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Original Contribution

Dietary B Vitamin and Methionine Intakes and Breast Cancer Risk Among Chinese Women

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Methionine, folate, vitamin B_6, vitamin B_{12}, niacin, and riboflavin intakes may be related to breast carcinogenesis. These associations may vary by breast cancer type. Using the prospective cohort Shanghai Women's Health Study (1997–2008) including 718 Chinese breast cancer cases, the authors evaluated baseline dietary intake of these factors and breast cancer risk and whether the associations varied by menopausal status and estrogen receptor (ER) and progesterone receptor (PR) status. They estimated associations using hazard ratios and 95% confidence intervals from Cox proportional hazards regression models and stratified analyses by menopausal status and ER/PR status. Lowest quantile of intake was used as the comparison group. For postmenopausal women, dietary intakes of methionine and B vitamins were not associated with breast cancer risk. For premenopausal women, higher intake of folate was associated with decreased breast cancer risk (hazard ratio = 0.58, 95% confidence interval: 0.34, 0.99 for the highest vs. lowest quintile of intake). Only niacin intake was associated with ER+/PR+ breast cancer risk (hazard ratio = 1.62, 95% confidence interval: 1.07, 2.46; *P* for trend = 0.04 for the highest vs. lowest quartile of intake). Findings support the hypothesis that high folate intake may reduce breast cancer risk and that the association may vary by menopausal and ER/PR status.

breast neoplasms; cohort studies; diet; estrogen receptor alpha; folic acid; niacin; premenopause

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

Folate, a B vitamin that is the major dietary source of one-carbon units, functions in methylation reactions and nucleotide synthesis. The epidemiologic literature is equivocal about the relation between folate and breast cancer. Generally, high folate intake has been associated with decreased breast cancer risk in case-control studies and is not associated with risk in cohort studies (1–3).

Methionine is an alternate dietary source of methyl groups. Riboflavin, vitamin B_6, and vitamin B_{12} are cofactors involved in one-carbon metabolism, and they have other physiologic roles. Niacin is a B vitamin involved in steroid hormone synthesis (4). Few studies have evaluated the relation of these dietary factors to breast cancer risk, although, similar to folate, there are reports of null (3, 5–10), inverse (8–12), and adverse (3) associations.

The amount of nutrients consumed varies between studies and may contribute to observed differences between studies. Another potentially important contributor may be a difference in the type of breast cancer predominant within and between populations and thus assessed in a particular study. For example, dietary risk factors for premenopausal and postmenopausal breast cancer may differ. It is also plausible that risk factors may differ by hormone receptor status, although few studies have evaluated the association of hormone receptor status with intake of B vitamins (9, 10, 13–15). None of these previous studies have been conducted among East Asian women, a population with a traditionally low breast cancer risk (16).

In this study, we evaluated whether dietary intakes of methionine, folate, and other B vitamins are related to breast cancer risk by using data from a large, population-based prospective cohort study of women in Shanghai, China. Few women in this population use vitamin supplements or drink alcohol, and the food supply is not fortified with folic

acid. Thus, the study population provides a unique resource to evaluate the relation between dietary factors and breast cancer risk. We also evaluated whether the relation between methionine and B vitamins and breast cancer risk varies by menopausal status and by estrogen receptor (ER) and progesterone receptor (PR) status.

MATERIALS AND METHODS

Participant recruitment and outcome ascertainment

The Shanghai Women's Health Study is a population-based prospective study of 74,942 Chinese women aged 40–70 years at cohort entry. Detailed descriptions of the study and validated food frequency questionnaire have been previously published (17, 18).

Briefly, from March 1997 to May 2000, a cohort of 74,942 adult Chinese women between the ages of 40 and 70 years was recruited from among 81,170 eligible women residing in 7 urban communities in Shanghai, with a 92% participation rate. Appropriate committees for the use of human participants in research approved the study protocol, and informed consent was obtained from all participants. The cohort was followed for cancer incidence and all-cause mortality by using a combination of follow-up surveys and annual record linkage to legally mandated cancer incidence data and death certificates. Nearly all cohort members were successfully followed, with response rates of 99.8% for the first in-person follow-up (2000–2002), 98.7% for the second (2002–2004), and 96.7% for the third (2004–2007). In this analysis, the date of the last follow-up was set as December 31, 2007, for study participants whose last in-person interview occurred before the censoring date, 6 months after the most recent record linkage (June 30, 2008). The cohort analysis evaluated 72,861 participants, including 718 breast cancer cases, after excluding those with a previous history of cancer ($n = 1{,}490$), reporting an unreasonably high (>3,500 kcal/day) or low (<500 kcal/day) energy intake ($n = 132$), or emigrating from Shanghai shortly after baseline recruitment ($n = 10$).

Data collection and nutrient estimation

All participants were interviewed in person at baseline by trained interviewers using structured questionnaires. Each participant was asked about her frequency of eating a specific food (daily, weekly, monthly, yearly, or never) and the raw amount typically eaten, that is, how many lians (1 lian = 50 g) or jins (1 jin = 500 g). The Chinese Food Composition Tables (19) were used to estimate daily intake levels of most nutrients. Folate content was not available for 5 food items (7%) in the Chinese Food Composition Tables. Niacin content was available for all food items. Methionine, vitamin B_6, and vitamin B_{12} were derived by using the US Department of Agriculture food composition tables, as previously described (3). Only one minor food item (conch) had no comparable food in the US Department of Agriculture database. All other foods had identical (82%) or equivalent (17%) food items in both databases. The Pearson correlation coefficients for riboflavin, niacin, and vitamin C intakes between the 2 databases were 0.91 or higher, providing assurance that use of the US Department of Agriculture database is appropriate and is unlikely to introduce substantial error.

A validation study was conducted for the food frequency questionnaire (18). Correlations between B vitamin intakes as measured on the food frequency questionnaire and multiple 24-hour recalls ranged from 0.56 to 0.59. Correlation between food frequency questionnaire folate intake and red blood cell folate level was 0.52. The interviewers also obtained standardized measurements for weight, standing and sitting height, and circumferences of the waist and hips. ER and PR status was abstracted from hospital medical records or determined from collected specimen slides.

Statistical analysis

In this cohort study population, we compared baseline characteristics of the women who were diagnosed with breast cancer in the follow-up period (cases) with characteristics of the comparison group of women who did not develop breast cancer during the follow-up period (noncases). These comparisons were conducted using *t* tests for nondietary continuous variables, χ^2 tests for categorical variables, and least-squares means for dietary variables adjusted for total energy intake. All continuous variables were log transformed.

We evaluated whether risk of developing breast cancer during the follow-up period differed between women consuming varying levels of B vitamins and methionine at baseline. To assess the association of baseline intake with breast cancer risk, we used Cox regression models to estimate the hazard ratio to compare higher quantile intake levels of these nutrients with the lowest quantile intake level (20). Nutrients were log transformed and energy adjusted by using the residual method before quantile cutpoints were determined (21). Quantile cutpoints were based on the intake distribution within the entire cohort. Models were stratified on birth cohort (5-year categories) (22). Tests for linear trend were performed by entering the categorical variables as continuous parameters in the models.

Risk factors for breast cancer in previous studies were evaluated for confounding and were included as covariates if the risk factor was related to both breast cancer and dietary intake or if inclusion of the factor in statistical models affected the parameter estimate by at least 10%. Evaluated risk factors included the following: age (years; continuous), age at menarche (years; continuous), parity (no, yes; categorical), age at first livebirth (years; continuous), menopausal status (no, yes; categorical), ever use of hormone replacement therapy at baseline (no, yes; categorical), personal history of fibroadenoma (no, yes; categorical), family history of breast cancer (no, yes; categorical), educational attainment (less than middle school, middle school, high school, college or above), household income (low, middle, high), lifetime smoking status defined as at least 1 cigarette per day for 6 months continuously (never, ever; categorical), lifetime alcohol use defined as at least 3 drinks per week for 6 months continuously (never, ever; categorical), physical activity level in the past 5 years (metabolic equivalent-hours/week per year; continuous), height

Table 1. Baseline Characteristics of Participants by Breast Cancer Diagnosis Status, Shanghai Women's Health Study, 1997–2008

Characteristic	Cases (n = 718)		Noncases (n = 72,519)		P Value[a]
	Mean (SD)	%	Mean (SD)	%	
Age at baseline, years	52.7 (8.8)		52.5 (9.1)		0.46
Age at menarche, years	14.7 (1.8)		14.9 (1.7)		0.003
Ever parous		95.4		96.7	0.05
Age at first livebirth, years	26.5 (4.14)		25.6 (4.1)		<0.0001
Postmenopausal		51.9		51.1	0.69
Age at menopause, years	49.3 (4.0)		49.1 (4.0)		0.37
Ever use of hormone replacement therapy		2.7		2.1	0.49
History of fibroadenoma		6.8		3.3	<0.0001
Family history of breast cancer		3.6		1.8	0.0004
Height, m	1.575 (0.055)		1.581 (0.056)		0.005
Waist-to-hip ratio	0.813 (0.052)		0.811 (0.054)		0.17
Body mass index, kg/m^2	24.2 (3.4)		24.0 (3.4)		0.08
Educational attainment					<0.0001
College or above		19.1		13.5	
High school		35.0		27.9	
Middle school		32.5		37.2	
Less than middle school		13.4		21.5	
Household income					
High		24.5		27.6	0.11
Middle		39.0		38.9	
Low		36.5		33.5	
Ever smoked regularly		2.0		2.8	0.18
Ever drank alcohol regularly		2.1		2.3	0.78
Physical activity, MET-hours/week per year	102 (44)		107 (45)		0.13
Ever used a B vitamin supplement		13.7		10.3	0.003
Daily dietary intake					
Energy, kcal	1,674 (373)		1,674 (394)		0.76
Vegetables, g	299 (162)		296 (169)		0.43
Fruits, g	265 (179)		264 (179)		0.83
Red meat, g	48.4 (33.4)		50.9 (36.1)		0.79
Fat, g29.2 (12.4)	29.4 (13.0)		0.87		

Abbreviations: MET, metabolic equivalent; SD, standard deviation.

[a] P values are from t tests for log-transformed continuous variables, F tests for energy-adjusted least-squares means for log-transformed dietary intakes, and χ^2 tests for categorical variables.

(meters, continuous), waist-to-hip ratio (continuous), body mass index (weight in kilograms/height in meters squared; continuous), use of a B-vitamin-containing supplement (no, yes; categorical), and daily intakes of energy (kilocalories; continuous), vegetables (grams/day; continuous), fruits (grams/day; continuous), red meat (grams/day; continuous), and fat (grams/day, continuous).

Potential effect modifiers, including menopausal status, body mass index, and ER/PR status, were evaluated in stratified analyses. ER/PR analyses were limited to the 586 breast cancer cases (81.6%) for whom these data were available.

Multivariate models were constructed to determine the joint association of folate intake with intakes of other nutrients. Tests for linear trend within strata for joint association models were conducted by performing stratified analyses by one of the dietary factors included in the joint analyses. Multiplicative variables were constructed by multiplying the log-transformed continuous variables after adding a positive constant. Tests for multiplicative interaction were conducted by including the multiplicative variables in the model and performing the likelihood ratio test. P values of <0.05 (2-sided probability) were interpreted as being statistically significant. Statistical analyses were conducted by

Table 2. International Comparison of Dietary Intake of B Vitamins and Methionine in Select Previous Studies of B Vitamins or Methionine and Breast Cancer Risk

Country (Reference No.)	Study, Study Design, Menopausal Status	% of the Population[a]		Measure Type	Folate, g		Methionine, g	Vitamin B_{12}, μg	Vitamin B_6, mg	Riboflavin, mg	Niacin, mg
		B Vitamin Supplement Users	Alcohol Drinkers		Diet	Total					
United States (42)				RDA[b]		400	0.011 g/kg Weight	2.4	1.5	1.1	14
China	SWHS, cohort, both	10.3	2.3	Mean	274		1.4	2.1	1.6	0.79	13.6
Australia (43)	MCS, cohort, both		69	Mean	330						
Brazil (5)	Sao paulo, case-control, both		21.8	Mean	529			7	0.9		
Denmark (44)	Diet, Cancer, and Health; nested case-control; both	48	97	Median	311	348					
France (30)	E3N, cohort, postmenopausal	9.7	95.4	Median		393					
Sweden (29)	MDC, cohort, postmenopausal	>19	91.4	Mean	237			13.2	3.7	2.7	
Sweden (15)	Swedish Mammography, Cohort, cohort, both	24.5		Mean	234						
Switzerland (6)	Swiss Canton of Vaud, case-control, both		41.2	Median	262				1.9	1.5	16.1
Mexico (11)	Mexico City, case-control, both			Median	310			4.58	1.27		
Canada (7, 25)	CNBSS, cohort, both			Third quintile	281–320		2.0–2.2			1.63–1.86	18.7–20.5
United States (32)	Case-control, premenopausal,			Median	231						
United States (13, 26)	IWHS, cohort, postmenopausal	>30	>36.1	Median	294	351		11.16	2.41	2.36	24.7
United States (24)	MEC, nested case-control, both		57.6	Median	316	437					
United States (8, 14)	NHS, cohort, both			Third quintile	246–284	294–381, 300–449	1.70–1.85				
United States (9, 23)	NHS II, cohort, premenopausal			Median	309	413	2	8	2.8		
United States (10)	VITAL, cohort, postmenopausal	"Most"		Median	346	776	1.4	10.8	3.05	2.7	
United States (27)	PLCO, RCT cohort, postmenopausal	85.4		Median		660					
United States (33)	WAFACS, RCT, both	23.2	45.0	Median		438.7		7.0	2.5		

United States (31)	Western New York, case-control, premenopausal	48.7	Median		367	
United States (28)	WHI, RCT cohort, postmenopausal	48.5	49.2	Mean	254	446

Abbreviations: CNBSS, Canadian National Breast Screening Study; E3N, Etude Epidémiologique auprès de femmes de la muteuelle Générale de l'Education Nationale; MCS, Melbourne Collaborative Study; MDC, Mälmo Diet and Cancer Cohort; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; RCT, randomized controlled trial; RDA, recommended dietary allowance; SWHS, Shanghai Women's Health Study; VITAL, VITamins And Lifestyle Cohort; WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study; WHI, Women's Health Initiative.

[a] Percentage of the population is based on the entire cohort population for cohort studies, the control population for case-control study designs, and the placebo group for WAFACS.

[b] Recommended dietary allowances for females aged 50–70 years.

using SAS statistical software (version 9.1; SAS Institute, Inc., Cary, North Carolina).

RESULTS

The median length of follow-up within the cohort was 9.2 years. The median age at enrollment of study participants was 50.5 years. Baseline characteristics of participants by breast cancer diagnosis status are presented in Table 1. Participants who developed breast cancer were younger at menarche and older at their first livebirth than participants who did not develop breast cancer. Cases were more likely to have a history of breast fibroadenoma or a family history of breast cancer and less likely to have given birth. They were also more likely to be taller, to have lower educational attainment, and to have used a B-vitamin-containing supplement.

Intake levels reported in the present study and in other international study populations, and the US recommended dietary allowance, are shown in Table 2. Most women in this study consumed B vitamins in quantities below the US recommended dietary allowance. In comparison to other study populations for whom data were reported, the Shanghai Women's Health Study population had a much lower alcohol intake; very few women had ever consumed alcohol regularly. This study population was not as likely as most other populations to have ever used a B-vitamin-containing supplement. Intake of folate in the Shanghai Women's Health Study was lower than in the North American populations and was comparable to that in some European populations. However, intakes of vitamin B_6, niacin, and, particularly, vitamin B_{12} were much lower in the present study population than in most other populations.

The associations of breast cancer risk with dietary intake quintiles for all women in the cohort and by menopausal status are shown in Table 3. Rice and vegetables were the main sources of folate, vitamin B_6, and riboflavin; rice and meat for niacin and methionine; and fish and meat for vitamin B_{12} (data not shown in table). Overall and among postmenopausal women, dietary intakes were not associated with risk of breast cancer. However, for premenopausal women, higher folate intake was associated with a 40% decreased breast cancer risk (hazard ratio = 0.58, 95% confidence interval: 0.34, 0.99 for high vs. low intake). Although there was no statistically significant linear trend, the relation between premenopausal risk and folate intake appeared to be a threshold effect because all other quintiles were associated with reduced risk in comparison to the lowest quintile. A sensitivity analysis was conducted by limiting the participants included in analysis to women who did not drink alcohol, women who did not smoke, women diagnosed with breast cancer more than 2 years after recruitment, or women who did not use B vitamin supplements. Results were similar to those that included the entire cohort (data not shown in table). Body mass index was evaluated as an effect modifier, and, as in the entire cohort, similar null results were observed (data not shown in table).

The joint associations between premenopausal breast cancer risk and folate intake with intakes of methionine

Table 3. Associations Between Dietary Intake and Breast Cancer Risk, Shanghai Women's Health Study, 1997–2008

Nutrient	Quintile of Dietary Intake					*P* for Trend
	1 (Low)	2	3	4	5	
			All Women[a,b]			
Folate						
Mean intake, μg/day	194	243	282	328	404	
Cases, *n*	146	142	143	160	127	
HR	1.0	0.90	0.89	0.99	0.79	0.32
95% CI	Ref	0.71, 1.15	0.69, 1.15	0.76, 1.28	0.59, 1.06	
Methionine						
Mean intake, g/day	1.13	1.34	1.48	1.65	1.97	
Cases, *n*	140	146	141	150	141	
HR	1.0	0.98	0.94	0.98	0.92	0.68
95% CI	Ref	0.76, 1.26	0.71, 1.23	0.73, 1.32	0.66, 1.28	
Vitamin B_{12}						
Mean intake, μg/day	1.00	1.83	2.44	3.11	4.60	
Cases, *n*	143	139	145	152	139	
HR	1.0	0.89	0.88	0.91	0.83	0.35
95% CI	Ref	0.69, 1.14	0.68, 1.15	0.69, 1.19	0.61, 1.12	
Vitamin B_6						
Mean intake, mg/day	1.23	1.50	1.67	1.88	2.23	
Cases, *n*	126	167	138	140	147	
HR	1.0	1.26	1.01	1.02	1.05	0.57
95% CI	Ref	0.98, 1.61	0.77, 1.33	0.76, 1.36	0.76, 1.46	
Niacin (vitamin B_3)						
Mean intake, mg/day	11.7	12.9	13.9	15.1	17.6	
Cases, *n*	132	146	148	147	145	
HR	1.0	1.06	1.06	1.05	1.05	0.81
95% CI	Ref	0.83, 1.35	0.82, 1.37	0.80, 1.39	0.78, 1.42	
Riboflavin (vitamin B_2)						
Mean intake, mg/day	0.55	0.72	0.84	0.98	1.16	
Cases, *n*	131	138	159	145	145	
HR	1.0	0.98	1.08	0.94	0.93	0.55
95% CI	Ref	0.76, 1.27	0.82, 1.42	0.70, 1.27	0.66, 1.30	
			Premenopausal Women[a]			
Folate						
Cases, *n*	55	31	45	42	40	
HR	1.00	0.45	0.63	0.59	0.58	0.22
95% CI	Ref	0.29, 0.72	0.40, 0.98	0.37, 0.95	0.34, 0.99	
Methionine						
Cases, *n*	41	38	41	45	48	
HR	1.00	0.72	0.73	0.80	0.81	0.82
95% CI	Ref	0.45, 1.16	0.44, 1.20	0.47, 1.35	0.45, 1.45	
Vitamin B_{12}						
Cases, *n*	30	38	42	49	54	
HR	1.00	0.93	0.93	1.03	1.02	0.73
95% CI	Ref	0.56, 1.52	0.56, 1.55	0.61, 1.73	0.59, 1.78	

Table continues

and other vitamins are presented in Table 4. Overall, there were no statistically significant interactions. For women with low folate intake, high methionine intake (hazard ratio = 1.47, 95% confidence interval: 0.88, 2.45) and high

Table 3. Continued

Nutrient	Quintile of Dietary Intake					*P* for Trend
	1 (Low)	2	3	4	5	
Vitamin B_6						
Cases, *n*	32	48	41	37	55	
HR	1.00	1.25	1.02	0.90	1.34	0.75
95% CI	Ref	0.78, 2.00	0.61, 1.71	0.52, 1.57	0.74, 2.42	
Niacin (vitamin B_3)						
Cases, *n*	36	32	47	38	60	
HR	1.00	0.73	1.01	0.80	1.28	0.24
95% CI	Ref	0.45, 1.20	0.63, 1.62	0.48, 1.33	0.76, 2.18	
Riboflavin (vitamin B_2)						
Cases, *n*	32	44	47	43	47	
HR	1.00	1.06	1.05	0.95	1.00	0.81
95% CI	Ref	0.65, 1.72	0.63, 1.76	0.54, 1.65	0.54, 1.85	
	Postmenopausal Women[a]					
Folate						
Cases, *n*	66	72	67	80	61	
HR	1.00	1.20	1.12	1.29	0.97	0.98
95% CI	Ref	0.84, 1.71	0.77, 1.63	0.89, 1.89	0.63, 1.49	
Methionine						
Cases, *n*	81	72	69	71	53	
HR	1.00	1.01	1.04	1.08	0.87	0.72
95% CI	Ref	0.71, 1.44	0.70, 1.54	0.71, 1.65	0.53, 1.42	
Vitamin B_{12}						
Cases, *n*	93	75	70	59	49	
HR	1.00	0.86	0.84	0.74	0.70	0.10
95% CI	Ref	0.62, 1.19	0.59, 1.20	0.50, 1.10	0.45, 1.09	
Vitamin B_6						
Cases, *n*	71	89	63	65	58	
HR	1.00	1.39	1.03	1.07	0.96	0.38
95% CI	Ref	0.99, 1.95	0.70, 1.52	0.70, 1.61	0.60, 1.54	
Niacin (vitamin B_3)						
Cases, *n*	78	77	69	68	54	
HR	1.00	1.09	1.04	1.11	0.93	0.82
95% CI	Ref	0.78, 1.52	0.72, 1.49	0.75, 1.64	0.60, 1.44	
Riboflavin (vitamin B_2)						
Cases, *n*	82	70	76	59	59	
HR	1.00	0.90	0.98	0.71	0.72	0.11
95% CI	Ref	0.64, 1.29	0.68, 1.43	0.47, 1.09	0.44, 1.17	

Abbreviations: CI, confidence interval; HR, hazard ratio; Ref, reference.

[a] Adjusted for age at baseline (years), age at menarche (years), parity (ever, never), age at first livebirth (years), educational attainment (college or above, high school, middle school, less than middle school), physical activity (metabolic equivalent-hours/week per year), use of a B vitamin supplement (never, ever), height (m), and total daily intakes of energy (kcal), vegetables (g), and fat (g).

[b] Additionally adjusted for menopausal status (no, yes).

niacin intake (hazard ratio = 1.57, 95% confidence interval: 0.96, 2.57) were associated with increased risks that were not statistically significant.

The associations between intake and breast cancer risk according to ER and PR status are presented in Table 5. Women with double-negative breast cancer had no statistically significant risk associated with any of the dietary factors, although risk appeared somewhat reduced for folate intake, particularly within the third quartile. However, for women with double-hormone-positive breast cancer,

Table 4. Joint Association of Folate and Dietary Factors With Risk of Premenopausal Breast Cancer for 32,377 Women Who Do Not Use B Vitamin Supplements, Shanghai Women's Health Study, 1997–2008

	Daily Mean Intake	Tertile of Daily Folate Intake[a]						*P* for Trend
		1 (Low)		2		3		
		HR	95% CI	HR	95% CI	HR	95% CI	
Mean intake, μg/day		211		283		377		
Methionine, g								
Low	1.28	1.0	Ref	0.74	0.44, 1.26	1.09	0.61, 1.96	0.88
High	1.75	1.47	0.88, 2.45	1.08	0.65, 1.81	1.10	0.64, 1.89	0.37
P for interaction		0.11						
Vitamin B_{12}, μg								
Low	1.59	1.0	Ref	0.81	0.47, 1.39	0.87	0.47, 1.59	0.64
High	3.60	1.19	0.72, 1.95	0.86	0.52, 1.42	1.05	0.62, 1.79	0.63
P for interaction		0.30						
Vitamin B_6, mg								
Low	1.42	1.0	Ref	0.72	0.44, 1.16	1.03	0.56, 1.92	0.63
High	1.99	0.85	0.47, 1.56	0.74	0.46, 1.21	0.80	0.48, 1.34	0.97
P for interaction		0.58						
Niacin (vitamin B_3), mg								
Low	12.6	1.0	Ref	1.10	0.67, 1.81	1.02	0.55, 1.89	0.77
High	15.9	1.57	0.96, 2.57	0.85	0.49, 1.45	1.18	0.67, 2.06	0.30
P for interaction		0.31						
Riboflavin (vitamin B_2), mg								
Low	0.67	1.0	Ref	0.64	0.39, 1.05	0.88	0.48, 1.61	0.36
High	1.03	0.74	0.41, 1.35	0.78	0.48, 1.26	0.82	0.49, 1.36	0.95
P for interaction		0.99						

Abbreviations: CI, confidence interval; HR, hazard ratio; Ref, reference.

[a] Adjusted for age at baseline (years), age at menarche (years), parity (ever, never), age at first livebirth (years), educational attainment (college or above, high school, middle school, less than middle school), physical activity (metabolic equivalent-hours/week per year), use of a B vitamin supplement (never, ever), height (m), and total daily intakes of energy (kcal), vegetables (g), and fat (g).

niacin intake was associated with a statistically significantly increased breast cancer risk (hazard ratio = 1.62, 95% confidence interval: 1.07, 2.46; *P* for trend = 0.04). The highest folate intake level was not associated with breast cancer risk, although intake in the third quartile was associated with increased risk (hazard ratio = 1.54, 95% confidence interval: 1.06, 2.23). Intake of other factors was not associated with breast cancer risk for women with ER+/PR+ breast cancers.

DISCUSSION

In this study, folate was associated with a reduced risk of premenopausal breast cancer but not overall or for postmenopausal women. Most previous cohort studies also have not observed an overall association with folate intake (7–10, 15, 23–30), although many case-control studies have found a modest inverse association (1). A few previous studies have evaluated folate and premenopausal breast cancer risk, and the cohort study results were null (8, 9, 23, 25), whereas the case-control studies generally observed a reduced risk (6, 31, 32) or an increased risk (5).

One possible contributing factor to the difference in the results between the present study and the other cohort studies is the intake level of folate within the study's unfortified population. Only 13% of the women in this population are at or exceed the US recommended dietary allowance for folate. In comparison to the previous null cohort studies of premenopausal women, this intake level is much lower than that of all of the other study populations (8, 9, 23, 25), and the food supply has not been fortified over the course of the study period. However, intake level in the present study is comparable to that in some of the case-control studies reporting an inverse association with premenopausal breast cancer risk (6, 32) and nearly half of the level in the case-control study reporting an increased risk (5).

Thus, it is possible that the observed relation with folate intake among premenopausal women may be due to a difference in folate insufficiency versus sufficiency. In support of this possibility, the present study appeared to have a threshold effect for folate intake that was achieved between the first and second quintiles of intake, with no added benefit beyond that level. Other studies have also observed effects within subgroups that may have higher folate needs,

Table 5. Association Between Dietary Intake and Risk of ER−/PR− and ER+/PR+ Breast Cancer, Shanghai Women's Health Study, 1997–2008

	Quartile of Daily Dietary Intake[a]				*P* for Trend
	1 (Low)	2	3	4	
ER−/PR− Breast Cancer					
Folate					
Mean intake, μg/day	200	258	310	393	
Events, *n*	43	53	30	44	
HR	1.0	1.09	0.60	0.84	0.19
95% CI	Ref	0.71, 1.68	0.36, 1.01	0.50, 1.43	
Methionine					
Mean intake, g/day	1.16	1.39	1.59	1.92	
Events, *n*	35	45	50	40	
HR	1.0	1.21	1.33	1.05	0.92
95% CI	Ref	0.74, 1.98	0.78, 2.27	0.56, 1.97	
Vitamin B_{12}					
Mean intake, μg/day	1.21	2.06	2.84	4.36	
Events, *n*	44	32	47	47	
HR	1.0	0.65	0.90	0.85	0.98
95% CI	Ref	0.40, 1.05	0.55, 1.45	0.50, 1.45	
Vitamin B_6					
Mean intake, mg/day	1.27	1.57	1.80	2.18	
Events, *n*	39	51	39	41	
HR	1.0	1.12	0.79	0.76	0.18
95% CI	Ref	0.71, 1.76	0.47, 1.34	0.42, 1.38	
Niacin (vitamin B_3)					
Mean intake, mg/day	11.9	13.3	14.6	17.2	
Events, *n*	41	46	43	40	
HR	1.0	0.98	0.86	0.76	0.27
95% CI	Ref	0.63, 1.52	0.53, 1.40	0.44, 1.31	
Riboflavin (vitamin B_2)					
Mean intake, mg/day	0.58	0.76	0.93	1.13	
Events, *n*	37	47	37	49	
HR	1.0	1.17	0.88	1.11	0.95
95% CI	Ref	0.73, 1.88	0.51, 1.51	0.61, 2.02	

Table continues

such as alcohol users (8, 10, 25, 26). It is further possible that premenopausal women, because of their child-bearing potential, may have higher folate needs than do postmenopausal women.

In the only known randomized trial of the effect of folic acid, vitamin B_6, and vitamin B_{12} on breast cancer risk, the study population's folic acid intake was substantially higher than in this present study, folate status was affected by fortification of the food supply, the participants were at high risk of cardiovascular disease, and over 91% of the participants were postmenopausal (33). Thus, it is difficult to compare the null findings in the trial study with the results in the present study. Two additional trials of B vitamins and cancer prevention also found no associations with total cancer incidence (34, 35), except for a possible increased risk of total cancer with folic acid supplementation, although this finding was not likely related to breast cancer because more than 75% of the participants were male (35). Other studies have used physiologic markers of folate status to evaluate the relation of folate levels to breast cancer risk. Similar to studies evaluating intake, there are reports of null (36), protective (37, 38), and adverse (39) associations in these populations with nondeficient levels.

Few studies have evaluated the relation among ER/PR status, folate, and breast cancer risk (9, 10, 13–15), and most of these studies have evaluated ER status only (9, 10) or have separately evaluated ER and PR status (13, 14). Findings from these cohort studies have been largely null, with no observed associations for ER+ (10, 13, 14), ER− (9, 13), and PR+ or PR− (13, 14). However, higher folate intake was associated with a lower risk of ER− breast cancer in the Nurses' Health Study (14), and the VITamins And Lifestyle (VITAL)

Table 5. Continued

	Quartile of Daily Dietary Intake[a]				*P* for Trend
	1 (Low)	2	3	4	
ER+/PR+ Breast Cancer					
Folate					
Events, *n*	60	76	93	62	
HR	1.0	1.26	1.54	1.09	0.55
95% CI	Ref	0.88, 1.80	1.06, 2.23	0.71, 1.67	
Methionine					
Events, *n*	79	76	93	62	
HR	1.0	0.81	0.80	0.87	0.66
95% CI	Ref	0.57, 1.16	0.54, 1.20	0.56, 1.37	
Vitamin B_{12}					
Events, *n*	81	66	68	76	
HR	1.0	0.87	0.88	0.93	0.81
95% CI	Ref	0.61, 1.24	0.60, 1.29	0.61, 1.40	
Vitamin B_6					
Events, *n*	74	82	56	79	
HR	1.0	1.02	0.68	0.93	0.40
95% CI	Ref	0.72, 1.44	0.45, 1.02	0.60, 1.45	
Niacin (vitamin B_3)					
Events, *n*	65	74	67	85	
HR	1.0	1.20	1.04	1.62	0.04
95% CI	Ref	0.84, 1.71	0.70, 1.55	1.07, 2.46	
Riboflavin (vitamin B_2)					
Events, *n*	68	74	82	67	
HR	1.0	1.01	1.04	0.83	0.46
95% CI	Ref	0.70, 1.46	0.70, 1.54	0.52, 1.33	

Abbreviations: CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; PR, progesterone receptor; Ref, reference.

[a] Adjusted for age at baseline (years), age at menarche (years), parity (ever, never), age at first livebirth (years), educational attainment (college or above, high school, middle school, less than middle school), physical activity (metabolic equivalent-hours/week per year), use of a B vitamin supplement (never, ever), height (m), and total daily intakes of energy (kcal), vegetables (g), and fat (g).

cohort (10). Although folate was not related to postmenopausal breast cancer risk regardless of ER or PR status in the Iowa Women's Health Study, low folate intake appeared to be related to an increased risk of ER− breast cancer when alcohol intake was also high (13). Thus, there is now evidence from 4 of 6 cohort studies, including this current one, that folate may be associated with ER− breast cancers. To our knowledge, only one previous study has evaluated ER and PR jointly (15). In the Swedish Mammography Cohort, folate intake was not related to risk of ER−/PR− or ER+/PR+ breast cancers. However, the authors did observe a statistically significant decreased risk of ER+/PR− breast cancer. To date, the Swedish study is the only one to report an association with ER+ breast cancer. The sample size for ER+/PR− in our study was not sufficient to evaluate this subtype.

Methionine, vitamin B_{12}, vitamin B_6, and riboflavin are involved in one-carbon metabolism, although they may also function as cofactors in other physiologic processes. In general, dietary intake of these factors was lower in the present study, particularly vitamin B_{12}, compared with levels reported in previous studies. Most women in the study population did not consume B vitamins at or exceeding the US recommended dietary allowance. However, the findings of no associations with intakes of these factors are consistent with most of those from previous studies of dietary or blood levels of methionine (3, 7, 9, 10), vitamin B_6 (9–12, 36), vitamin B_{12} (3, 5, 9, 10), and riboflavin (6, 7, 10), with few exceptions (3, 6, 11, 12, 36), despite variations in intake levels. Thus, the present study supports the hypothesis that these factors do not have strong, independent roles in breast carcinogenesis.

Physiologically, niacin is a cofactor in redox reactions and is a substrate for several adenosine 5'-diphosphate–ribosyl transferases involved in DNA repair, cell death, transcription, and other cellular processes (40, 41). Niacin is involved in steroid hormone synthesis and is a common pharmacotherapy for dyslipidemia. Niacin deficiency causes pellagra and toxicity, including flushing and hepatotoxicity. Three previous studies have evaluated the association of niacin with breast cancer risk, and all observed no association (6, 7, 26). The present study found no overall association, although high niacin intake was associated with

an increased risk of ER+/PR+ breast cancer. The increased risk we observed was statistically significant for only those in the upper quartile of intake. In our study, the median niacin intake was nearly equal to the US recommended dietary allowance. In the previous studies that evaluated niacin and breast cancer, median population intakes were larger than the US recommended dietary allowance. It is thus possible that the positive relation between niacin intake and ER+/PR+ breast cancer may be evident only in a population whose intake is relatively insufficient. Because of niacin's involvement in steroid hormone synthesis, and ER+/PR+ breast cancer is hormone dependent, it is possible that niacin could accentuate estrogen synthesis and then, in turn, cancer progression. However, to our knowledge, no study has evaluated niacin intake and breast cancer according to ER/PR status; therefore, further studies are needed to understand a possible mechanism.

In the present study, several factors related to one-carbon metabolism were evaluated in a population with no vitamin fortification of the food supply and a low prevalence of alcohol and vitamin supplement use. The study population had a wide range of dietary intakes, although the high intake levels achieved only through supplementation could not be evaluated. The prospective design, high participation rate, and high follow-up rates minimized the possibility of recall and selection biases. Foods rich in folate, B vitamins, and methionine also contain other nutrients and dietary factors. Evaluation and adjustment were conducted for several of these factors, including vegetable and fat intakes, but it is possible that the observed associations or lack of associations in this study could be due to the relation of other nutrients with breast cancer. Residual confounding is also a possibility. For example, it is possible that our measure of physical activity did not sufficiently account for potential confounding by physical activity even though it included occupational, daily living, and leisure-time activities.

As with nearly all studies, it is possible that the timing of the diet assessment did not capture the appropriate exposure window. To minimize the probability of measurement error regarding self-reported dietary intake, a validated food frequency questionnaire specifically designed for this population was used (18). However, it is possible that some nondifferential misclassification occurred, which usually leads to attenuation of associations. Although data in the Chinese Food Composition Tables were not available for some nutrients, the correlations between the US Department of Agriculture database and the Chinese database for multiple nutrients were very high, minimizing the probability of error due to a non-Chinese source of dietary data.

The relatively short follow-up time for the cohort could have also affected the findings. A sensitivity analysis was conducted by excluding cases diagnosed within 2 years of baseline, and results remained consistent with those found in the entire cohort. Finally, the case sample size for some of the subanalyses was small.

In summary, this study evaluated B vitamins and methionine intakes in relation to breast cancer risk in a population whose food supply is not fortified. In the study, high folate intake was related to a lower risk of premenopausal and possibly ER−/PR− breast cancer. To our knowledge, the finding that niacin intake is related to an increased risk of ER+/PR+ breast cancer is new and should be evaluated in other studies. These findings support the hypothesis that high dietary folate intake may reduce breast cancer risk.

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