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Folate, vitamin B12 and postmenopausal breast cancer in a prospective study of French women.

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Abstract

Objective: Adequate folate intake may be important for breast cancer prevention. Its protective effect may be influenced by factors associated with folate metabolism. We sought to evaluate folate intake in relation to breast cancer risk and examine whether the relation is affected by alcohol and intake of vitamin B_2 and B_{12}.

Methods: A prospective cohort analysis of folate intake was conducted among 62,739 postmenopausal women in the French E3N cohort who had completed a validated food frequency questionnaire in 1993. During nine years' follow-up, 1,812 cases of pathology-confirmed breast cancer were documented through follow-up questionnaires. Nutrients were categorized in quintiles and energy-adjusted using the regression-residual method. Cox model-derived relative risks (RRs) were adjusted for known breast cancer determinants.

Results: The multivariate RR for extreme quintiles of folate intake was was 0.78 (95% CI: 0.67–0.90; *p*-trend = 0.001) [Median intake for Q_1 = 296 µg/day and Q_5 = 522 µg/day]. There was no evidence to support effect modification by alcohol or B_2 intake. The decreasing trend was most marked in women with higher folate and vitamin B_{12} intake. However, test for interaction was not statistically significant (p = 0.29)

Conclusions: High folate intake was associated with decreased breast cancer risk. Vitamin B_{12} intake may modify this association.

Keywords: Breast cancer, Women, Nutrition, Folate, Vitamin B_{12}, Vitamin B_2 Alcohol, Diet.

Introduction

Substantial experimental and epidemiologic research has shown that low folate intake can increase cancer occurrence [1]. Diminished folate status may disrupt DNA synthesis and repair mechanisms and may influence gene expression through abnormal DNA and RNA methylation [1–3]. Furthermore, the metabolic pathway involved in DNA methylation requires the presence of other micronutrients like vitamins B_2 and B_{12} as cofactors and may be inhibited by ethanol [4]. With the exception of one study [5], several large prospective epidemiologic studies suggest that the risk of breast cancer in women who consume alcohol can be reduced by adequate folate intake [6–9]. In addition, two population-based case–control studies suggest that if folate intake is to have a protective effect against breast cancer, an adequate intake of vitamin B_{12} may also be necessary [10, 11]. We therefore conducted a prospective analysis of a large sample of French women to evaluate folate intake in relation to breast cancer risk and examined whether the relation was affected by alcohol and vitamin B_2 and B_{12} intake.

Materials and methods

The methodology of the E3N (Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale) study has been described elsewhere [12]. Briefly, in 1990–1991, 98,995 women born between 1925 and 1950 and insured with the Mutuelle Générale de l'Education Nationale, a French health insurance scheme primarily covering teachers, completed a mailed questionnaire on their lifestyle and medical

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history. Regular follow-up questionnaires were sent out to update information. A dietary questionnaire was included in 1993 and was completed by 77,613 participants (81.1%). Women who did not consent to external health follow-up by the insurer in case of dropout, questionnaires containing miscoded answers or individuals in the top and bottom 1% of the ratio of energy intake to basal metabolic rate computed on the basis of age, height, and weight were excluded [13]. Of the remaining 73,034 questionnaires, those of 4,500 women who had previously reported a diagnosis of cancer were excluded along with those of 901 women for whom subsequent follow-up information was not available, yielding a sample of 67,633 women.

The questionnaire used for dietary assessment was a previously validated food frequency questionnaire covering the daily consumption of 208 food items, beverages, and recipes [14]. Nutrient intakes were calculated using a food composition table derived from the updated French national database [15]. Cases were ascertained through follow-up questionnaires in 1994, 1997, 2000, and 2002. Participants who reported cancer diagnosis were asked to provide their physician's address for confirmation. Deaths in the cohort were identified by reports from family members, the postal service, and the health insurance database. Cause of death was obtained from the French National Service of Deaths. A total of 2,323 cases of breast cancer (2,054 invasive and 269 in situ) were identified, 96.6% of which were confirmed by pathology reports. As the number of false positives was < 5%, all cases were included. Menopausal status was updated after each follow-up questionnaire and the analyses were restricted to postmenopausal women as postmenopausal breast cancer is considered to have a stronger association with environmental exposures [16]. The final analysis included 62,739 postmenopausal women, 1,812 of whom had breast cancer.

Person-years were calculated from the date of return of the 1993 dietary questionnaire to the date of cancer diagnosis, the date of the last questionnaire returned or 4 July 2002, whichever occurred first. Participants who died during follow were censored. Relative risk (RR) estimates were obtained using Cox's proportional hazard model with age as the time scale. Nutrients were energy-adjusted using the regression-residual method [17]. Folate intake was categorized in quintiles based on the distribution in the sample and the RRs of breast cancer were calculated by comparison with the lowest quintile. To test for trend, the quintile median value for dietary folate was assigned to each subject in that quintile and the values were used as a continuous variable. In multivariate analyses, adjustment was made for the covariates listed in the footnotes to Table 1. As folate intake may be correlated with that of other nutrients and to exclude the possibility of nutrients other than folate being responsible for the observed result, we conducted analyses including betacarotene, retinol, vitamins B_2, B_6, B_{12}, C, D, E, and fiber in the models. No information was available on the content of folate or vitamin B supplements. Participants were asked on drug section ''Do you currently take, at least three times a week, vitamin supplements?'' with options for vitamins A, C, D, E, B group, folic acid, beta-carotene, and other vitamins. Because vitamin B complex supplements may contain folate, we conducted the analyses without adjusting for this variable. The analyses were repeated excluding individuals who reported the use of folate or vitamin B supplements (9.7%). Because breast cancer cases diagnosed in the first 2 years of follow-up may have been present at the time of the dietary assessment, we excluded them and repeated the analyses. To evaluate consistency with our folate intake models, we also examined the associations between specific foods high in folate and breast cancer risk. Analyses were stratified by median alcohol intake (none, < 6.2 g/day, ≥6.2 g/day) and by tertiles of vitamin B_2 and B_{12} intake. Log-likelihood tests were used to evaluate interaction with alcohol and vitamins B_2 and B_{12}. The SAS statistical software (version 9.02, SAS Institute Inc., Cary, NC) was used for data analysis. All tests of statistical significance were two-sided.

Results

Median folate intake for the whole sample was 393 μg/ day and median intakes in energy-adjusted quintiles ranged from 296 μg/day to 522 μg/day. Folate intake was inversely associated with postmenopausal breast cancer risk; the RR for the highest quintile of intake compared to the lowest was 0.78 (95% CI: 0.67–0.90; *p*-trend = 0.001) (Table 1). Alcohol intake was associated with an increased risk of breast cancer; the multivariate RR for women with an alcohol intake of at least 6.2 g/day compared to women with no alcohol intake was 1.62 (95% CI: 1.22–2.15; *p*-trend = 0.002). No association was observed between vitamin B_2 [the $RR_{Q1–Q5}$ was 0.95 (95% CI: 0.81–1.10; *p*-trend = 0.42)] and B_{12} intake and breast cancer risk [the $RR_{Q1–Q5}$ was 1.05 (95% CI: 0.90–1.20; *p*-trend = 0.80)]. The main contributors to folate intake inversely associated to breast cancer risk were lettuce, spinach, and vegetable soup.

Table 1 **Relative risk (95% CI) of postmenopausal breast cancer according to quintiles of energy-adjusted folate**

Folate	Quintiles of intake					
	1	2	3	4	5	*p* for trend
Cases	395	355	366	347	349	
Person-years	77,779	80,108	82,379	83,957	86,091	
Median intake (μg/day)[a]	296	350	392	440	522	
Age-adjusted	1.00	0.86 (0.75–1.00)	0.86 (0.75–0.99)	0.80 (0.69–0.92)	0.78 (0.67–0.90)	0.0006
Multivariable-adjusted[b]	1.00	0.85 (0.73–0.98)	0.85 (0.73–0.98)	0.79 (0.68–0.91)	0.78 (0.67–0.90)	0.001

[a] Nutrients are adjusted for total energy intake

[b] Adjusted for age, two-year follow-up period, region of residence, years of education (< 12, 12–15 or ≥15), family breast cancer (0, 1 or >1 first degree relatives), history of benign breast disease (yes or no), age at menarche (< 12, 12 13 or ≥14), parity (0, ≤2 and age at first birth ≤30 years, ≥3 and age at first birth ≤30 years or age at first birth >30 years), breastfeeding (none, < 7, 7–12 or ≥12 months), years since last use of oral contraceptives (never, unknown date of last use, < 25, 25–29 or ≥30), age at menopause (< 45, 45–49, 50–54 or ≥55), years of hormone replacement therapy use (never, unknown duration, < 3, 3–5.9 or ≥6), regular mammographic evaluation (yes or no), height in cm, body mass index (quartiles), vitamin (other than vitamin B) supplement use (yes or no), alcohol intake (0, < 6, 2 or ≥6. 2 gr/day) and physical activity (quartiles). Regular mammographic evaluation defined as a report of a recent mammogram in 1990, 1992, and 1993

An inverse association was observed between folate intake and breast cancer risk in women who consumed alcohol. Among those with an alcohol intake of less than 6.2 g/day, there was a decreased risk in the second quintile of folate intake and the inverse association remained stable with increasing folate intake. Among women with an alcohol intake of at least 6.2 g a day, there was a significant decreasing trend in breast cancer risk with increasing folate intake, with the strongest association in the two highest quintiles (*p*-trend = 0.006). When extreme quintiles of folate intake were compared, the RR was 0.76 (95% CI: 0.63– 0.94). However, a formal test for interaction between folate and alcohol intake was not statistically significant (*p*-value = 0.30). We also analyzed the relation between folate and breast cancer risk by vitamin B_{12} intake (Table 2). The inverse association of folate intake was stronger in the two highest tertiles of vitamin B_{12} intake; the RRs for the extreme quintiles of folate intake were 0.73 (95% CI: 0.56–0.97; *p*-trend = 0.01) and 0.62 (95% CI: 0.47–0.81; *p*-trend = 0.02), compared to 0.92 (95% CI: 0.70–1.20; *p*-trend = 0.44) in the first tertile of vitamin B_{12} intake. The log-likelihood ratio test for interaction did not yield statistically significant results (*p* = 0.28). When the joint effects of different levels of alcohol and vitamin B_{12} were explored, there was a suggestion that the inverse association between folate intake and breast cancer risk was stronger among women with high intake of alcohol and vitamin B_{12}; however, results were not statistically significant. The inverse association between folate intake and breast cancer risk did not differ by vitamin B_2 intake.

Because intakes of folate, fiber, and vitamins may be correlated, we conducted analyses including two nutrients at a time to identify the one responsible for the observed inverse association. The apparent protective effect of folate intake was essentially the same after further adjustment one at a time for beta-carotene, retinol, vitamin B_2, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E, and fiber. In the subanalyses excluding women who reported folate or vitamin B supplement use or cancer cases diagnosed within the first two years of follow-up, the RRs did not materially change. The $RR_{Q1–Q5}$ for folate intake after exclusion of these cases was 0.76 (95% CI: 0.65–0.89; *p*-trend 0.009). For the B_{12} stratified analyses the $RR_{Q1–Q5}$ for folate intake in the highest tertile of B_{12} intake was 0.67 (95% CI: 0.49–0.90; *p*-trend 0.05).

Discussion

In our prospective cohort, we observed an inverse association between folate intake and postmenopausal breast cancer. There was no evidence to support effect modification by alcohol or vitamin B_2 intake. However, the inverse association between folate intake and breast cancer appeared to be somewhat stronger among women who reported high intakes of vitamin B_{12}.

Table 2 **Relative risk (95% CI) of postmenopausal breast cancer according to quintiles of energy-adjusted folate by intake of energy-adjusted vitamin B12**

Median intake[a]	Tertiles of energy-adjusted vitamin B_{12} intake								
	1			2			3		
	4.2 µg/day			6.7 µg/day			11.6 µg/day		
Quintiles of folate intake	Cases	Age-adjusted	Multivariate[b]	Cases	Age-adjusted	Multivariate[b]	Cases	Age-adjusted	Multivariate[b]
1	180	1.00	1.00	135	1.00	1.00	80	1.00	1.00
2	138	0.96 (0.77–1.20)	0.92 (0.74–1.15)	134	0.90 (0.71–1.14)	0.90 (0.71–1.14)	83	0.63 (0.47–0.86)	0.62 (0.45–0.84)
3	97	0.77 (0.60–0.99)	0.75 (0.59–0.97)	155	1.04 (0.82–1.31)	1.02 (0.81–1.29)	114	0.66 (0.49–0.88)	0.64 (0.48–0.86)
4	107	0.96 (0.75–1.22)	0.92 (0.72–1.18)	104	0.76 (0.59–0.98)	0.75 (0.58–0.97)	136	0.62 (0.47–0.82)	0.62 (0.47–0.82)
5	79	0.92 (0.70–1.20)	0.92 (0.70–1.20)	87	0.75 (0.57–0.98)	0.73 (0.56–0.97)	183	0.62 (0.47–0.80)	0.62 (0.47–0.81)
p-value trend		0.46	0.44		0.01	0.01		0.01	0.02

[a] Adjusted for total energy intake

[b] Adjusted for age, two-year follow-up period, region of residence, years of education (< 12, 12–15, or ≥15), family breast cancer (0, 1, or >1 first degree relatives), history of benign breast disease (yes or no), age at menarche (< 12, 12 13, or ≥14), parity (0, ≤2 and age at first birth ≤30 years, ≥3 and age at first birth ≤30 years or age at first birth >30 years), breastfeeding (none, < 7, 7–12, or ≥12 months), years since last use of oral contraceptives (never, unknown date of last use, < 25, 25–29, or ≥30), age at menopause (< 45, 45–49, 50–54, or ≥55), years of hormone replacement therapy use (never, unknown duration, < 3, 3–5.9, or ≥6), regular mammographic evaluation (yes or no), height in cm, body mass index (quartiles), vitamin (other than vitamin B) supplement use (yes or no), and physical activity (quartiles). Regular mammographic evaluation defined as a report of a recent mammogram in 1990, 1992, and 1993

Several large prospective studies evaluating the relation between folate intake and breast cancer risk found no overall association [5, 6, 8, 9, 18]. However, three of them noted an inverse association between folate intake and breast cancer risk among women with a high alcohol intake [6, 7, 9]. A large nested case–control study using prospectively collected blood reported an indication of an overall association between circulating levels of folate and breast cancer risk [19]. We did not observe a more evident inverse association between folate intake and breast cancer among women who regularly consumed alcohol. However, in our study the power to detect the interaction between folate and alcohol intake was low as only 53 breast cancer cases reported no alcohol consumption. In contrast to other studies, most (95.4%) of the women in our cohort drink alcohol and few (9.7%) use vitamin B complex or folate supplements. These two factors may possibly explain why we found that folate had an overall protective effect on breast cancer risk. The protective effect differed according to the level of vitamin B_{12} intake, with a stronger inverse association at higher intakes. These results are consistent with two population-based breast cancer case-control studies [10, 11], which reported that an adequate intake of vitamin B_{12} appeared to be necessary if the folate intake was to have a protective effect. Furthermore, a prospective cohort study reported a strong joint protective effect of high folate and high vitamin B_{12} intake on colon cancer risk [20].

Folate is involved in nucleotide synthesis and DNA and RNA methylation [1]. There is consistent evidence that low folate intake may have procarcinogenic effects [21–23]. Adequate folate intake is necessary for the conversion of homocysteine into methionine for DNA methylation via methionine synthase [1]. This enzyme requires vitamin B_{12} as a cofactor to convert homocysteine into methionine and determines the methylation capacity of the cell. The protective effect of high folate intake on breast cancer risk may therefore be of greater significance in individuals with adequate intake of vitamin B_{12}.

The prospective design and the high follow-up rates in the E3N study limit the possibility of serious bias as an explanation for our results. Although residual confounding may be present, the minimal effect observed in our estimates after adjustment for several recognized risk factors for breast cancer makes this unlikely. Given that our findings for folate are independent of the consumption of fiber and several vitamins, our results suggest that folate intake is the primary factor involved. Dietary assessment was limited to a baseline measurement; it is possible that participants changed their diets during follow-up, so some misclassification of exposure may be present. Any non-differential misclassification would weaken the observed association. Another potential limitation of this study was the lack of information on the amounts of folate and vitamin B complex derived from supplements. However, only 9% of participants reported the use of vitamin B complex supplements and the estimates remained unchanged when these individuals were excluded from the analysis.

In summary, our findings suggest that high dietary folate intake lowers the risk of breast cancer in postmenopausal women. The protective effect of folate on breast cancer risk may be influenced by vitamin B_{12}

intake. The association between folate and breast cancer at different levels of vitamin B_{12} intake should be explored in other prospective studies.

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