Null Results in Brief

Nutrients Involved in One-Carbon Metabolism and Risk of Breast Cancer among Premenopausal Women

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Abstract

Folate, vitamin B6, vitamin B12, methionine, choline, and betaine are nutrients involved in one-carbon metabolism and have been hypothesized to reduce the risk of breast cancer. However, previous epidemiologic studies on most of these nutrients and breast cancer risk have been inconclusive and have included primarily postmenopausal women. No study has examined choline and betaine in relation to breast cancer risk. Therefore, we examined the intake of these nutrients in relation to breast cancer risk among 90,663 premenopausal women ages 26 to 46 years in 1991 in the Nurses' Health Study II. Nutrient intake was assessed with a validated food frequency questionnaire in 1991, 1995, and 1999. During 12 years of follow-up from 1991 to 2003, we documented 1,032 incident cases of invasive breast cancer. Overall, none of the nutrients was associated with risk of breast cancer. The results were similar by levels of alcohol intake and folate intake and for estrogen receptor–negative breast cancer. In conclusion, we found no evidence that higher intakes of nutrients involved in one-carbon metabolism reduce risk of breast cancer among premenopausal women. (Cancer Epidemiol Biomarkers Prev 2007;16(12):2787–90)

Introduction

One-carbon metabolism is a network of biochemical reactions that transfer methyl groups from one compound to another (1). Folate and betaine donate methyl groups to homocysteine to produce methionine, which in turn donates a methyl group to generate *S*-adenosylmethionine, a compound involved in methylation of DNA and RNA and influencing gene stability and expression. One-carbon metabolism also mediates nucleotide synthesis, and perturbation of the metabolism may lead to chromosome breaks and disruption of DNA repair. Besides folate, betaine, and methionine, other nutrients influence one-carbon metabolism, including vitamin B6, vitamin B12, and choline. Previous epidemiologic studies of intakes of these nutrients and breast cancer risk have been limited for most of the nutrients except folate; have included primarily postmenopausal women; and, to our knowledge, have not examined choline and betaine (2-8). We therefore examined intakes of these nutrients in relation to breast cancer risk among premenopausal women.

Materials and Methods

The Nurses' Health Study II is a prospective cohort study of 116,671 female registered nurses who were 25 to 42 years of age in 1989. For the current analysis, we started follow-up from 1991 when diet was first measured. We excluded women who had an implausible dietary intake, who had reported a diagnosis of cancer, or who were postmenopausal at baseline. We censored women after they reached either natural or surgical menopause (including hysterectomy without bilateral oophorectomy) during follow-up (9).

A semiquantitative food frequency questionnaire with ~130 foods was sent to women in 1991, 1995, and 1999 to assess usual dietary intake in the past year(10). Participants also reported their current use and dose of multivitamins and vitamin supplements biennially. Nutrient intake per individual was calculated based on data from the U.S. Department of Agriculture (11, 12) or other sources (13, 14). We used the regression-residual method to adjust nutrient intakes for total energy intake (15) and calculated cumulative averaged intake of nutrients using the three dietary assessments (16).

Biennial follow-up questionnaires were used to identify newly diagnosed cases of breast cancer. Deaths were documented by responses to follow-up questionnaires by family members or the postal service and by a search of the National Death Index. Participants contributed person-time from the date of return of the 1991 questionnaire until the date of breast cancer diagnosis, death, or June 2003, whichever came first. Participants were divided into quintiles according to their nutrient intakes. Relative risks of breast cancer (and 95% confidence intervals) for quintiles of dietary variables were calculated. We used Cox proportional hazards regression (17) stratified jointly by age and calendar year. Multivariate models were adjusted simultaneously for potential confounding variables listed in the footnotes to Table 1.

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All covariates except height, age at menarche, and family history of breast cancer were updated in each questionnaire cycle. SAS PROC PHREG (18) and the Anderson-Gill data structure (17) were used to handle time-varying covariates efficiently. Tests for trend were conducted using the median value for each category of nutrient as a continuous variable. We also conducted analyses stratified by alcohol and folate intake and restricted analyses to estrogen receptor−negative tumors in secondary analyses. All P values were two sided. We had a power of 98% to detect a significant linear trend for the relative risk of 0.8 for the highest versus the lowest quintile of nutrient intake ($\alpha = 0.05$; ref. 19).

Results

During 12 years (862,509 person-years) of follow-up, we documented 1,032 cases of invasive breast carcinoma among 90,663 premenopausal women (mean age, 36 years at baseline). Intakes of folate, vitamin B6, vitamin B12, methionine, choline, and betaine were not associated with breast cancer risk (Table 1). The results were similar for estrogen receptor−negative breast cancer cases ($n = 221$; Table 2). There were no inverse associations when the intakes were examined by levels of alcohol consumption [nondrinker (case $n = 319$), <5 ($n = 462$), and ≥5 g/d ($n = 251$)]. There were no inverse associations when intakes of vitamin B6, vitamin B12, methionine, choline, and betaine were examined by total folate intake [<250 ($n = 114$), 250-<400 ($n = 364$), and ≥400 μg/d ($n = 554$)].

Discussion

In this prospective study of premenopausal women, intakes of folate, vitamin B6, vitamin B12, methionine, choline, and betaine were not inversely related to breast cancer risk.

Table 1. Relative risk and 95% confidence interval of breast cancer ($n = 1,032$) according to quintile of cumulative averaged energy-adjusted nutrients related to one-carbon metabolism in women 26 to 46 y of age at baseline

Nutrient	Quintile of intake					P_{trend}
	1	2	3	4	5	
Total folate						
Median intake (μg/d)	237	317	413	568	822	
Age-adjusted RR (95% CI)	1.00	1.07 (0.88-1.30)	0.85 (0.69-1.04)	1.03 (0.85-1.26)	1.10 (0.90-1.35)	0.23
Multivariate* RR (95% CI)	1.00	1.06 (0.87-1.30)	0.83 (0.67-1.02)	1.00 (0.82-1.23)	1.09 (0.88-1.34)	0.31
Folate from food only						
Median intake (μg/d)	217	268	309	354	436	
Age-adjusted RR (95% CI)	1.00	1.17 (0.96-1.44)	1.14 (0.93-1.40)	1.15 (0.94-1.40)	1.05 (0.85-1.29)	>0.99
Multivariate* RR (95% CI)	1.00	1.18 (0.96-1.44)	1.15 (0.93-1.41)	1.16 (0.94-1.43)	1.08 (0.86-1.35)	0.77
Total vitamin B6						
Median intake (mg/d)	1.7	2.2	2.8	4.1	13.3	
Age-adjusted RR (95% CI)	1.00	1.11 (0.91-1.35)	1.09 (0.90-1.33)	1.08 (0.88-1.32)	1.15 (0.95-1.40)	0.29
Multivariate* RR (95% CI)	1.00	1.10 (0.90-1.34)	1.07 (0.88-1.31)	1.06 (0.86-1.30)	1.11 (0.91-1.35)	0.53
Vitamin B6 from food only						
Median intake (mg/d)	1.6	1.9	2.1	2.3	2.7	
Age-adjusted RR (95% CI)	1.00	1.18 (0.97-1.43)	1.10 (0.90-1.34)	0.96 (0.78-1.17)	1.16 (0.95-1.41)	0.50
Multivariate* RR (95% CI)	1.00	1.16 (0.95-1.41)	1.09 (0.89-1.33)	0.96 (0.78-1.18)	1.18 (0.96-1.44)	0.38
Total vitamin B12						
Median intake (μg/d)	4	6	8	10	18	
Age-adjusted RR (95% CI)	1.00	1.01 (0.83-1.23)	0.98 (0.81-1.19)	0.96 (0.80-1.16)	0.95 (0.75-1.15)	0.53
Multivariate* RR (95% CI)	1.00	0.99 (0.81-1.20)	0.96 (0.79-1.17)	0.94 (0.77-1.13)	0.92 (0.76-1.12)	0.37
Vitamin B12 from food only						
Median intake (μg/d)	4	5	6	7	9	
Age-adjusted RR (95% CI)	1.00	1.10 (0.91-1.34)	1.09 (0.90-1.32)	1.14 (0.94-1.38)	0.96 (0.79-1.18)	0.99
Multivariate* RR (95% CI)	1.00	1.09 (0.90-1.34)	1.08 (0.88-1.32)	1.13 (0.93-1.39)	0.96 (0.78-1.19)	0.61
Total methionine						
Median intake (g/d)	1.6	1.8	2.0	2.2	2.5	
Age-adjusted RR (95% CI)	1.00	0.93 (0.76-1.13)	1.04 (0.86-1.25)	0.87 (0.71-1.05)	1.10 (0.91-1.34)	0.47
Multivariate* RR (95% CI)	1.00	0.92 (0.76-1.13)	1.03 (0.85-1.25)	0.86 (0.70-1.06)	1.10 (0.89-1.36)	0.47
Total choline						
Median intake (mg/d)	263	301	327	354	397	
Age-adjusted RR (95% CI)	1.00	0.83 (0.68-1.01)	0.95 (0.78-1.14)	0.82 (0.68-1.00)	0.93 (0.77-1.13)	0.49
Multivariate* RR (95% CI)	1.00	0.80 (0.66-0.97)	0.91 (0.75-1.10)	0.79 (0.64-0.96)	0.88 (0.72-1.07)	0.26
Total betaine						
Median intake (mg/d)	114	155	190	230	305	
Age-adjusted RR (95% CI)	1.00	1.09 (0.89-1.34)	1.05 (0.85-1.29)	1.23 (1.01-1.50)	1.01 (0.82-1.24)	0.93
Multivariate* RR (95% CI)	1.00	1.07 (0.87-1.32)	1.01 (0.82-1.25)	1.19 (0.97-1.46)	0.99 (0.79-1.22)	0.88

Abbreviations: RR, relative risk; 95% CI, 95% confidence interval.

*Multivariate model was stratified by age in months at start of follow-up and calendar year of the current questionnaire cycle and was simultaneously adjusted for smoking (never, past <25, past 25+, current <25, and current 25+ cigarettes/d); height (<62, 62-<65, 65-<68, 68+ inches); parity and age at first birth (nulliparous, parity ≤2 and age at first birth <25 y, parity ≤2 and age at first birth 25-<30 y, parity ≤2 and age at first birth 30+ y, parity 3+ and age at first birth <25 y, parity 3+ and age at first birth 25+ y); body mass index (<18.5, 18.5-19.9, 20.0-22.4, 22.5-24.9, 25.0-29.9, 30.0+ kg/m^2); age at menarche (<12, 12, 13, ≥14 y); family history of breast cancer (yes, no); history of benign breast disease (yes, no); oral contraceptive use (never, past <4 y, past 4+ y, current <8 y, current 8+ y); and intakes of alcohol (continuous), energy (continuous), and animal fat (continuous).

Table 2. Multivariate relative risk and 95% confidence interval of estrogen receptor negative breast cancer (*n* = 221) according to quintile of cumulative averaged energy-adjusted nutrients related to one-carbon metabolism in women 26 to 46 y of age at baseline

Nutrient	Quintile of intake					P_{trend}
	1	2	3	4	5	
Total folate	1.00	1.09 (0.72-1.64)	0.77 (0.49-1.21)	0.86 (0.55-1.33)	1.08 (0.70-1.66)	0.85
Folate from food only	1.00	1.10 (0.72-1.70)	1.09 (0.70-1.69)	1.17 (0.75-1.82)	1.16 (0.73-1.85)	0.53
Total vitamin B6	1.00	1.15 (0.76-1.74)	0.81 (0.52-1.26)	0.86 (0.55-1.35)	1.18 (0.78-1.78)	0.26
Vitamin B6 from food only	1.00	1.03 (0.67-1.57)	1.26 (0.84-1.89)	0.88 (0.57-1.37)	0.93 (0.60-1.44)	0.56
Total vitamin B12	1.00	0.98 (0.63-1.51)	0.93 (0.61-1.44)	1.08 (0.72-1.60)	1.05 (0.70-1.58)	0.71
Vitamin B12 from food only	1.00	1.29 (0.82-2.02)	1.23 (0.78-1.93)	1.51 (0.97-2.35)	1.24 (0.78-1.98)	0.39
Total methionine	1.00	0.64 (0.40-1.02)	1.11 (0.74-1.68)	1.02 (0.67-1.56)	0.86 (0.54-1.36)	0.89
Total choline	1.00	0.65 (0.42-1.00)	0.84 (0.56-1.25)	0.74 (0.49-1.13)	0.86 (0.57-1.30)	0.67
Total betaine	1.00	0.93 (0.61-1.43)	0.90 (0.59-1.38)	0.94 (0.61-1.45)	0.85 (0.54-1.33)	0.55

NOTE: The models were adjusted for the same covariates as the multivariate model in Table 1.

Our findings on folate are in accordance with recent meta-analyses of prospective studies, which found no overall association with breast cancer risk (2, 3). We previously did not find any association in this population of premenopausal women (20) and, with an additional 300 breast cancer cases, we still found no association between folate intake and breast cancer. There are several possible explanations. Some of the previous prospective studies reported inverse associations specifically among moderate to high alcohol consumers (21, 22) or with estrogen receptor–negative breast cancer cases (23, 24). Because alcohol intake was relatively low in our population (our highest category of alcohol was ≥5 g/d), we were not able to examine the association among higher alcohol drinkers. In addition, we had relatively few estrogen receptor–negative breast cancer cases. Finally, our population was relatively well nourished with folate, partly due to folic acid fortification of grain products that largely started in 1996. For example, median intakes of folate (both from food only and total) for the 1st quintiles in our population were within the ranges of the 2nd quintiles in the Nurses' Health Study where reverse association between folate intake and estrogen receptor–negative breast cancer risk was found (23).

Besides folate, other nutrients involved in one-carbon metabolism have not been evaluated extensively. Most studies of dietary or plasma levels of vitamins B6 (4-7) and methionine (8) have not supported inverse associations with breast cancer risk. Dietary (7, 25) or plasma (5, 6) levels of vitamin B12 were inversely association with breast cancer, except in one study (4). We are not aware of any studies examining choline and betaine intakes in relation to breast cancer.

This study had several strengths. The prospective nature of our study avoided recall and selection biases and provided a unique opportunity to evaluate nutrient intakes and breast cancer in younger women.

In conclusion, we found no evidence that higher intakes of nutrients involved in one-carbon metabolism reduce breast cancer risk in premenopausal women.

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