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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 $Q_1 = 296 \ \mu g/day$ and $Q_5 = 522 \ \mu g/day$]. 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 B₁₂, Vitamin B₂ 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 guintiles 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 B₂ and B₁₂ intake. Log-likelihood tests were used to evaluate interaction with alcohol and vitamins B₂ and B₁₂. 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 μ g/ day and median intakes in energy-adjusted quintiles ranged from 296 μ g/day to 522 μ g/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 B₂ [the RR_{Q1-Q5} was 0.95 (95% CI: 0.81–1.10; *p*-trend = 0.42)] and B₁₂ 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-adju	sted
folate								

Folate	Quintiles of intake						
	1	2	3	4	5	<i>p</i> for trend	
Cases	395	355	366	347	349		
Person-years	77,779	80,108	82,379	83,957	86,091		
Median intake $(\mu g/day)^a$	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 ($< 12, 12 \ 13 \ or \ge 14$), 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 B₁₂ 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 loglikelihood 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 B₁₂; however, results were not statistically significant. The inverse association between folate intake and breast cancer risk did not differ by vitamin B₂ 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	Cases	Age-adjusted	Multivariate ^b	Cases	Age-adjusted	Multivariate ^b	Cases	Age-adjusted	Multivariate ^b
folate intake									
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 \geq 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 \geq 14), parity (0, \leq 2 and age at first birth \leq 30 years, \geq 3 and age at first birth \leq 30 years or age at first birth \geq 30 years), breastfeeding (none, <7, 7–12, or \geq 12 months), years since last use of oral contraceptives (never, unknown date of last use, < 25, 25–29, or \geq 30), age at menopause (<45, 45–49, 50–54, or \geq 55), years of hormone replacement therapy use (never, unknown duration, < 3, 3–5.9, or \geq 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 B₁₂ 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 B₁₂ 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 B₁₂ 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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References

- 1. Mason JB, Choi SW (2000) Folate and carcinogenesis: developing a unifying hypothesis. Advan Enzyme Regul 40:127–141
- 2. Duthie SJ, Narayanan S, Blum S, Pirie L, Brand GM (2000) Folate deficiency in vitro induces uracil misincorporation and DNA hypomethylation and inhibits DNA excision repair in immortalized normal human colon epithelial cells. Nutr Cancer 37:245–251
- 3. Szyf M, Pakneshan P, Rabanni SA (2004) DNA methylation and breast cancer. Biochem Pharm 68:1187–1197
- 4. Mason JB, Choi SW (2005) Effects of alcohol on folate metabolism: implications for carcinogenesis. Alcohol 35:235-241
- Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ, Calle EE (2003) Alcohol, folate, methionine and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. Cancer Epidemiol Biomark Prev 12:161–164
- 6. Zhang S, Hunter DJ, Hankinson SE, et al (1999) A prospective study of folate intake and the risk of breast cancer. JAMA 281:1632–1637
- 7. Rohan TE, Jain MG, Howe GR, Miller AB (2000) Dietary folate consumption and breast cancer risk. JNCI 92:266–269
- 8. Sellers TA, Kushi LH, Cerhan JR, et al (2001) Dietary folate intake, alcohol and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology 12:420–428
- 9. Biglietto L, English DR, Gertig DM, Hopper JL, Giles GL (2005) Does dietary folate intake modify effect of alcohol consumption on breast cancer risk? Prospective cohort study. BMJ 331:807–810
- 10. Shrubsole MJ, Jin F, Dai Q, et al (2001) Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Res 61:7136–7141
- 11. Lajous M, Lazcano-Ponce E, Hernandez-Avila M, Willett W, Romieu I (2006) Folate, vitamin B6 and vitamin B12 intake and the risk of breast cancer among Mexican women. Cancer Epidemiol Biomark Prev 15:443–448
- 12. Touvier M, Kesse E, Clavel-Chapelon F, Boutron-Ruault MC (2005) Dual association of beta-carotene with risk of tobacco-related cancers in a cohort of French women. J Natl Cancer Inst 97:1338–1344
- Schofield WN (1985) Predicting basal metabolic rate, new standards, review of previous work. Hum Nutr Clin Nutr 39C:5–41
- 14. van Liere MJ, Lucas F, Clavel F, Slimani N, Villeminot S (1997) Relative validity and reproducibility of a French dietary history questionnaire. Int J Epidemiol 26(Suppl1):S128–S136
- 15. Favier JC, Ireland-Ripert J, Toque C, Feinberg M (1995) Répertoire Général des Aliments. Table de composition (Composition tables). INRA, CIQUAL-REGAL, Tec & Doc Lavoisier, Paris
- 16. Gaudet MM, Britton JA, Kabat GC, et al (2004) Fruits, vegetables, and micronutrients in relation to breast cancer modified by menopause and hormone receptor status. Cancer Epidemiol Biomark Prev 13:1485–1494
- 17. Willett W, Stampfer M (1998) Implications of total energy intake for epidemiologic analysis. In: Willett W (ed) Nutritional epidemiology, 2nd edn. Oxford University Press, New York, pp 273–301
- 18. Cho E, Spiegelman D, Hunter DJ, et al (2003) Premenopausal intakes of vitamins A, C, and E, folate, and carotenoids, and risk of breast cancer. Cancer Epidemiol Biomark Prev 12:713–720
- 19. Zhang S, Willet WC, Selhub J, et al (2003) Plasma folate, vitamin B6, vitamin B12, homocysteine and risk of breast cancer. JNCI 95:373–380
- 20. Harnack L, Jacobs DR Jr, Nicodemus K, Lazovich D, Anderson K, Folsom AR (2002) Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. Nutr Cancer. 43(2):152–158
- Fowler BM, Giuliano AR, Piyathilake C, Nour M, Hatch K (1998) Hypomethylation in cervical tissue: is there a correlation with folate status? Cancer Epidemiol Biomark Prev 7:901–906

- 22. Jacob RA, Gretz DM, Taylor PC, et al. (1998) Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J Nutr 128:1204–1212
- 23. Fenech M, Baghurst P, Luderer W, et al. (2005) Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, betacarotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability results from a dietary intake and micronucleus index survey in South Australia. Carcinogenesis 26:991–999