Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial[1–3]

Rachael Z Stolzenberg-Solomon, Shih-Chen Chang, Michael F Leitzmann, Karen A Johnson, Christine Johnson, Saundra S Buys, Robert N Hoover, and Regina G Ziegler

ABSTRACT

Background: Several epidemiologic studies suggest that higher folate intakes are associated with lower breast cancer risk, particularly in women with moderate alcohol consumption.

Objective: We investigated the association between dietary folate, alcohol consumption, and postmenopausal breast cancer in women from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial cohort.

Design: Dietary data were collected at study enrollment between 1993 and 2001. Folate content was assigned on the basis of prefortification (ie, pre-1998) databases. Of the 25 400 women participants with a baseline age of 55–74 y and with complete dietary and multivitamin information, 691 developed breast cancer between September 1993 and May 2003. We used Cox proportional hazard models with age as the underlying time metric to generate hazard ratios (HRs) and 95% CIs.

Results: The adjusted HRs were 1.19 (95% CI: 1.01, 1.41; *P* for trend = 0.04) for women reporting supplemental folic acid intake ≥400 μg/d compared with subjects reporting no supplemental intake. Comparison of the highest with the lowest quintile gave adjusted HRs of 1.04 (95% CI: 0.83, 1.31; *P* for trend = 0.56) and 1.32 (95% CI: 1.04, 1.68; *P* for trend = 0.03) for food and total folate intake, respectively. Alcohol consumption was positively associated with breast cancer risk (highest compared with lowest quintile: HR = 1.37; 95% CI: 1.08, 1.76; *P* for trend = 0.02); the risk was greatest in women with lower total folate intake.

Conclusions: Our results do not support the hypothesis that high folate intake reduces breast cancer risk; instead, they suggest that a high intake, generally attributable to supplemental folic acid, may increase the risk in postmenopausal women. However, our results confirm previous studies showing positive associations between moderate alcohol consumption and breast cancer. *Am J Clin Nutr* 2006;83:895–904.

KEY WORDS Folate, alcohol, breast cancer, prospective study, cohort, epidemiology, diet

INTRODUCTION

Over the past 10 y, evidence has been mounting that folate inadequacy may be an important risk factor for cancer (1). Low folate intakes and status have been positively associated with several cancers, including colorectal, cervical, lung, esophageal, and pancreas, and the evidence is most compelling for colorectal cancer (1). In addition, some but not all retrospective and prospective studies suggest that high folate intake or circulating folate concentrations may be associated with a lower risk for postmenopausal breast cancer, particularly in women with moderate or high alcohol consumption (2–21). Alcohol use has been consistently associated with increased breast cancer risk (22, 23). Although increases in sex hormones have been proposed to explain the association of alcohol and breast cancer (24), chronically high alcohol consumption, such as that of alcoholics, is also associated with inadequate folate status (25).

The 2 most commonly hypothesized, but yet unproven, mechanisms by which folate inadequacy may contribute to carcinogenesis are *1*) DNA hypomethylation and subsequent proto-oncogene activation and *2*) misincorporation of uracil during DNA synthesis, which leads to DNA instability (1). Methionine, in the form *S*-adenosylmethionine, is the principal methyl donor for >100 biologic methylation reactions, including DNA methylation. *S*-adenosylhomocysteine, the product of these reactions, is hydrolyzed to homocysteine in a reversible reaction with equilibrium dynamics that favor synthesis of *S*-adenosylhomocysteine. High *S*-adenosylhomocysteine concentrations inhibit methyltransferases and thus methylation reactions (26). Folate and vitamin B-12 are coenzymes needed to regenerate methionine from homocysteine. Deficiencies in one-carbon related nutrients, particularly folate, may lead to lower cellular *S*-adenosylmethionine, diminished or altered DNA methylation (eg, DNA hypomethylation), and accumulation of *S*-adenosylhomocysteine and homocysteine. Thus, both higher homocysteine concentrations and less methionine regeneration

[1] From the Nutritional Epidemiology Branch (RZS-S, S-CC, and MFL) and the Epidemiology and Biostatistics Program (RNH and RGZ), Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD; the Breast and Gynecologic Cancer Research Group, Division of Cancer Prevention, National Institutes of Health, Rockville, MD (KAJ); the Henry Ford Health Sciences Center, Detroit, MI (CJ); and the University of Utah Health Sciences Center, Salt Lake City, UT (SSB).

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[3] Address reprint requests to RZ Stolzenberg-Solomon, 6120 Executive Boulevard, Suite 320, Rockville, MD 20852. E-mail: rs221z@nih.gov.

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may lead to DNA hypomethylation, which may increase the susceptibility of genes to mutations or alter gene expression, each of which has been hypothesized to increase cancer risk. Although there is evidence to support an influence of folate deficiency on global methylation, the influence of folate on gene-specific methylation is not yet understood (27). Folate, in the form 10-formyl tetrahydrofolate, is also required to synthesize purines for DNA synthesis, whereas folate in the form 5,10-methylenetetrahydrofolate is required to convert deoxyuridylate into deoxythymidylate. Therefore, folate inadequacy may increase cancer susceptibility through greater misincorporation of uracil for thymine in DNA and impaired DNA excision repair, both of which lead to DNA strand breaks and chromosome damage (1). The purpose of the current study was to better understand the association between folate intake (food folate, the natural polyglutamate forms in foods, synthetic folic acid supplements, and total folate), alcohol consumption, the interaction of these factors, and postmenopausal breast cancer in a prospective analysis of women participating in the screening arm of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial.

SUBJECTS AND METHODS

Study design and population

Subjects in this study were women randomly assigned to the intervention arm of the PLCO Cancer Screening Trial, a randomized multicenter trial investigating whether screening for prostate, lung, colorectal, and ovarian cancer can reduce cancer-specific incidence and mortality. Details of the study have been described elsewhere (28). Briefly, women aged 55–74 y were recruited between November 1993 and July 2001 in 10 US centers. Participants with a personal history of 1 of the 4 PLCO cancers, with a recent history of screening procedures for one of the cancers, or with current treatment for any cancer except nonmelanoma skin cancer were excluded from the trial.

Women randomly assigned to the intervention arm underwent periodic cancer screening tests, including chest X-ray, flexible sigmoidoscopy, digital rectal examination, cancer antigen 125 screening, and transvaginal ultrasound. Women randomly assigned to the control arm were instructed to follow their usual medical practice.

Of the 77 376 women enrolled in the PLCO trial, only those in the intervention arm were given the dietary history questionnaire at baseline (n = 38 660 women); of that group, 31 411 (81%) completed the questionnaire. We excluded 6011 of those women for the following reasons: history of any cancer other than nonmelanoma skin cancer (n = 2338); > 8 items missing from the food-frequency questionnaire (n = 319); extreme values for energy intake (lowest or highest 1% of the distribution, n = 544); and missing data on multivitamin use (n = 2810). The current analysis included the remaining 25 400 women. Given the age of the women at randomization (55–74 y), we estimated that <0.3% of them may have been premenopausal at baseline, and we not exclude any women on the basis of menopausal status.

Each eligible participant provided written informed consent. The study was approved by the institutional review boards of the National Cancer Institute and each of the participating centers.

Breast cancer case ascertainment

Participants were sent an annual health survey questionnaire and were asked whether they had been diagnosed by a health care provider as having cancer and, if so, what was the type of cancer. Incident breast cancer cases were identified by self-report in the annual mail-in survey, state cancer registries, death certificates, physician reports, and (for deceased persons) reports from the next of kin. Medical record abstracts containing pathology reports were sought for confirmation of breast cancer diagnosis. Between September 1993 and June 2003, 691 incident breast cancer cases were identified. Five hundred cases (72.4%) were confirmed though medical review, of which 96 were in situ cancers. Analyses that excluded nonconfirmed and in situ cancers did not noticeably differ from those using all of the cases; therefore, we included all ascertained cases in our analysis to increase the statistical power.

Assessment of diet, vitamin supplement and alcohol use, and other baseline characteristics

At randomization, all study subjects were asked to complete a self-administered baseline questionnaire that included questions on demographic factors, medical history, and health-related behaviors. In addition, all participants randomly assigned to the PLCO Trial intervention arm were given the PLCO food-frequency questionnaire (FFQ), which was designed to be self-administered and to characterize usual dietary intake over the past 12 mo (http://www.cancer.gov/prevention/plco/DQX.pdf). With the use of a grid format, frequency of consumption was asked for 137 food items; in addition, usual portion size (small, medium, or large) was queried for 77 items. For beer, wine, and spirits, both frequency and portion size were assessed. Descriptive data for calculating nutrients and food groups were derived from the two 24-h recalls administered in the 1994–1996 Continuing Survey of Food Intake by Individuals (CSFII; 29), a nationally representative survey from the US Department of Agriculture that was conducted during the period when the FFQ was being used. In particular, the cutoffs between small and medium and between medium and large intakes correspond to the 25th and 75th percentiles for portion sizes reported by participants aged ≥51 y in the 1994–1996 CSFII (29). The choice of food items, the wording, and the assumptions for estimating nutrients and food groups for the PLCO FFQ incorporated elements of both cognitive (30, 31) and database (29) research. Supplemental vitamin and mineral use was assessed for 12 types of supplements by asking for the name of each supplement ever taken since age 25 y, the years of use, and the number of pills and whether the supplement was taken currently, 2 y ago, or 5 y ago. Supplemental folic acid use and dose were derived from recent use (current or 2 y ago) of 4 multivitamins: One-a-Day (100% RDA; Bayer Corp, Pittsburgh, PA), a therapeutic or high-dose type (>100% RDA; eg, Theragran; Bristol-Myers Squibb, New York, NY), Stresstabs (B-complex + vitamin C; Inverness Medical Inc, Waltham, MA), and B-complex. The B-complex multivitamin was assigned a 200-μg folic acid dose, whereas the other multivitamins were assigned a 400-μg folic acid dose.

Statistical analysis

The follow-up period for each participant was from the date of randomization until diagnosis of breast cancer, death, or last completed annual health survey. The analysis reported here included follow-up to June 2003 (median follow-up time: 4.94 y;

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maximum: 9.33 y), which represents 127 261 person-years of observation.

Analyses were performed separately for folate from foods, supplements, and foods and supplements combined (total) and for the natural folate in foods. The folate species occurring naturally in foods such as green vegetables and oranges are polyglutamates. Also found in food is folic acid, a synthetic folate used to fortify breakfast cereal and grain products. In 1998, in an effort to reduce the incidence of neural tube defects, the FDA instituted in the United States mandatory folic acid fortification of grains at 140 μg folic acid/100 g grain products (32). Therefore, our analyses examined both prefortification and postfortification values for food and total folate. Nutrients were analyzed both as continuous and categorical variables. Continuous variables were categorized on the basis of distribution in our cohort. Trend tests across categorical variables were analyzed by calculating a score variable based on the median values of each category. Spearman correlations were calculated to assess the correspondence between study variables in the cohort. Because most dietary variables were correlated with total energy, nutrient and food variables were energy-adjusted by using the residual method (33). The use of alcohol, supplemental folic acid, and multivitamins was not highly correlated with energy intake, and energy did not change risk estimates when included in these models; therefore, values for alcohol, supplemental folic acid, and multivitamin use were not energy-adjusted.

Generalized linear models were used to estimate the means, standard errors, and *P* for trend within and across the total folate quintiles for the continuous population characteristic variables in **Table 1**. For the categorical variables, we show frequency proportions, and we used the Cochran-Armitage test to calculate the *P* for trend. Cox proportional hazard models with age as the underlying time metric were used to generate hazard ratios (HRs) and 95% CIs. Entry time was defined as a subject's age in days at randomization, and exit time was defined as a subject's age in days at cancer diagnosis or censoring. Our multivariable best-fit models were developed separately for each folate variable and alcohol use by entering covariates individually into the model. Variables were included in the model if they were associated with both the disease and exposure and if they either changed the risk estimate by 10% or increased the precision of the risk estimate by narrowing the range of the CI. Variables examined as potential confounders included dietary intakes of energy; vitamins A, B-6, C, D, and E and carotenoids from foods, supplements, and both sources; and protein, fat, carbohydrate, and fiber; alcohol and multivitamin use (ever or never); height (median quintiles or data missing); current body mass index [(BMI; in kg/m^2) median quintiles or data missing]; physical activity (<4 h/wk, ≥4 h/wk, or data missing); age at menarche (<12, 12–13, or ≥14 y); age at menopause (<40, 40–49, 50–54, or ≥55 y); age at first birth and number of live births (nulliparous; <20 y and 1, 2, or ≥3 births; 20–29 y and 1, 2, or ≥ 3 births; and ≥30 y and 1, 2, or ≥3 births); oral contraceptive use (no, yes, or data missing); hormone replacement therapy (HRT) use (never; former; current and 1–5 y, >5–10 y, or >10 y; unknown; or data missing); mammography history (no, once, more than once, or unknown); history of benign breast disease (no, yes, or data missing): family history of breast cancer (no, yes, or data missing); smoking history (never, quit >10 y ago and smoked ≤20 cigarettes/d, quit >10 y ago and smoked >20 cigarettes/d, smoked in past 10 y and smoked ≤20 cigarettes/d, smoked in past 10 y and smoked >20 cigarettes/d, or data missing); and education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate). Nutrients were entered into the models as their score quintile trend variable, and other variables were entered as continuous or categorical. In addition to the variables in the best-fit model, the full multivariable models included putative risk factors for breast cancer (ie, education, energy intake, HRT use, oral contraceptive use, mammography history, history of benign breast disease, family history of breast cancer, age at menarche, age at menopause, and age at first birth and number of live births), although they were not necessarily confounders.

Because 85% of our population reported having used multivitamins that contained folic acid and because the inclusion of these subjects could obscure risk associations with dietary folate if they exist, we examined the association of food folate and natural polyglutamate folate with breast cancer in the subset of our population that reported never using multivitamins (n = 3706, n = 91 breast cancer cases). Among the women in our cohort, orange juice (9.2%), fiber-rich breakfast cereals fortified at 25% of the recommended dietary allowance (6.6%), and breakfast cereals fortified at 100% RDA (6.1%) were the greatest contributors to food folate; therefore their association with breast cancer was also examined in those who never used multivitamins. Because the food variables were highly skewed, the foods were log transformed before energy adjustment. More than 20% of the women reported never consuming the 2 categories of breakfast cereals; therefore, the lowest reported intake of each cereal was added to the zero values before the log transformation, and the foods were categorized by quartiles instead of quintiles.

We examined the effect of folic acid fortification of grain by testing whether the calendar year modified the association of folate and breast cancer (binary variable: <1998 and ≥1998; continuous calendar year from 1993 to 2003) and by ascertaining the association between postfortification food and total folate intakes and breast cancer in those who continued to be part of the cohort (n = 25 152) after 1997. Effect modification of the folate and breast cancer association by alcohol use, BMI, and HRT use and effect modification of the alcohol and breast cancer association by BMI, total folate intake, and HRT use were tested by using cross-product terms in the multivariable model and by comparing estimates of the stratum-specific HRs. Effect modification of the folate and alcohol variables by time during follow-up was also evaluated by using cross-product terms of the binary time variable (eg, <3 and ≥3 y of follow-up). We used SAS software (version 8.2; SAS Institute, Cary, NC) for these analyses. All tests were 2-sided.

RESULTS

Women in our cohort reported a wide range of total folate intake, averaging 263 mg/d in the lowest quintile and 1154 mg/d in the highest quintile, according to the prefortification food composition data (Table 1). Total folate came primarily from food in the 2 lowest quintiles, supplements in the highest quintile, and both sources in the third and fourth quintiles. Of the women in the lowest quintile, 60% had used multivitamins, whereas >97% of the women in the top 3 quintiles had used multivitamins. Statistically significant trends across increasing quintiles of total folate intake were seen for all breast cancer risk factors except family history. Specifically, with increasing total folate

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TABLE 1
Baseline characteristics of women in the intervention arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial by energy-adjusted total dietary folate intake based on values before folic acid fortification[1]

	Quintile of energy-adjusted total folate intake					
Characteristics	1 (n = 5080)	2 (n = 5080)	3 (n = 5080)	4 (n = 5080)	5 (n = 5080)	p[2]
Total folate intake (μg/d)	≤335.5	>335.5 to ≤543.0	>543.0 to ≤715.5	>715.5 to ≤853.0	>853.0	
Age at randomization (y)	62.4 ± 0.07[3]	63.4 ± 0.07	62.5 ± 0.07	63.3 ± 0.07	62.8 ± 0.07	<0.0001
Folate intake (μg/d)						
Natural folate	241.2 ± 1.01	302.5 ± 1.01	264.6 ± 1.01	308.5 ± 1.01	327.2 ± 1.01	<0.0001
Food						
Before fortification	260.6 ± 1.39	361.5 ± 1.39	303.7 ± 1.39	370.7 ± 1.39	416.5 ± 1.39	<0.0001
After fortification	336.5 ± 1.31	424.1 ± 1.31	374.7 ± 1.31	430.6 ± 1.31	472.6 ± 1.31	<0.0001
Supplemental, recent[4]	2.8 ± 2.32	54.6 ± 2.32	346.7 ± 2.32	401.7 ± 2.32	738.1 ± 2.32	<0.0001
Total (food and supplements)						
Before fortification	262.7 ± 1.98	416.9 ± 1.98	649.8 ± 1.98	773.9 ± 1.98	1153.6 ± 1.98	<0.0001
After fortification	338.7 ± 2.02	479.5 ± 2.02	720.7 ± 2.02	833.7 ± 2.02	1209.7 ± 2.02	<0.0001
Multivitamin use, ever [n (%)]	3029 (59.6)	3585 (70.6)	4942 (97.3)	5064 (99.7)	5074 (99.9)	<0.0001[5]
Height (cm)	163.3 ± 0.93	163.0 ± 0.93	163.2 ± 0.93	163.2 ± 0.93	163.5 ± 0.93	0.0008
Current BMI (kg/m^2)	27.6 ± 0.08	26.9 ± 0.08	27.2 ± 0.08	26.5 ± 0.08	26.5 ± 0.08	<0.0001[5]
Physical activity ≥ 4 h/wk [n (%)]	738 (15.4)	1078 (21.2)	931 (18.3)	1194 (23.5)	1301 (25.6)	<0.0001[5]
Family history of breast cancer [n (%)]	73 (1.4)	57 (1.1)	58 (1.1)	55 (1.1)	61 (1.2)	0.38[5]
History of benign breast disease [n (%)]	1301 (25.6)	1444 (28.4)	1410 (27.8)	1509 (29.7)	1622 (31.9)	<0.0001[5]
Age at menarche [n (%)][6]						
≤11 y	908 (17.9)	1072 (21.2)	967 (19.1)	1006 (19.9)	1050 (20.7)	0.03[5]
12–13 y	2779 (54.9)	2732 (53.9)	2825 (55.7)	2794 (55.1)	2751 (54.3)	0.95[5]
≥14 y	1380 (27.2)	1259 (24.9)	1278 (25.2)	1266 (25.0)	1266 (25.0)	0.05[5]
Age at menopause [n (%)][7]						
≤49 y	2619 (52.0)	2540 (50.3)	2558 (50.8)	2527 (50.2)	2602 (51.7)	0.92[5]
50–54 y	1856 (36.9)	1943 (38.5)	1916 (38.0)	1909 (37.9)	1805 (35.9)	0.11[5]
≥55 y	557 (11.1)	567 (11.2)	565 (11.2)	596 (11.8)	624 (12.4)	0.02[5]
Live births (n)	3.39 ± 0.03	3.32 (0.03)	3.30 (0.03)	3.23 (0.03)	3.26 (0.03)	<0.0001
Aged >30 y at first live birth [n (%)]	353 (7.0)	382 (7.5)	338 (6.7)	331 (6.5)	289 (5.7)	0.0006[5]
Oral contraceptive use, ever [n (%)]	2783 (54.8)	2631 (51.8)	2891 (56.9)	2751 (54.2)	2914 (57.4)	<0.0001[5]
HRT use, ever [n (%)]	3194 (62.9)	3377 (66.5)	3526 (69.6)	3634 (71.5)	3766 (74.1)	<0.0001[5]
HRT use, current [n (%)]	2334 (44.9)	2552 (50.2)	2703 (53.2)	2877 (56.6)	2949 (58.1)	<0.0001[5]
>1 Mammography during the past 3 y [n (%)]	3433 (67.6)	3713 (73.1)	3744 (73.7)	3944 (77.6)	3803 (74.9)	<0.0001[5]
College or postgraduate (or both) education [n (%)]	1302 (25.7)	1654 (32.6)	1477 (29.1)	1735 (34.1)	1722 (33.9)	< 0.0001[5]
Daily nutrient intakes						
Energy (kcal/d)	1787.3 ± 8.40	1698.4 ± 8.40	1789.1 ± 8.40	1657.2 ± 8.40	1807 ± 8.40	<0.0001
Protein (g/d)	68.2 ± 0.16	69.5 ± 0.16	69.0 ± 0.16	70.3 ± 0.16	70.0 ± 0.16	<0.0001
Total fat (g/d)	59.6 ± 0.16	53.2 ± 0.16	57.0 ± 0.16	52.1 ± 0.16	51.1 ± 0.16	<0.0001
Carbohydrate (mg/d)	231.8 ± 0.48	251.8 ± 0.48	239.2 ± 0.48	253.9 ± 0.48	257.9 ± 0.48	<0.0001
Total fiber (g/d)	18.1 ± 0.08	22.7 ± 0.08	19.7 ± 0.08	23.1 ± 0.08	24.4 ± 0.08	<0.0001
Alcohol (g/d)	7.2 ± 0.18	4.9 ± 0.18	6.6 ± 0.18	4.7 ± 0.18	4.9 ± 0.18	<0.0001
Vitamin A (μg RAE)	1347.3 ± 8.47	1738.9 ± 8.47	1480.5 ± 8.47	1783.6 ± 8.47	1893.7 ± 8.47	<0.0001
Total vitamin A (μg RAE)[8]	10 719.2 ± 92.25	14 736.3 ± 92.25	15 927.2 ± 92.25	19 088.8 ± 92.25	22 931.1 ± 92.25	<0.0001
Carotenoids (μg RAE)	856.4 ± 8.09	1156.6 ± 8.09	950.6 ± 8.09	1182.1 ± 8.09	1262.7 ± 8.09	<0.0001
Vitamin C (mg/d)	129.2 ± 1.14	181.0 ± 1.14	150.1 ± 1.14	185.6 ± 1.14	205.4 ± 1.14	<0.0001
Total vitamin C (mg/d)[8]	284.5 ± 5.78	406.9 ± 5.78	481.3 ± 5.78	536.5 ± 5.78	938.8 ± 5.78	<0.0001
Vitamin E (IU/d)	10.3 ± 0.07	12.1 ± 0.07	11.5 ± 0.07	12.2 ± 0.07	14.5 ± 0.07	<0.0001
Total Vitamin E (IU/d)[8]	140.7 ± 0.07	185.7 ± 0.07	240.7 ± 0.07	255.9 ± 0.07	368.2 ± 0.07	<0.0001

[1] n = 25 400. HRT, hormone replacement therapy; RAE, retinol activity equivalents.
[2] Generalized linear models were used to estimate means, SEs, and P value trends for the continuous variables within and across each quintile of total folate intake.
[3] $\bar{x}$ ± SE (all such values).
[4] Folic acid use: current or past 2 y.
[5] The Cochran-Armitage test was used to calculate trends for dichotomous proportions across each quintile of total folate intake.
[6] n = 67 women with missing data on age at first menstrual period.
[7] n = 216 women with missing data on age at last menstrual period.
[8] Total intake from diet and supplements combined.

intake, women were older, had lower BMIs and greater height, were more physically active and more educated, were younger at menarche and older at menopause, were more likely to report fewer live births, and were more likely to have a history of benign breast disease, oral contraceptive use, HRT use, and more than one mammography; they also less often reported

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age > 30 y at first birth (P for trend < 0.05). In addition, women in the highest quintile of total folate intake tended to consume more energy, protein, carbohydrate, fiber, carotenoids, and vitamins A, C, and E and less fat and alcohol than did women in the other quintiles.

The breast cancer HRs and 95% CIs for dietary folate from foods, supplements, total folate from foods and supplements, multivitamin use, and alcohol consumption are shown in **Table 2**. Given the age of women in our cohort, we show only results using the prefortification folate values, because most of our population's exposure occurred before fortification in 1998. We report the results from the best-fit multivariable models because they did not differ substantially from the models that adjusted for all putative breast cancer risk factors. Folate from foods alone and natural folate were not significantly associated with postmenopausal breast cancer. Women consuming ≥400 μg supplemental folic acid/d had a 19% greater risk (95% CI: 1.01, 1.41; P for trend = 0.04) of postmenopausal breast cancer than did women not taking supplemental folic acid. Those in the highest quintile of total folate had a 32% greater risk (95% CI: 1.04, 1.68; P for trend = 0.03) than did those in the lowest quintile. A 5-knot spline was used to model a nonlinear relation between energy-adjusted total folate and breast cancer risk. The test for nonlinearity did not reach statistical significance (chi-square test: 3.18, $P > 0.05$), which implies the relation is nearly linear. There was no significant interaction of these associations by calendar year. In addition, in women who remained in the cohort after 1997 and thus were exposed to and using postfortification folic acid concentrations (n = 25 152; 592 incident breast cancers), food folate was not positively associated with breast cancer (highest compared with lowest quintile: HR = 0.87; 95% CI: 0.68, 1.12; P for trend = 0.46), whereas total folate was positively but marginally not significantly associated (highest compared with lowest quintile: HR = 1.29; 95% CI: 0.99, 1.68; P for trend = 0.22).

The 18% (nonsignificant) increase in breast cancer risk in women who reported ever multivitamin use was similar in magnitude to that observed in those with recent use of ≥400 μg supplemental folic acid. Alcohol use was positively associated with breast cancer: women in the highest quintile (>7.62 g alcohol/d, or ≈0.5 servings/d) had a significantly (37%) greater risk than did those in the lowest quintile (<0.01 g alcohol/d), and there was a significant trend (P for trend = 0.02).

In the women in our analysis who reported never taking multivitamins (n = 3706), folate from foods and natural folate intake were not associated with breast cancer (**Table 3**). When the major sources of food folate before 1998 were considered, breast cancer risk increased with the intake of highly fortified cereals: intakes of 100% RDA generated a significant trend (4th compared with the lowest quintile; HR = 1.69; 95% CI: 0.92, 3.10; P for trend = 0.03), whereas orange juice and fiber-rich fortified cereals showed no association. The fortified-cereal model, when adjusted for energy without using the residual method, also showed a strong positive association with breast cancer when nonconsumers were compared with consumers of >0 to < 1 g/d and consumers of ≥ 1 g/d (HR = 1.25; 95% CI: 0.74, 2.11 and 1.87; 95% CI: 1.13, 3.11, respectively; P for trend = 0.02). The associations between any of the folate, alcohol, or high folate food variables and breast cancer were not modified by follow-up time (<3 and >3 y follow-up after baseline).

Alcohol consumption did not significantly modify the association of any of the folate variables; however, total folate intake qualitatively modified the association with alcohol consumption (total folate × alcohol interaction, P = 0.05; **Table 4**). The greater risk with greater alcohol consumption tended to be stronger in women with low total folate intake; those in the highest quintile of alcohol use had a risk of breast cancer approximately twice that of those in the lowest quintile. Neither BMI nor HRT modified the associations between breast cancer and total folate intake (BMI × total folate and HRT × total folate interactions, $P > 0.05$) or alcohol intake (BMI × alcohol and HRT × alcohol interactions, P > 0.05).

DISCUSSION

The current study is the first to observe a significant positive association between total folate and supplemental folic acid intakes and postmenopausal breast cancer. However, food folate was not associated with risk. Alcohol use significantly increased the risk of postmenopausal breast cancer. The positive association with greater alcohol use was modified by total folate intake, so that the risk associated with greater alcohol use was stronger in women who had lower folate intake.

The strengths of our study include its prospective design, in which diet and other exposures are assessed before the diagnosis of disease, which eliminates recall bias and reverse causation. Our questionnaire likely is comparable to other FFQs in that we relied on nationally representative dietary data to assign the database values used to calculate nutrient estimates from foods reported on the FFQ (29).

Several retrospective and prospective epidemiologic studies support the hypothesis that folate may play a role in postmenopausal breast cancer, but not all study results are consistent (2–21). We reviewed the results of cohort studies because their design is most similar to the current study. The Nurses' Health Study (NHS) has observed a significant inverse association between total folate intake and postmenopausal breast cancer (>600 ug/d compared with 150–299 μg/d total folate intake: RR = 0.86; 95% CI: 0.76, 0.98; P for trend = 0.02) that was limited to the highest category of folate, which included intakes that could be achieved only by taking vitamin supplements (2). The NHS and the Canadian National Breast Screening study both showed stronger protective folate associations in women who reported alcohol consumption of ≥1 drink (or 15 g)/d (2, 4). In addition, a nested case-control study in the NHS that measured plasma folate showed associations (>14.0 compared with < 4.6 ng/mL: OR = 0.73; 95% CI: 0.50, 1.07; P for trend = 0.06) and interactions with alcohol consumption similar to those observed in the dietary studies; however, no association was observed with plasma homocysteine, a functional biomarker for folate status (3). The Iowa Women's Health Study showed that greater folate intake attenuated the risk of breast cancer in women with a family history of breast cancer but only in those who did not drink alcohol, whereas no association was observed for folate intake in women who did not have a family history of breast cancer (34). In contrast, the American Cancer Society's Cancer Prevention Study did not observe main effects for total dietary folate (>603.7 compared with < 209 μg total folate intake/d: RR = 1.10; 95% CI: 0.94, 1.29) or interactions with alcohol use (8); nor did a nested case-control study of the Washington County CLUE

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TABLE 2
Hazard ratios and 95% CIs for breast cancer according to baseline folate intakes and alcohol consumption in postmenopausal women participating in the intervention arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial[1]

Daily intake	Cases (n = 691)	Person-years	Hazard ratio (95% CI)[2] Univariable model	Multivariable full adjusted model[3]	Best-fit multivariable adjusted model
Natural folate from foods (μg)	*n*				
≤233.6	134	24 502	1.00 (reference)	1.00 (reference)	1.00 (reference)[4]
>233.6 to ≤265.6	140	25 377	1.05 (0.82, 1.33)	0.99 (0.78, 1.26)	1.00 (0.78, 1.27)
>265.6 to ≤295.5	135	25 608	1.00 (0.78, 1.28)	0.90 (0.71, 1.16)	0.92 (0.72, 1.18)
>295.5 to ≤337.0	126	26 066	0.91 (0.71, 1.17)	0.80 (0.63, 1.03)	0.81 (0.64, 1.05)
>337.0	156	25 709	1.10 (0.87, 1.38)	0.97 (0.76, 1.22)	0.98 (0.78, 1.24)
P for trend			0.65	0.53	0.63
Folate from foods (μg)[5]					
≤261.3	135	24 475	1.00 (reference)	1.00 (reference)	1.00 (reference)[4]
>261.3 to ≤307.2	135	25 195	1.00 (0.78, 1.27)	0.93 (0.73, 1.19)	0.95 (0.74, 1.21)
>307.2 to ≤350.5	112	25 666	0.81 (0.63, 1.04)	0.73 (0.56, 0.94)	0.75 (0.58, 0.97)
>350.5 to ≤411.9	149	26 060	0.89 (0.77, 1.25)	0.87 (0.68, 1.11)	0.89 (0.70, 1.13)
>411.5	169	25 865	1.16 (0.93, 1.46)	1.01 (0.80, 1.27)	1.04 (0.83, 1.31)
P for trend			0.13	0.72	0.56
Supplemental folic acid (μg)[6]					
0	223	46 652	1.00 (reference)	1.00 (reference)	1.00 (reference)[7]
<400	71	13 467	1.12 (0.86, 1.46)	1.08 (0.83, 1.41)	1.11 (0.85, 1.45)
≥400	397	67 143	1.25 (1.06, 1.47)	1.16 (0.98, 1.37)	1.19 (1.01, 1.41)
P for trend			0.009	0.08	0.04
Multivitamin use					
Never	91	19 156	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ever	600	108 106	1.18 (0.95, 1.48)	1.11 (0.89, 1.38)	1.18 (0.95, 1.48)
Total folate (μg)[8]					
≤335.5	115	25 868	1.00 (reference)	1.00 (reference)[9]	1.00 (reference)[10]
>335.5 to ≤543.0	134	26 645	1.13 (0.88, 1.46)	1.05 (0.82, 1.35)	1.07 (0.84, 1.38)
>543.0 to ≤715.5	153	24 767	1.39 (1.09, 1.77)	1.32 (1.03, 1.68)	1.33 (1.04, 1.70)
>715.5 to ≤853.0	130	25 292	1.16 (0.90, 1.49)	1.03 (0.80, 1.33)	1.06 (0.82, 1.37)
>853.0	159	24 689	1.45 (1.14, 1.84)	1.27 (1.00, 1.62)	1.32 (1.04, 1.68)
P for trend			0.003	0.05	0.03
Alcohol consumption (g)					
≤0.01	104	22 816	1.00 (reference)	1.00 (reference)	1.00 (reference)[11]
>0.01 to ≤0.43	138	24 747	1.23 (0.95, 1.59)	1.21 (0.94, 1.57)	1.23 (0.95, 1.58)
>0.43 to ≤1.39	158	28 187	1.22 (0.95, 1.56)	1.18 (0.92, 1.51)	1.20 (0.94, 1.54)
>1.39 to ≤7.62	118	25 734	1.02 (0.78, 1.32)	0.94 (0.72, 1.22)	0.97 (0.75, 1.26)
>7.62	173	25 778	1.48 (1.16, 1.89)	1.30 (1.02, 1.67)	1.37 (1.08, 1.76)
P for trend			0.003	0.065	0.02

[1] n = 25 400.

[2] Cox proportional hazard models were used to calculate hazard ratios. All models should be considered adjusted for age because age is the time metric. All nutrients were adjusted for energy by using the residual method with energy additionally in the model except supplemental folic acid, multivitamin use, and alcohol consumption, because they were not highly correlated with energy.

[3] Multivariable model adjusted for energy, education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate), hormone replacement therapy (never, former, current <5 y, current ≥5 and <10 y, or current ≥10 y), mammography screening history (no, once, more than once, or don't know), birth control pill use (no, yes, or data missing) history of benign breast disease (no, yes, or data missing), family history of breast cancer (no, yes, or data missing), age at menarche (<12 y, 12–13 y, or ≥14 y), age at menopause (<40 y, 40–49 y, 50–54 y, or ≥55 y), and age at first birth and number of live births (nulliparous; <20 y and 1, 2, or ≥3 births, respectively; 20–29 y and 1, 2, or ≥ 3 births, respectively; or ≥30 y and 1, 2, or ≥3 births, respectively).

[4] Adjusted for energy, hormone replacement therapy (never, former, current <5 y, current ≥5 and <10 y, or current ≥10 y), and education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate).

[5] Food folate includes natural folate and added folic acid, before mandatory folic acid fortification in 1998.

[6] Recent supplemental folic acid use, ie, current or past 2 y.

[7] Adjusted for hormone replacement therapy (never, former, current <5 y, current ≥5 and <10 y, or current ≥10 y).

[8] Folate from food and supplemental folic acid.

[9] Additionally adjusted for BMI (continuous).

[10] Adjusted for energy, hormone replacement therapy (never, former, current <5 y, current ≥5 and <10 y, or current ≥10 y), education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate), and BMI (continuous).

[11] Adjusted for education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate).

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TABLE 3
Hazard ratios and 95% CIs for breast cancer according to baseline folate intake from food in postmenopausal women participating in the intervention arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial who never took multivitamins[1]

Daily intake	Cases (n = 91)	Person-years	Hazard ratio (95% CI)[2] Univariable model	Multivariable full adjusted model[3]	Best-fit multivariable adjusted model[4]
Natural folate from foods (μg)	n				
≤221	19	3615	1.00 (reference)	1.00 (reference)	1.00 (reference)
>221 to ≤251	13	3783	0.67 (0.33, 1.37)	0.65 (0.32, 1.33)	0.64 (0.31, 1.31)
>251 to ≤280	21	3855	1.06 (0.56, 2.00)	0.96 (0.51, 1.83)	1.00 (0.52, 1.89)
>280 to ≤322	15	3953	0.69 (0.34, 1.39)	0.65 (0.32, 1.32)	0.65 (0.32, 1.33)
>322	23	3950	1.06 (0.58, 1.96)	0.99 (0.53, 1.25)	1.01 (0.54, 1.89)
P for trend			0.72	0.88	0.81
Folate from foods (μg)[5]					
≤244	19	3633	1.00 (reference)	1.00 (reference)	1.00 (reference)
>244 to ≤288	15	3758	0.77 (0.39, 1.54)	0.72 (0.36, 1.45)	0.73 (0.37, 1.47)
>288 to ≤333	11	3855	0.53 (0.25, 1.13)	0.46 (0.21, 1.00)	0.50 (0.23, 1.08)
>333 to ≤395	18	3929	0.85 (0.44, 1.64)	0.83 (0.42, 1.61)	0.80 (0.41, 1.56)
>395	28	3980	1.29 (0.71, 2.33)	1.16 (0.63, 2.12)	1.20 (0.66, 2.19)
P for trend			0.16	0.25	0.23
Orange or grapefruit juice or both (g)					
0 and ≤5.61	17	3768	1.00 (reference)	1.00 (reference)	1.00 (reference)
>5.61 to ≤29.7	18	3776	1.07 (0.55–2.08)	1.02 (0.53–2.00)	1.06 (0.55–2.06)
>29.7 to ≤100.3	19	3830	1.03 (0.53–1.99)	1.01 (0.52–1.97)	1.00 (0.51–1.93)
>100.3 to ≤180.4	21	3947	1.14 (0.60–2.16)	1.07 (0.56–2.04)	1.10 (0.58–2.10)
>180.4	16	3835	0.90 (0.45–1.78)	0.82 (0.41–1.65)	0.86 (0.43–1.72)
P for trend			0.90	0.72	0.79
Fiber cereals, 25% RDA fortified (g)					
≤0.01	21	4392	1.00 (reference)	1.00 (reference)	1.00 (reference)
>0.01 to ≤0.20	19	4623	0.96 (0.49–1.88)	0.93 (0.47–1.83)	0.95 (0.49–1.87)
>0.20 to ≤0.94	25	5131	0.98 (0.53–1.82)	1.00 (0.54–1.87)	0.99 (0.53–1.84)
>0.94	33	5009	1.15 (0.64–2.07)	1.14 (0.63–2.06)	1.15 (0.64–2.07)
P for trend			0.53	0.53	0.52
Cereals, 100% RDA fortified (g)					
0	21	4410	1.00 (reference)	1.00 (reference)	1.00 (reference)
>0 to ≤0.02	19	4361	1.02 (0.47–2.21)	1.00 (0.46–2.18)	1.00 (0.46–2.18)
>0.02 to ≤0.64	24	5469	1.18 (0.62–2.26)	1.11 (0.58–2.14)	1.18 (0.62–2.25)
>0.64	27	4916	1.74 (0.95–3.20)	1.66 (0.90–3.06)	1.69 (0.92–3.10)
P for trend			0.03	0.04	0.03

[1] n = 3706. RDA, recommended dietary allowance.

[2] Cox proportional hazard models were used to calculate hazard ratios. All models should be considered adjusted for age because age is the time metric, and nutrients and foods were adjusted for energy by using the residual method with energy additionally in the model.

[3] Multivariable model adjusted for energy, education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate), hormone replacement therapy (never, former, current <5 y, current ≥5 and <10 y, or current ≥10 y), birth control pill use (no, yes, or data missing), mammography screening history (no, once, more than once, or don't know), history of benign breast disease (no, yes, or data missing), family history of breast cancer (no, yes, or data missing), age at menarche (<12 y, 12–13 y, or ≥14 y), age at menopause (<40 y, 40–49 y, 50–54 y, or ≥55 y), and age at first birth and number of live births (nulliparous; <20 y and 1, 2, or ≥3 births, respectively; 20–29 y and 1, 2, or ≥3 births, respectively; or ≥30 y and 1, 2, or ≥3 births, respectively).

[4] Adjusted for energy, hormone replacement therapy (never, former, current <5 y, current ≥5 and <10 y, or current ≥10 y), and education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate).

[5] Food folate includes natural folate and added folic acid, before mandatory folic acid fortification in 1998.

1 and CLUE 2 cohorts that measured serum folate (lowest compared with highest quintile of serum folate: RR = 0.66; 95% CI: 0.17, 2.60; P for trend = 0.79) (9). Finally, there was no association between dietary folate and breast cancer (RR = 1.01; 95% CI: 0.93, 1.10; P for trend = 0.79) in the Melborne Collaborative Cohort (17).

There are several differences between our study and these earlier prospective studies. The postmenopausal women from the PLCO Trial have considerably higher total folate intake (median intake: ≈660 μg/d) than do other cohorts (≈300–350 μg/d; 2, 4, 5, 8), particularly from folic acid, the more biologically available form. Therefore, our population may have not had sufficiently low folate range to allow the observation of protective associations if they exist. The cohorts in which significant protective folate associations were observed (2–4) began in the 1980s with baseline measures and a greater proportion of follow-up occurring before public awareness of the potential health benefits of folate intake, and they had a longer duration of follow-up.

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TABLE 4
Hazard ratios and 95% CIs of baseline alcohol consumption for breast cancer risk stratified by total folate intake in postmenopausal women[1]

	Total folate intake ≤335.5 μg/d			Total folate intake >335.5 μg/d		
Alcohol consumption	Cases	HR (95% CI)[2]	HR (95% CI)[3]	Cases	HR (95% CI)[2]	HR (95% CI)[3]
	n			*n*		
≤0.01 g/d	12	1.00 (reference)	1.00 (reference)	92	1.41 (0.77, 2.58)	1.00 (reference)
>0.01 and ≤0.43 g/d	23	1.32 (0.66, 2.65)	1.36 (0.67, 2.76)	115	1.68 (0.93, 3.06)	1.18 (0.90, 1.56)
>0.43 and ≤1.39 g/d	24	1.30 (0.65, 2.60)	1.31 (0.65, 2.63)	134	1.66 (0.93, 3.05)	1.17 (0.90, 1.5)
>1.39 and ≤7.62 g/d	13	0.78 (0.35, 1.70)	0.81 (0.37, 1.80)	105	1.39 (0.76, 2.54)	0.98 (0.74, 1.30)
>7.62 g/d	43	1.95 (1.03, 3.72)	2.10 (1.08, 4.07)	130	1.76 (0.97, 3.19)	1.23 (0.93, 1.62)
P for trend			0.004			0.30
P for interaction[4]						0.05

[1] Cox proportional hazard models were used to calculate HRs. All models should be considered adjusted for age because age is the time metric. Total folate was adjusted for energy by using the residual method. The model is further adjusted for energy, education (≤11 y, completed high school, some college or vocational training, or college graduate or postgraduate), hormone replacement therapy (never, former, current <5 y, current ≥5 and <10 y, or current ≥10 y), and current body mass index (continuous).

[2] Reference group: low folate intake with low alcohol use.

[3] Reference group: low alcohol consumption.

[4] Alcohol use × total folate intake interaction for breast cancer.

Finally, many studies did not control for socioeconomic status or education, both of which are important potential confounders for postmenopausal breast cancer (2–4) that attenuated risk estimates in our analysis.

The positive association between alcohol consumption and breast cancer as modified by folate intake in the current study is consistent with findings from other studies. A meta-analysis of 53 studies examining the association between alcohol consumption and breast cancer showed that, compared with nondrinkers, women consuming 35–44 g alcohol/d (≈2–3 drinks) had a 32% greater risk for breast cancer, and those consuming ≥45 g alcohol/d (>3 drinks) had a 46% greater risk, both of which were significant (22). The biological mechanism that may be the most important explanation of the alcohol and breast cancer association—namely, an increase in sex hormone concentrations—is fairly well established and further supported by randomized controlled alcohol feeding trials (24, 35). The NHS (2), Iowa Women's Health Study (5), and the Melborne Collaborative Cohort (17) also showed stronger positive associations with increasing alcohol consumption in women who had low folate intake. Additional analyses within the Iowa Women's Study and the NHS showed that significant positive associations with low folate intake and high alcohol consumption were observed only for estrogen receptor–negative tumors (7, 36). The biological mechanisms that may explain the interactions between alcohol and folate and breast cancer risk, as well as the association with estrogen receptor–negative status, remain unclear.

The positive association that we observed between breast cancer and total folate intake and supplemental folic acid is biologically plausible, but it should be interpreted with caution. Along with previous experimental and epidemiologic observations, our results suggest that the role of folate in breast cancer development may be more complex than previously appreciated. The 3 rodent studies that have examined the effect of folic acid on breast cancer development suggest that folic acid deficiency decreases chemically induced mammary cancer (37–39). Among participants in a large ($n = 2928$) trial of folic acid supplementation during pregnancy, women who were randomly assigned to the highest folic acid dose, 5 mg/d, had a 70% greater risk of total cancer ($n = 112$), which was significant, and a 2-fold greater risk of breast cancer ($n = 31$), which was not significant, than did women in the placebo group during follow-up that lasted up to 36 y (40). The folic acid dose used in that trial was considerably higher than that currently prescribed to pregnant women, and the greater risk of cancer could have been due to chance, given that the trial was not powered for a cancer outcome (40). In addition, folic acid deprivation with antifolate chemotherapy such as 5-fluorouracil and methotrexate, which interferes with thymidylate uptake and ultimately DNA synthesis, has been commonly used as an effective treatment for patients with breast cancer (41), and it is possible that very high folic acid intakes could promote the growth of an existing breast neoplasm or cancer (42). Compared with matched noncancerous breast tissue, breast cancer tissue contained higher folate concentrations but was more hypomethylated (27). High folic acid concentrations could contribute to epigenetic changes in gene-regulatory mechanisms, which may result in gene silencing and enhanced cancer development (43), or they could promote the growth of tumors expressing folate receptor α (FR-α), which is present in ≈25% of breast cancers (44). However, these mechanisms are speculative, and folate's effect on these molecular processes during breast cancer development is unknown.

In conclusion, our study does not support a simple protective association between dietary folate and breast cancer. Although our results confirm previous positive associations between alcohol consumption and breast cancer, particularly in subjects with lower folate intake, they also suggest that high folate intake, which is generally attributable to folic acid from supplements, may increase the risk of breast cancer in postmenopausal women. Because of the complexity of folate function, it is hypothetically possible that both deficiency and abundance of folate may contribute to breast carcinogenesis at different stages of tumor development or in different neoplastic or tumor phenotypes. It is also possible that our observed positive associations may be due to chance or uncontrolled

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confounding. The associations could be explained by other components of multivitamins and vitamin-fortified breakfast cereals or by behaviors associated with the intake of these products. In particular, residual confounding from HRT, which is known to increase breast cancer risk, is possible. However, the positive association with the intake of ≥400 μg supplemental folic acid/d remained when the analysis was limited to women reporting no HRT use (n = 7903; HR = 1.48; 95% CI: 1.06, 2.05; P for trend = 0.02). The positive association with total folate intake that the current study found should be interpreted with great caution, and replication of our findings is important. Further research is needed to confirm our results and to elucidate the mechanisms by which folate modifies the association between alcohol consumption and breast cancer development.

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RZS-S, S-CC, MFL, RGZ, and RNH contributed to study design. RZS-S, S-CC, MFL, RNH, and RGZ contributed to data analysis. RZS-S was responsible for writing and revising the manuscript. S-CC, MFL, KAJ, CJ, SSB, RNH, and RGZ provided substantive editorial comments on manuscript drafts. None of the authors had any personal or financial conflict of interest.

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