week). Ten-year average daily supplemental intakes of folate and other micronutrients were calculated by summing intakes from multivitamin preparations and individual vitamin sources: $\sum$(dose per day) $\times$ (days per week/7) $\times$ (years/10). Supplemental riboflavin was assessed from multivitamins only. We computed the 10-y average of multivitamins as (days per week of use) $\times$ (years of use)/10. To summarize intakes of micronutrients that act as cofactors in the one-carbon pathway, we generated a ''one-carbon micronutrient score'' by summing the z scores of total folate, dietary methionine, total riboflavin, and vitamins B-6 and B-12 and then dividing this score into quartiles for categorical analyses. Additional details are provided in the

table footnotes. Women were excluded from the dietary and total (diet plus supplement) nutrient analyses if they did not complete all pages of the FFQ or if their reported total energy intake was <600 or >4000 kcal.

Other breast cancer risk factors were obtained as follows. PMH use was computed from questions about prescription estrogen and progestin as pills or patches, excluding oral contraceptives. Past hormone therapy use for <1 y was considered ''never use.'' Body mass index (BMI; in kg/m^2) was computed from self-reported weight at baseline and height when participants were tallest. Total recreational physical activity in MET-hours/wk [further described in (31)] was computed from reported regular activity in the past 10 y.

Ascertainment of breast cancer

The cohort was followed for breast cancer diagnoses from baseline to 31 December 2006, for a mean follow-up time of 5 y. Incident breast cancer cases were identified though annual linkage of the VITAL cohort database to the SEER cancer registry, as maintained by the Fred Hutchinson Cancer Research Center. All incident cancer cases (except nonmelanoma skin cancer) diagnosed within the 13-county area of western Washington state were reported to SEER along with stage and other tumor characteristics. Cases were ascertained through all area hospitals, offices of pathologists, oncologists, and radiotherapists and from state death certificates; extensive quality-control procedures were followed to ensure that registry data were accurate and complete. Linkage to SEER was based on ranking of the agreement between variables common to VITAL and SEER (eg, name, social security number, date of birth, and address). Using these data, we identified 743 incident cases of invasive breast cancer (n = 527 ductal, 152 lobular, and 64 other) among eligible women in the cohort.

Statistical analyses

Cox proportional hazards models were used to estimate age- and multivariable-adjusted relative risks (RRs) of breast cancer and 95% CIs. Age was treated as the time variable with participants entering at their baseline age and exiting at their age at breast cancer diagnosis (event) or censor date (last date for follow-up). Censor date was defined as the earliest date of: withdrawal from the study (0.04%), death (3.4% as ascertained from Washington State death files), move from the 13 county catchment area of the SEER registry (5.2% as identified by linkage to the National Change of Address System and other follow-up procedures), or the end of follow-up on 31 December 2006.

For our multivariable models, we adjusted for established risk factors for breast cancer: age (y), race (white or other), family history of breast cancer (none, 1 affected mother or sister, ≥2 affected mother or sisters), mammography within 2 y preceding baseline (no or yes), history of breast biopsy (no or yes), age at menarche (≤ 11, 12, 13, or ≥14 y), age at first birth (nulliparous or ≤19, 20–24, 25–34, or ≥35 y), age at menopause (≤44, 45–49, or ≥50 y), years of combined estrogen and progestin PMH (never or <1, 1–4, 5–9, or ≥10 y), BMI (<25, 25 to <30, or ≥30), height (<62, 62 to <65, 65 to <68, or ≥68 in; 1 inch = 2.54 cm), total physical activity (none and tertiles of metabolic equivalent hours/wk), and alcohol intake in the past year (<1.5, 1.5–4.9, 5.0–9.9, or ≥10 g/d). We additionally adjusted for total energy intake (kcal/d) in analyses of dietary and total nutrient intakes. Tests for linear trend were performed by modeling categories of the nutrient factor as a single ordinal variable.

In addition, stratified analyses were used to examine whether the association between 10-y average total folate consumption in tertiles (main exposure) and breast cancer risk were modified by intakes of methionine (median categories), riboflavin (median categories), vitamin B-6 (median categories), and vitamin B-12 (median categories), one-carbon micronutrient score (median categories, without folate), and alcohol (<10 and ≥10 g/d). Tertiles of folate rather than quartiles were used to minimize sparse cells. Tests for interaction were performed by entering the product term of the ordinal folate variable and dichotomous effect modifier in a multivariable-adjusted model and using Wald statistic to obtain a P value. To make joint effects clear, we used a single reference group (ie, lowest intake of folate and dietary effect modifier) for the RRs.

Last, we examined the association between 10-y average total folate intake and risk of breast cancer defined by estrogen-receptor (ER) status or tumor size (<1.5 and ≥1.5 cm). We evaluated differences in risk estimates by subgroups of cases (eg, by breast cancer ER status) by excluding all noncases and fitting a multivariable unconditional logistic regression model comparing the 2 case groups of interest; a Wald statistic was used to obtain a P value. All statistical analyses were performed by using SAS (version 9.1; SAS Institute Inc, Cary, NC). P values <0.05 were considered statistically significant, and all statistical tests were 2-sided.

RESULTS

The average age of the women at baseline was 62 y (range: 50–76 y), and the average BMI was 27 (range: 15–55). Ninety-one percent of the women had had a mammogram in the past 2 y, 15% had a mother or sister with a diagnosis of breast cancer, and 63% were current or former users of PMH. The most common food contributors of nonfortified dietary folate were green salad, broccoli, and citrus foods or juices.

Ten-year intake of total folate was statistically significantly associated with a modest decrease in breast cancer risk (RR: 0.78 for quartile 4 compared with quartile 1; 95% CI: 0.61,0.99; P for trend = 0.05; **Table 1**). The distribution of total folate intakes was wide, with a median of 345 DFE/d in the lowest quartile and 1272 DFE/d in the highest quartile. For supplemental folate averaged over 10 y, the RR for quartile 4 compared with quartile 1 was inverse but not statistically significant (RR: 0.87; 95% CI: 0.72, 1.06; P for trend = 0.10). No statistically significant associations were observed for dietary, current supplemental, and current total folate intakes (Table 1).

We next examined the relation between other micronutrients that have a role in the association between the one-carbon pathway and breast cancer risk (Table 1). We observed suggestive lower risks of breast cancer for intakes of dietary methionine (RR: 0.78 for quartile 4 compared with quartile 1; 95% CI: 0.56, 1.09; P for trend = 0.09); however, the RRs were not statistically significant (Table 1). Associations for vitamins B-6 and B-12 were not statistically significant (Table 1).

TABLE 1
Relative risks (RRs) of postmenopausal breast cancer by daily intakes of B vitamins, methionine, multivitamins, and alcohol among 35,023 postmenopausal women in the VITAL (VITamins And Lifestyle) Study between 2000 and 2006[1]

	Quartile of intake[2]				
	1	2	3	4	*P* for trend[3]
Current folate intake					
Dietary, natural and synthetic from foods					
Median intake (μg/d)	210 (37 to <260)	303 (260 to <346)	392 (346 to <450)	541 (450–1483)	
Cases	165	161	180	157	
Multi-RR (95% CI)	1.00	0.94 (0.75, 1.18)	1.04 (0.82, 1.33)	0.91 (0.68, 1.22)	0.76
Supplemental					
Median intake category (μg/d)	None	86 (86 to <200)	314 (200 to <400)	400 (400–1400)	
Cases	251	13	90	384	
Multi-RR (95% CI)		0.98 (0.55, 1.75)	0.87 (0.67, 1.13)	1.00 (0.84, 1.19)	0.95
Total[4]					
Median intake category (DFE/d)	325 (45 to <472)	604 (472 to <776)	960 (776 to <1082)	1302 (1082–3663)	
Cases	153	91	193	222	
Multi-RR (95% CI)	1.00	0.92 (0.70, 1.20)	1.02 (0.82, 1.27)	0.90 (0.72, 1.12)	0.52
10-y Average folate intake					
Supplemental					
Median intake (μg/d)	None	80 (9 to <200)	251 (200 to <400)	400 (400–1400)	
Cases	202	167	135	234	
Multi-RR (95% CI)	1.00	1.00 (0.81, 1.22)	0.87 (0.70, 1.08)	0.87 (0.72, 1.06)	0.10
Total[4,5]					
Median intake (DFE/d)	345 (45 to <472)	609 (472 to <776)	942 (776 to <1082)	1272 (1082–3620)	
Cases	169	164	177	149	
Multi-RR (95% CI)	1.00	0.96 (0.77, 1.20)	0.95 (0.77, 1.18)	0.78 (0.61, 0.99)	0.05
Methionine intake					
Dietary					
Median intake (g/d)	0.82 (0.12 to <1.03)	1.22 (1.03 to <1.40)	1.59 (1.40 to <1.82)	2.17 (1.82–6.41)	
Cases	171	179	160	153	
Multi-RR (95% CI)	1.00	0.98 (0.78, 1.22)	0.84 (0.65, 1.08)	0.78 (0.56, 1.09)	0.09
Riboflavin					
Dietary					
Median intake (mg/d)	1.10 (0.21 to <1.36)	1.60 (1.36 to <1.83)	2.09 (1.83 to <2.42)	2.93 (2.42–9.52)	
Cases	161	164	188	150	
Multi-RR (95% CI)	1.00	0.97 (0.78, 1.22)	1.07 (0.84, 1.36)	0.86 (0.64, 1.15)	0.54
10-y Average supplemental					
Median intake (mg/d)	None	0.34 (0.07 to <0.67)	1.07 (0.67–1.36)	1.70 (1.70–1.70)	
Cases	226	160	142	215	
Multi-RR (95% CI)	1.00	1.02 (0.83, 1.25)	0.83 (0.67, 1.03)	0.87 (0.72, 1.06)	0.07
Total[5]					
Median intake (mg/d)	1.46 (0.29 to <1.90)	2.32 (1.90 to <2.70)	3.07 (2.70 to <3.47)	4.04 (3.47–10.0)	
Cases	165	165	173	160	
Multi-RR (95% CI)	1.00	0.96 (0.77, 1.20)	0.95 (0.76, 1.18)	0.83 (0.65, 1.07)	0.17
Vitamin B-6 intake					
Dietary					
Median intake (mg/d)	0.96 (0.20 to <1.18)	1.39 (1.18 to <1.58)	1.79 (1.58 to <2.05)	2.45 (2.05–7.26)	
Cases	159	172	181	151	
Multi-RR (95% CI)	1.00	1.04 (0.83, 1.30)	1.08 (0.84, 1.38)	0.90 (0.66, 1.21)	0.61
10-y Average supplemental					
Median intake (mg/d)	None	0.50 (0.09 to <1.26)	2.00 (1.26 to <2.89)	10.7 (2.89–261)	
Cases	204	159	194	184	
Multi-RR (95% CI)	1.00	0.88 (0.72, 1.09)	0.90 (0.73, 1.09)	0.90 (0.73, 1.10)	0.31
Total[5]					
Median intake (mg/d)	1.34 (0.29 to <1.83)	2.37 (1.83 to <3.05)	3.75 (3.05 to <4.84)	12.6 (4.84–263)	
Cases	162	168	164	169	
Multi-RR (95% CI)	1.00	1.00 (0.80, 1.25)	0.90 (0.71, 1.13)	0.99 (0.79, 1.24)	0.69
Vitamin B-12 intake					
Dietary					
Median intake (μg/d)	2.71 (0.02 to <3.60)	4.42 (3.60 to <5.23)	6.14 (5.23 to <7.35)	9.33 (7.35–62.9)	
Cases	169	178	151	165	
Multi-RR (95% CI)	1.00	0.99 (0.80, 1.23)	0.84 (0.66, 1.06)	0.91 (0.70, 1.18)	0.28

(*Continued*)

TABLE 1 (*Continued*)

	Quartile of intake[2]				
	1	2	3	4	*P* for trend[3]
10-y Average supplemental					
Median intake (μg/d)	None	2.40 (0.13–4.80)	6.25 (4.84 to <12.5)	25.0 (12.5–300)	
Cases	197	144	199	193	
Multi-RR (95% CI)	1.00	0.84 (0.68, 1.04)	0.97 (0.80, 1.18)	0.96 (0.78, 1.17)	0.97
Total[5]					
Median intake (μg/d)	4.26 (0.23 to 6.31)	8.46 (6.31 to <10.8)	14.1 (10.8 to <19.6)	32 (19.6–310)	
Cases	163	162	159	179	
Multi-RR (95% CI)	1.00	0.95 (0.76, 1.19)	0.89 (0.71, 1.12)	0.99 (0.79, 1.23)	0.83
Multivitamin use					
By category (d/wk of use)	None	0.30 to <2.80 (tertile 1)	2.80 to <7.00 (tertile 2)	7.00 (tertile 3)	
Cases	226	160	142	215	
Multi-RR (95% CI)	1.00	1.02 (0.83, 1.25)	0.83 (0.67, 1.03)	0.87 (0.72, 1.06)	0.07
Total alcohol intake					
By category (g/d)	<1.5 (category 1)	1.5–4.9 (category 2)	5.0–9.9 (category 3)	≥10 (category 4)	
Cases	379	100	80	171	
Multi-RR (95% CI)	1.00	1.01 (0.80, 1.29)	1.22 (0.95, 1.57)	1.60 (1.31, 1.94)	<0.0001

[1] Age- and multivariable-adjusted RRs (multi-RRs) were similar; thus only multi-RRs are reported. Multi-RRs and associated 95% CIs were estimated by using a Cox regression model adjusted for age (y), race (white or other), family history of breast cancer (none, 1 affected mother or sister, or ≥2 affected mother or sisters), mammography within 2 y preceding baseline (no or yes), history of breast biopsy (no or yes), age at menarche (≤11, 12, 13, or ≥14 y), age at first birth (nulliparous, or ≤19, 20–24, 25–34, or ≥35 y), age at menopause (≤44, 45–49, or ≥50 y), years of combined estrogen and progestin postmenopausal hormone use (never or <1, 1–4, 5–9, or ≥10), BMI (in kg/m^2; <25, 25 to <30, or ≥30), height (<62, 62 to <65, 65 to <68, or ≥68 in.; 1 in. = 2.54 cm), total physical activity (none, 1–3 tertiles of metabolic equivalent hours/wk), and alcohol intake in the past year (<1.5, 1.5–4.9, 5.0–9.9, or ≥10 g/d). We additionally adjusted for total energy intake (kcal/d) in analyses of dietary and total nutrient intakes. Case numbers do not sum to the total number of cases because of missing data. For median intakes, ranges are in parentheses.

[2] Exposure data reported by quartiles, except for multivitamin use (reported as none or by tertile categories) and alcohol consumption (reported based on a priori–defined categories as indicated). Current supplemental and current total folate categories were based on cutoffs of 10-y folate intakes to facilitate comparison.

[3] Calculated by modeling categories of the exposure as a single ordinal variable in a Cox regression; a Wald test was used to obtain a *P* value.

[4] Total folate reported in dietary folate equivalents (DFE) = synthetic folate (μg) × 1.7 + food folate (μg). Dietary and synthetic folate do not add up to total folate because a conversion factor was used to take into account the higher bioavailability of synthetic folate.

[5] Total intake was calculated as the sum of dietary intakes over the year before baseline plus the 10-y average from supplements.

We examined the association between multivitamin use averaged over 10 y and breast cancer risk (Table 1), because multivitamins are a major source of folate and other B vitamins, and 71% of women in this cohort reported ever taking a multivitamin. Multivitamin use was potentially associated with lower breast cancer risk, but the associations were not statistically significant (RR: 0.87 for 7d/wk of use compared with no use; 95% CI: 0.72, 1.06; *P* for trend = 0.07; Table 1). In addition, because folate, methionine, and the vitamins riboflavin, B-6, and B-12 are involved in one-carbon metabolism and because they were highly correlated (range: r = 0.64–0.80, $P \leq 0.0001$), we combined these nutrients into a summary "one-carbon micronutrient score"; the association with breast cancer risk was not statistically significant (RR: 0.80 for quartile 4 compared with quartile 1; 95% CI: 0.62, 1.04; *P* for trend = 0.13; data not shown in the table). In stratified analyses, the potential benefit of 10 y of total folate consumption did not differ significantly by intakes of these one-carbon pathway micronutrients (**Table 2**).

Alcohol, a known folate antagonist (32, 33), is thought to increase breast cancer risk, partly by influencing the absorption and metabolism of folate and interfering with one-carbon metabolism. When we compared women consuming ≥10 g alcohol/d (≈0.75 g/drink) with those reporting <1.5 g/d, we observed a statistically significant 60% increase in breast cancer risk (95% CI: 1.31, 1.94; *P* for trend ≤ 0.0001; Table 1). In stratified analyses, higher folate intakes did not have a clear breast cancer risk benefit among women with high alcohol intakes (≥10 g/d) compared with women consuming lower amounts of alcohol (*P* for interaction = 0.78) (Table 2). Only 16% reported drinking ≥10 g alcohol/d, and consumers of alcohol typically drank wine. However, our analyses of the joint associations between alcohol (<10 or ≥10 g/d) and multivitamin (none or some) intakes suggested a benefit of multivitamin use among daily alcohol drinkers. Relative to those who drank <10 g alcohol/d and those who did not use multivitamins over the 10 y period before baseline (reference group), women who took multivitamins but did not drink had an RR of breast cancer of 1.02 (95% CI: 0.84, 1.24), those who drank and did not take multivitamins had an RR of 2.05 (95% CI: 1.51, 2.78), and those who drank and took multivitamins had an RR of 1.36 (95% CI: 1.04, 1.77). Moreover, the test of interaction between alcohol (<10 or ≥10 g/d) and multivitamin (none, some) intake was statistically significant (*P* for interaction = 0.02). Taken together, these results suggest that multivitamin use attenuated the increased risk of breast cancer from daily alcohol consumption.

In **Table 3**, we evaluated whether the association between total folate intake over 10 y and breast cancer risk differed by breast tumor characteristics. The protective association of total folate over 10 y was stronger for estrogen receptor negative (ER−) breast cancers (RR: 0.38; 95% CI: 0.18, 0.80; *P* for

TABLE 2
Multivariable-adjusted joint associations between total daily intakes of folate and other dietary factors and postmenopausal breast cancer risk among women in the VITAL (VITamins And Lifestyle) Study between 2000 and 2006[1]

	Tertile of 10-y total folate intake							
	1 (45 to <560 DFE/d)		2 (560 to <989 DFE/d)		3 (989–3620 DFE/d)			
	Cases	Multi-RR (95% CI)	Cases	Multi-RR (95% CI)	Cases	Multi-RR (95% CI)	*P* for trend[2]	*P* for interaction[3]
Dietary methionine								
Median, low	144	1.00	115	0.93 (0.73, 1.19)	90	0.88 (0.67, 1.15)	0.38	
High	78	0.83 (0.62, 1.12)	116	0.87 (0.65, 1.16)	116	0.67 (0.50, 0.90)	0.09	0.58
Total riboflavin								
Median, low	203	1.00	109	0.95 (0.75, 1.20)	17	0.91 (0.55, 1.49)	0.65	
High	19	1.01 (0.62, 1.62)	122	1.02 (0.80, 1.28)	189	0.82 (0.67, 1.02)	0.10	0.42
Total vitamin B-6								
Median, low	207	1.00	116	0.90 (0.72, 1.14)	6	0.69 (0.31, 1.56)	0.19	
High	15	0.80 (0.47, 1.35)	115	1.04 (0.82, 1.31)	200	0.82 (0.67, 1.01)	0.25	0.98
Total vitamin B-12								
Median, low	194	1.00	102	0.91 (0.71, 1.16)	27	0.86 (0.57, 1.29)	0.25	
High	28	1.01 (0.68, 1.51)	129	1.05 (0.84, 1.32)	179	0.83 (0.66, 1.03)	0.13	0.54
Modified micronutrient score, without folate[4]								
Median, low	183	1.00	115	1.00 (0.79, 1.26)	33	0.93 (0.64, 1.35)	0.57	
High	39	1.15 (0.80, 1.64)	116	1.02 (0.79, 1.31)	173	0.84 (0.67, 1.06)	0.05	0.22
Alcohol								
<10 g/d	170	1.00	164	0.92 (0.74, 1.14)	165	0.86 (0.69, 1.08)	0.19	
≥10 g/d	52	1.47 (1.07, 2.01)	67	1.73 (1.29, 2.32)	41	1.05 (0.73, 1.50)	0.16	0.78

[1] Multivariable-adjusted relative risks (multi-RRs) and associated 95% CIs were estimated by using a Cox regression model adjusted for age (y), race (white or other), family history of breast cancer (none, 1 affected mother or sister, or ≥2 affected mother or sisters), mammography within 2 y preceding baseline(no or yes), history of breast biopsy (no or yes), age at menarche (≤11, 12, 13, or ≥14 y), age at first birth (nulliparous, or ≤19, 20–24, 25–34, or ≥35 y), age at menopause (≤44, 45–49, or ≥50 y), years of combined estrogen and progestin postmenopausal hormone use (never or <1, 1–4, 5–9, or ≥10), BMI (in kg/m^2; <25, 25 to <30, or ≥30), height (<62, 62 to <65, 65 to <68, or ≥68 in.; 1 in. = 2.54 cm), total physical activity (none, 1–3 tertiles of metabolic equivalent hours/wk), and alcohol intake in the past year (<1.5, 1.5–4.9, 5.0–9.9, or ≥10 g/d). We additionally adjusted for total energy intake (kcal/d) in analyses of dietary and total nutrient intakes. Total intake was calculated as the sum of dietary intakes over the year before baseline plus the 10-y average from supplements.

[2] Calculated by modeling categories of total folate as a single ordinal variable in a Cox regression; a Wald test was used to obtain a *P* value.

[3] *P* for interaction tested whether the association between total folate intake and breast cancer risk was modified by nutrient or alcohol intake. The Wald test was used to assess the significance of the interaction term.

[4] Modified micronutrient score computed by summing the z scores of methionine, riboflavin, and vitamins B-6 and B-12 and then dividing this sum into tertiles.

trend = 0.02) than for ER positive (ER+) breast cancers (RR: 0.88; 95% CI: 0.68, 1.14; *P* for trend = 0.43) in a comparison of quartile 4 with quartile 1 intakes. Formal testing showed a significant difference by ER status (*P* for difference = 0.02). The association between folate and breast cancer risk did not differ by tumor size (*P* for difference = 0.57).

With respect to the timing of nutrient intakes, we found no significant associations of breast cancer risk with current intakes of folate (Table 1), riboflavin, and vitamins B-6 and B-12; because of space limitations, nonfolate data are presented elsewhere (*see* Appendix under ''Supplemental data'' in the online issue). The correlation coefficients between current and longer-term (10 y) intakes of folate, riboflavin, and vitamins B-6 and B-12 ranged from 0.53 to 0.91.

DISCUSSION

In this cohort of mostly supplement users, we examined the association between intakes of folate and other nutrients involved in one-carbon metabolism and the risk of breast cancer in postmenopausal women. Women who consumed ≥1272 DFE/d of total folate for >10 y had a 22% lower risk of breast cancer than did women in the lowest category of folate consumption. Folate intake appeared particularly protective for women with ER− breast cancers.

Folate may lower breast cancer risk through several mechanisms. Folate is the principal component of one-carbon metabolism, where it is an essential cofactor for the de novo synthesis of purines and thymidylate—nucleotides needed for DNA synthesis and repair; aberrations in these processes can increase cancer risk (34, 35). Furthermore, folate, in the form of 5-methyltetrahydrofolate, is important for the remethylation of methionine and, thus, for the production of *S*-adenosylmethionine—the donor of methyl groups for methylation reactions, including DNA (36). DNA methylation is an important determinant of gene expression, particularly gene silencing, and aberrations are associated with carcinogenesis.

Our results are consistent with some but not all, epidemiologic studies (13, 14). Similar to our results, the French E3N cohort

TABLE 3
Association between total folate intake and postmenopausal breast cancer risk, by tumor characteristics[1]

	Quartile of 10-y total folate intake					
	1	2	3	4	*P* for trend[2]	*P* for difference[3]
Tumor size						
Cases with tumors <1.5 cm	82	89	88	76		
Multivariable-adjusted RR (95% CI)	1.00	1.09 (0.80, 1.49)	0.97 (0.71, 1.31)	0.84 (0.60, 1.17)	0.20	
Cases with tumors ≥1.5 cm	85	72	84	70		
Multivariable-adjusted RR (95% CI)	1.00	0.83 (0.60, 1.15)	0.89 (0.65, 1.22)	0.69 (0.49, 0.98)	0.07	0.57
ER status						
Cases with ER+ status	139	132	151	136		
Multivariable-adjusted RR (95% CI)	1.00	0.95 (0.74, 1.21)	0.99 (0.78, 1.26)	0.88 (0.68, 1.14)	0.43	
Cases with ER− status	24	27	26	11		
Multivariable-adjusted RR (95% CI)	1.00	1.08 (0.61, 1.90)	0.95 (0.54, 1.68)	0.38 (0.18, 0.80)	0.02	0.02

[1] Multivariable relative risks (RRs) and associated 95% CIs were estimated by using a Cox regression model adjusted for age (y), race (white or other), family history of breast cancer (none, 1 affected mother or sister, or ≥2 affected mother or sisters), mammography within 2 y preceding baseline (no or yes), history of breast biopsy (no or yes), age at menarche (≤11, 12, 13, or ≥14 y), age at first birth (nulliparous, or ≤19, 20–24, 25–34, or ≥35 y), age at menopause (≤44, 45–49, or ≥50 y), years of combined estrogen and progestin postmenopausal hormone use (never or <1, 1–4, 5–9, or ≥10), BMI (in kg/m^2; <25, 25 to <30, or ≥30), height (<62, 62 to <65, 65 to <68, or ≥68 in.; 1 in. = 2.54 cm), total physical activity (none, 1–3 tertiles of metabolic equivalent hours/wk), and alcohol intake in the past year (<1.5, 1.5–4.9, 5.0–9.9, or ≥10 g/d). We additionally adjusted for total energy intake (kcal/d) in analyses of dietary and total nutrient intakes. Total folate intake was calculated as the sum of diet over year before baseline plus the 10 y-average from supplements. ER, estrogen receptor.

[2] Calculated by modeling categories of total folate as a single ordinal variable in a Cox regression; a Wald test was used to obtain a *P* value.

[3] Tests for differences were calculated by excluding all noncases and fitting a multivariable unconditional logistic regression model, with comparison of case groups by tumor characteristic; a Wald statistic was used to obtain a *P* value.

observed a statistically significant 22% lower risk in breast cancer among French women consuming the highest quintile (median: 522 μg/d) than in those consuming the lowest quintile (median: 296 μg/d) of folate intake primarily from diet; only 9% reported taking supplements (37). Moreover, the Malmo study observed a statistically significant 44% lower risk among Swedish women in the highest quintile (median: 456 μg/d) than in those with the lowest quintile (median: 160 μg/d) of total folate intake (38). Among case-control studies, dietary or total folate was significantly negatively associated with breast cancer risk in 6 (39–44) investigations (RR: 0.47–0.71). In a nested case-control study of the Nurses' Health Study (45), there were suggestive negative associations between plasma folate and breast cancer risk (>14 ng/mL compared with <4.6 ng/mL; RR: 0.73; 95% CI: 0.73, 1.07; *P* for trend = 0.06). Other cohort (46–52) and case-control (53–59) studies have not observed statistically significant main effects between folate intake and breast cancer risk. However, suggestive of a benefit of folate, some studies have reported that higher intakes of folate or multivitamin use attenuated the increased risk of breast cancer from alcohol (13), which is consistent with our findings.

Our results are of interest because the median intake in the highest category of self-reported total folate was much higher than that in most previous studies. In the Women's Health Study, plasma folate was marginally positively associated with breast cancer risk (>15.8 ng/mL compared with ≤5.1 ng/mL plasma folate; RR: 1.42; 95% CI: 1.00, 2.02; *P* for trend = 0.21); however, total folate, as assessed from an FFQ, was not significantly associated with breast cancer risk (60). Our highest category of folate intake was similar to that of the Prostate Lung, Colorectal and Ovarian Screen Trial (12), which reported a greater risk of breast cancer among women consuming >853 μg total folate/d than among women consuming ≤335 μg/d (RR: 1.32; 95% CI: 1.04, 1.68; *P* for trend = 0.03). In support of our finding of no such increase, folic acid supplementation 4–20 times above the basal dietary requirement did not increase the development or progression of mammary tumors in rats (61–63).

In subanalyses, we observed a greater benefit of total folate intake for ER− breast cancers than for ER+ breast cancers. This result is consistent with that of the Nurses' Health Study (64). The Iowa Women's Health Study did not observe an overall association between folate intake and risk of breast cancer by ER status, but did observe a higher risk of developing ER− cancers among women with a low intake of folate and a high intake of alcohol (65). Folate is involved in DNA methylation, and data from breast cells and tissue suggest that methylation of CpG islands of the ER gene is associated with a lack of ER gene transcription (eg, gene silencing) (66–70). Our case numbers by ER status were small, and the results may have been due to chance. Confirmation of these results and the underlying mechanisms are needed.

Our results regarding tumor size suggest that folate may not be enhancing tumor growth, because the risk reduction was not statistically different between large compared with small tumors. However, our analyses of current folate intakes closer to the time of cancer diagnosis showed no indication of the risk reduction seen for longer-term intakes. This suggests that there is no protective effect of either supplemental or total intakes at the later stages of breast carcinogenesis. However, our measures of timing of nutrient intakes were not very specific, and further investigation regarding the timing of folate intake on breast cancer risk would be useful.

Methionine, riboflavin, and vitamins B-6 and B-12 were not significantly associated with breast cancer risk, although there were suggestive negative associations for methionine. Results from other epidemiologic studies have been mixed. Several

studies have reported inverse breast cancer associations for vitamin B-6 and/or vitamin B-12 intakes (37, 40, 42, 43, 45). One case-control study reported increased breast cancer risk by methionine and vitamin B-6 intakes (39). Other studies have not observed statistically significant associations (52, 60, 71). Variation between populations with regard to dietary intakes, supplement use, alcohol intake, and polymorphisms in genes related to one-carbon metabolism may have contributed to differences in the results.

Our study had several strengths. First, our supplement questionnaire section was considerably longer (6 pages) and more detailed than typical supplement assessments used in other epidemiologic studies and has good measurement properties (29). Moreover, because, by study design, participants were mostly supplement users, we were able to examine higher folate intakes (eg, from diet, dietary fortification, and supplement use) than examined in previous studies. Furthermore, few studies have examined intakes of other nutrients involved in one-carbon metabolism, and our study is one of the very few that has ascertained information on intake of supplemental B vitamins from individual supplements and from mixtures such as B-complex vitamins and multivitamins.

This study also had limitations. First, there were likely errors in our estimate of total folate intake, which was the sum of folate from diet at baseline and from the 10-y average of supplemental folate (or folic acid) intake. The 10-y average was used for supplements to reflect the long induction period of cancer; however, for diet it is only feasible to ask questions on an FFQ about a short time period, in this case, the year before baseline. Thus, there was likely measurement error due to poor recall and to the fact that the diet at baseline did not accurately represent a person's long-term diet over the induction period. Another source of measurement error was the change in the folate content of food over time. Food manufacturers began fortifying grain products starting in 1996–1998 in response to US governmental regulations—a few years before the VITAL baseline questionnaire (2000–2002). Thus, the high intakes in this population represent the postfortification period. Examination of serum folate concentrations measured at multiple time points would have been ideal, although expensive for this large-sized cohort. Second, the collinearity between the total intake of most of the total B vitamins studied would have made it difficult to separate their individual effects; however, we did not observe any significant associations with any of the other B vitamins or for a summary score.

There is no consensus regarding a safe upper limit of folate intake, and a study of high doses of folate intake is particularly relevant given the common use of supplements containing folate, given the relatively recent US and Canadian fortification mandate, and because several European countries are also considering folate-fortification programs (5). In summary, our study of supplement users does not support recent reports that very high intakes of folate increase breast cancer risk; rather, it suggests that longer-term folate intakes may be protective, especially against ER− breast cancers. However, our results do not preclude the possibility that high folate intakes more closely before cancer diagnosis could be less beneficial; further investigation of the importance of the timing of intake is warranted. Additional studies of the effects of supplement use exceeding 10 y and lifetime alcohol consumption on breast cancer risk would also be useful. High intakes of multiple B vitamins in a recent randomized controlled trial were associated with a nonsignificant reduction in postmenopausal breast cancer risk (hazard ratio: 0.83; 95% CI; 0.60, 1.14). Our risk estimates among individuals with comparable high intakes of multiple B vitamins are consistent with these findings (72).

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