Folate, Vitamin B_6, and Vitamin B_{12} Intake and the Risk of Breast Cancer Among Mexican Women

Martin Lajous,[1] Eduardo Lazcano-Ponce,[1] Mauricio Hernandez-Avila,[1] Walter Willett,[2] and Isabelle Romieu[1]

[1]Center for Population Health Research, Instituto Nacional de Salud Publica, Cuernavaca, Mexico and [2]Departments of Nutrition and Epidemiology, Harvard School of Public Health; The Channing Laboratory; Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Abstract

Background: **High intake of folate, vitamin B_6, and vitamin B_{12} have been hypothesized to lower the risk for breast cancer. We conducted a population-based case-control study to evaluate the risk for breast cancer among Mexican women with relatively low vitamin intakes.**

Methods: **We included 475 women (median age, 53 years; range, 23-87 years) diagnosed with incident breast cancer through six hospitals in Mexico City and interviewed them to obtain data on breast cancer risk factors and their usual diet using a food frequency questionnaire. We selected 1,391 (median age, 49 years; range, 18-82 years) controls from the Mexico City population using a national sampling frame.**

Results: **Compared with women in the lowest quartile, the odds ratio for breast cancer for women in the highest quartile of folate intake was 0.64 [95% confidence intervals (CI), 0.45-0.90; *P*, test for trend = 0.009] and 0.32 (95% CI, 0.22-0.49; *P*, test for trend < 0.0001) for vitamin B_{12} intake. Among postmenopausal women, intakes of folate and vitamin B_{12} were associated with a lower risk of breast cancer and those associations were stronger than among premenopausal women. The inverse association of folate and breast cancer was stronger among women who consumed a high level of vitamin B_{12} as compared with women consuming diets low in vitamin B_{12}. No association was observed for vitamin B_6 intake.**

Conclusions: **In this population, high intakes of folate and vitamin B_{12} were independently associated with decreased breast cancer risk, particularly among postmenopausal women.** (Cancer Epidemiol Biomarkers Prev 2006;15(3):443–8)

Introduction

Various dietary factors have been hypothesized to be determinants of breast cancer, but few have been unequivocally associated with the disease. In contrast with most known risk factors for breast cancer, dietary factors are potentially modifiable, making their identification essential. The incidence of breast cancer in Mexico is still relatively low as compared with Western countries (1). However, recent changes in reproductive patterns, diet, and lifestyle are likely to contribute to increasing breast cancer incidence in Mexico. From 1979 to 2000, age-standardized mortality almost doubled from 6.4 to 12.2 deaths per 100,000 women (2).

Low vegetable and fruit intake (97% of Mexican women consume <400 g/d or less than four servings per day) and increasing consumption of processed foods may account for the high prevalence of micronutrient deficiencies observed in Mexican women (3). According to the 1999 National Nutrition Survey, median folate intake among Mexican women was 221 μg/d and median vitamin B_{12} intake was 1.6 μg/d, both close to half of the U.S. recommended dietary allowance (4). Based on a subsample of 461 Mexican women from the National Nutrition Survey, 35% of Mexican women had serum folate levels below the normal 3 ng/mL threshold, and 17% had vitamin B_{12} serum levels below the reference range.[3]

Inadequate folate intake has been associated with several cancers and low levels of serum folate, vitamin B_6, and vitamin B_{12} have been associated with increased breast cancer risk (5, 6). These vitamins all participate as coenzymes in the synthesis of purines and thymidylate for DNA synthesis. Altered DNA methylation, disruption of DNA integrity, and interference with DNA repair are hypothesized mechanisms by which imbalances in folate and other B vitamins may influence nucleic acid metabolism and participate in carcinogenesis (7, 8). Given the high prevalence of vitamin deficiencies in the Mexican population, we conducted a case-control study among women living in Mexico City to examine dietary intakes of folate, vitamin B_6, and vitamin B_{12} in relation to the risk for breast cancer.

Materials and Methods

Study Population. Participants in this study were enrolled in a population-based case-control study to assess the relationships of dietary and reproductive factors with breast cancer risk among residents of Mexico City (9). Cases that had been resident for at least 1 year in metropolitan Mexico City were recruited using a network of six hospitals that are part of the two major healthcare providers in Mexico City, the social security system and the Ministry of Health. These hospitals provided medical care to 80% of breast cancer cases reported to the Mexico City Tumor Registry. From 1990 to 1995, incident confirmed breast cancer cases without previous treatment were identified among women ages 20 to 75 years attending gynecologic clinics for the biopsy of a breast lump. Only

Received 7/21/05; revised 12/6/05; accepted 1/5/06.

Grant support: American Institute for Cancer Research, the Ministry of Health of Mexico, and the National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: This work has been presented at the meeting of the Society for Epidemiologic Research, June 2005.

Requests for reprints: Isabelle Romieu, Instituto Nacional de Salud Publica, Av. Universidad 655, Col. Santa Maria Ahuacatitlan, Cuernavaca, Morelos 62508, Mexico.
Phone: 52-777-101-2935; Fax: 52-777-311-1148. E-mail: iromieu@correo.insp.mx

doi:10.1158/1055-9965.EPI-05-0532

[3] Personal communication Miriam Aracely Anaya-Loyola, Universidad Autónoma de Querétaro.

women whose biopsy histologically confirmed the diagnosis of breast cancer were eligible. A total of 537 cases were identified. Of those, 88% (n = 475) agreed to participate and provided dietary information. Controls were an age-stratified random sample of metropolitan Mexico City residents. Households were first randomly selected using the National Household Sampling Frame. Study personnel ascertained whether the selected units contained a woman in the intended age group and then visited these households and determined willingness to be interviewed and to provide a blood sample. Only one eligible control was included per household. From 1,534 eligible controls, 90% (n = 1,391) agreed to participate in the study.

Interviewers administered a questionnaire on sociodemographic variables and potential risk factors for breast cancer including reproductive and lactation history and diet. Cases were interviewed at the gynecologic clinics of the study hospitals before they were aware of the diagnosis; the lag time between interview and biopsy was between 1 and 3 months. Controls were interviewed in their homes. Self-reported height and weight have proven unreliable in this population; we therefore sent participants to their health center to obtain actual measurements. These were available for only 226 cases (48% of all cases) and 699 controls (50% of all controls) that attended the health center.

Semiquantitative Food Frequency Questionnaires. We used a dietary questionnaire developed by Willett et al. (10), which was adapted to the Mexican population (11). We first identified relevant foods using a dietary survey from Mexico City residents. We then contacted a group of Mexican dietitians to include additional food items and made a pilot study in a small sample of women before conducting the validation study. Our modified questionnaire included 104 items and 10 multiple choice frequency categories of consumption: 6 or more per day, 4 to 5 per day, 2 to 3 per day, 1 per day, 5 to 6 per week, 2 to 4 per week, 1 per week, 2 to 3 per month, 1 per month, or less and never. For each food in the questionnaire, a commonly used unit or portion size (specified serving size: slice, glass, or natural unit such as one apple) was specified, and women were asked how often on average over the previous year they had consumed that amount of each food. Nutrient intakes were computed by multiplying the frequency response by the nutrient content of specified portion sizes using a program developed at the National Institute of Public Health, Mexico. The database for calculating the nutrient intakes used information from the U.S. Department of Agriculture food composition tables (12) complemented, when necessary, by a nutrient database developed by the Mexican National Institute of Nutrition (13). This questionnaire has been validated against 16 24-hour recalls among a sample of 134 women in Mexico City comparable to our present population, and has been shown to perform well. Correlations between the food frequency questionnaires and dietary records for total energy carbohydrate, protein, and total fat intakes were 0.52, 0.57, 0.32, and 0.63, respectively (11).

Statistical Analysis. We used χ^2 tests for categorical variables and t tests or Kruskal-Wallis tests for continuous variables for comparisons between cases and controls. Folate, vitamin B_6, and vitamin B_{12} intakes were adjusted for total energy intake using the regression-residual method (14). These variables were categorized as quartiles based on the distribution among the control group, and odds ratios (OR) for breast cancer were determined by comparison with the lowest quartile. Socioeconomic status was defined using an index for the Mexican population developed by Bronfman et al. (15). This variable was categorized as tertiles to define low, medium, and high socioeconomic status. Logistic regression was used to calculate OR and their 95% confidence intervals (CI) which were used to estimate relative risks. Crude models were age-adjusted. For multivariate analyses, we further controlled for socioeconomic status, family history of breast cancer, parity, total caloric intake, dietary fiber, carbohydrate, and polyunsaturated fat. In addition, because women for whom body mass index (BMI) was available may differ from those for whom it was not, we included an indicator variable in the models for availability of BMI [BMI = weight (kg) / height^2 (m)]. Dietary fiber, carbohydrates, and polyunsaturated fat were considered confounders because in a previous study they seemed to be strong predictors of breast cancer and were associated with intake of the micronutrients of interest (16). To test for linear trend, the quartile median value for dietary folate, vitamin B_{12}, and vitamin B_6 was assigned to each subject in that quartile and these values were used as a continuous variable. Spearman correlation coefficients were used to evaluate correlations between nutrients. Because BMI is a predictor of breast cancer (17), analyses were repeated in the subsample of women who had BMI data, with and without controlling for BMI in addition to the variables previously mentioned and compared with the results. Data were stratified by menopausal status because the effect of dietary intake of vitamins may be modified by hormonal status, and postmenopausal breast cancer is thought to be more susceptible to environmental exposures (18). Information on type of menopause (natural or surgical) was obtained by questionnaire. Natural menopause was defined as 12 consecutive months of amenorrhea without an obvious cause. Family history of breast cancer may modify nutrient intake and breast cancer risk (19). Similarly, because vitamins B_6 and B_{12} serve as important cofactors in folate metabolism and their availability for metabolic processing may affect folate metabolites, we evaluated whether the observed association with folate intake differed by family history of breast cancer and by levels of vitamin B_6 and vitamin B_{12} intake. Intakes of vitamins B_6 and B_{12} were categorized by the median intake of controls. Log-likelihood tests were used to evaluate interaction terms. We used SAS statistical software (version 9.0, SAS Institute, Inc., Cary, NC) for data analysis. All tests of statistical significance were two-sided.

Results

Cases were 475 women with histologically confirmed breast cancer, 189 of which occurred in premenopausal and 286 in postmenopausal women. A total of 1,391 women frequency-matched on 5-year strata served as controls. Table 1 presents the demographic, gynecologic, and nutritional characteristics of cases as compared with their controls. Cases were older, had a higher socioeconomic status, more often had a family history of breast, were more often nulliparous, had less children, and were less often premenopausal than controls. Total caloric and carbohydrate intake were higher among cases, whereas their dietary fiber intake seemed to be lower than controls. For the whole population, median folate intake was 310 μg/d (5th-95th percentile, 132-604), vitamin B_6 intake was 1.27 mg/d (5th-95th percentile, 0.65-2.15), and vitamin B_{12} intake was 4.58 μg/d (5th-95th percentile, 1.29-9.48).

For all women, greater intakes of folate, vitamin B_6, and vitamin B_{12} were associated with lower risk of breast cancer (Table 2). After adjusting for age, socioeconomic status, family history of breast cancer, menopausal status, parity, availability of BMI, total caloric intake, dietary fiber, animal fat intake, total carbohydrate, and polyunsaturated fat intake; the ORs for the highest quartile of intake compared with the lowest (OR_{Q1-Q4}) were 0.64 (95% CI, 0.45-0.90) for folate intake, 0.84 (95% CI, 0.61-1.18) for vitamin B_6 intake, and 0.32 (95% CI, 0.22-0.49) for

Table 1. Characteristics of Mexican women with histologically confirmed breast cancer and their population-based controls

Characteristic	Case patients (n = 475)	Controls (n = 1,391)	
	Mean (SD)		
			P*
Age (y)	53.5 (12.8)	49.4 (13.4)	<0.01
Parity	3.8 (3.2)	4.4 (3.4)	<0.01
BMI (kg/m^2) †	26.9 (4.8)	27.2 (4.8)	0.34
	Percentage		
			P ‡
Family history of breast cancer (%)	4	2	<0.01
Nulliparous women (%)	17	11	<0.01
Postmenopausal women (%)	60	51	<0.01
Women with high socioeconomic status (%)	30	20	<0.01
	Median (5th-95th percentile)		
			P§
Caloric intake (kcal/d)	1,696 (812-2,683)	1,565 (847-2,735)	0.02
Carbohydrate (g/d)	246 (106-424)	222 (113-412)	<0.01
Animal fat (g/d)	36.2 (10.9-79.7)	36.9 (13.8-79.5)	0.16
Polyunsaturated fat (g/d)	5.3 (2.7-8.7)	5.4 (3.3-9.5)	0.09
Dietary fiber (g/d)	23.8 (9.2-42.7)	27.5 (10.1-44.2)	<0.01

*t test.
†BMI available for 226 cases and 669 controls.
‡χ^2 test.
§Kruskal-Wallis test.

vitamin B_{12} intake. However, a significant inverse trend was observed only between folate intake (P, test for trend = 0.009) and vitamin B_{12} intake (P, test for trend ≤0.0001) and breast cancer risk.

Because intakes of folate, vitamin B_6, and vitamin B_{12} may be correlated; we conducted analyses including two of the nutrients at a time to identify the nutrient responsible for the observed inverse association. When we included both folate and vitamin B_{12} (r = 0.39) in the multivariate model, the inverse associations previously observed remained present for both nutrients. No significant association was observed with vitamin B_6 after inclusion of the other nutrients in the model one at a time.

The apparent protective effect of folate intake was slightly strengthened after further adjustment one at a time for β-carotene, lutein, total vitamin A, vitamin E, and vitamin D intake and the $OR_{Q1\text{-}Q4}$ ranged between 0.44 and 0.61. After inclusion of all these nutrients simultaneously in the model, the OR somewhat shifted away from the null ($OR_{Q1\text{-}Q4}$ = 0.44; 95% CI, 0.29-0.65). For vitamin B_{12}, the OR for extreme quartiles did not appreciably change after adjustment for β-carotene, lutein, total vitamin A, vitamin E, and vitamin D. Therefore, these nutrients are unlikely to explain the association we observed with folate and vitamin B_{12}.

Based on observations made by others, we examined whether the association between these nutrients and breast cancer differed by menopausal status and family history of breast cancer (18-23). Among premenopausal women, intakes of folate and vitamin B_6 were not appreciably associated with breast cancer risk (Table 3). For vitamin B_{12}, the multivariate $OR_{Q1\text{-}Q4}$ was 0.54 (95% CI, 0.29-0.99; P, test for trend = 0.01) Among postmenopausal women, folate and vitamin B_{12} intake were associated with a lower risk of breast cancer and this effect seemed to be stronger than among all women combined. For folate intake, the multivariate $OR_{Q1\text{-}Q4}$ was 0.55 (95% CI, 0.35-0.86; P, test for trend = 0.008; P, for interaction with menopausal status = 0.16). After multivariable adjustment, postmenopausal women in the lowest quartile of vitamin B_{12} intake had a 4.8 times higher breast cancer risk when compared with women in the highest quartile ($OR_{Q1\text{-}Q4}$ = 0.21; 95% CI, 0.12-0.37; P, test for trend <0.0001). No association was observed with vitamin B_6 intake. We found no evidence of effect modification after stratifying by family history of breast cancer (folate; P, for interaction = 0.24).

Because vitamin B_6 and vitamin B_{12} serve as important cofactors in folate metabolism, we evaluated whether the observed association between folate intake differed by levels of vitamin B_{12} and vitamin B_6 intake (Table 4), as previously reported by Shrubsole et al. (22). Low vitamin B_{12} intake seemed to reduce the apparent protection in the risk for breast cancer conferred by folate. Among women in the two lowest quartiles of vitamin B_{12} intake, folate intake was not related to the risk for breast cancer. In contrast, among women in the highest quartile of vitamin B_{12} intake, we observed a significant inverse trend (P = 0.006) in the risk for breast cancer with increasing folate intake (P, for interaction <0.0001). The joint effect of folate and vitamin B_{12} comparing women in the highest quartiles of both folate and vitamin B_{12} intake with those in the lowest quartiles was an OR of 0.16 (95% CI, 0.07-0.41). There was no evidence that vitamin B_6 intake modified the observed association between folate and breast cancer risk.

Table 2. OR (95% CI) of breast cancer according to quartiles of energy-adjusted folate, vitamin B_6, and vitamin B_{12} intake

	Quartiles of intake				
	1	2	3	4	P trend
Folate					
Cases/controls	162/347	113/348	97/349	103/347	
Median intake (μg/d)	224	303	362	454	
Age-adjusted	1.00	0.65 (0.49-0.87)	0.56 (0.42-0.76)	0.62 (0.46-0.83)	0.0007
Multivariable-adjusted*	1.00	0.77 (0.56-1.05)	0.68 (0.48-0.94)	0.64 (0.45-0.90)	0.009
Vitamin B_6					
Cases/controls	161/348	100/350	103/337	111/356	
Median intake (mg/d)	1.06	1.26	1.40	1.60	
Age-adjusted	1.00	0.56 (0.42-0.75)	0.61 (0.45-0.82)	0.69 (0.52-0.91)	0.01
Multivariable-adjusted*	1.00	0.67 (0.49-0.92)	0.76 (0.55-1.05)	0.84 (0.61-1.13)	0.41
Vitamin B_{12}					
Cases/controls	159/352	139/343	107/348	70/348	
Median intake (μg/d)	2.61	4.03	5.68	7.46	
Age-adjusted	1.00	0.83 (0.63-1.10)	0.62 (0.46-0.83)	0.40 (0.29-0.55)	<0.0001
Multivariable-adjusted*	1.00	0.80 (0.59-1.10)	0.51 (0.36-0.73)	0.32 (0.22-0.49)	<0.0001

*Adjusted for age, socioeconomic status, family history of breast cancer, menopausal status, parity, availability of BMI, total caloric intake, dietary fiber carbohydrate intake, and polyunsaturated fat intake.

Table 3. OR (95% CI) of breast cancer in premenopausal and postmenopausal women according to quartiles of energy-adjusted folate, vitamin B_6, and vitamin B_{12} intake

	Quartiles of intake				
	1	2	3	4	*P* trend
Folate					
Premenopausal women					
Cases/controls	58/189	47/155	41/161	43/181	
Median intake (μg/d)	218	303	360	460	
Multivariable-adjusted*	1.00	0.88 (0.53-1.44)	0.80 (0.47-1.36)	0.73 (0.42-1.27)	0.26
Postmenopausal women					
Cases/controls	104/158	66/193	56/188	60/166	
Median intake (mg/d)	229	302	363	458	
Multivariable-adjusted*	1.00	0.72 (0.47-1.09)	0.59 (0.38-0.91)	0.55 (0.35-0.86)	0.008
Vitamin B_6					
Premenopausal women					
Cases/controls	71/183	33/145	37/152	48/206	
Median intake (μg/d)	1.05	1.26	1,40	1.60	
Multivariable-adjusted*	1.00	0.57 (0.34-0.95)	0.66 (0.40-1.10)	0.76 (0.46-1.26)	0.34
Postmenopausal women					
Cases/controls	90/165	67/205	66/185	63/150	
Median intake (mg/d)	1.07	1.26	1.39	1.59	
Multivariable-adjusted*	1.00	0.80 (0.53-1.22)	0.89 (0.57-1.37)	0.94 (0.59-1.49)	0.87
Vitamin B_{12}					
Premenopausal women					
Cases/controls	64/206	54/156	44/167	27/157	
Median intake (μg/d)	2.6	4	5.6	7.7	
Multivariable-adjusted*	1.00	1.11 (0.69-1.80)	0.65 (0.38-1.10)	0.54 (0.29-0.99)	0.01
Postmenopausal women					
Cases/controls	95/146	85/187	63/181	43/191	
Median intake (μg/d)	2.6	4.1	5.7	7.3	
Multivariable-adjusted*	1.00	0.64 (0.42-0.98)	0.35 (0.21-0.58)	0.21 (0.12-0.37)	<0.0001

*Adjusted for age, socioeconomic status, family breast cancer, parity, availability of BMI, total caloric intake, dietary fiber, carbohydrate intake, and polyunsaturated fat intake.

Because BMI could confound the association between these nutrients and the risk of breast cancer, we repeated these analyses in the subsample of our study population for which information on height and weight was available. Including BMI in the models did not substantially change the apparent protective effect of folate and vitamin B_{12} intake. Thus, in this population, BMI did not seem to confound the association between vitamin intake and the risk of breast cancer.

Discussion

In this population-based case-control study in Mexican women, we observed inverse associations between intakes of folate and vitamin B_{12} and the risk of breast cancer. The observed associations were stronger among postmenopausal women and the inverse relation of folate to breast cancer risk was strengthened among participants with high intakes of vitamin B_{12}. We observed no association with vitamin B_6 intake.

The relation of folate intake to breast cancer risk has been investigated in five large prospective cohorts, most of which have not found an overall association of folate intake and breast cancer risk (20, 21, 24-26). However, in three of these studies, low folate intake seemed to increase the risk of breast cancer among women with high alcohol intake (20, 21, 24). In addition, two case-control studies did not find the association (27, 28). Our study is consistent with various case-control studies (22, 23, 29-32) that have reported a protective relationship and a large nested case-control study using prospectively collected blood that reported a strong inverse association between circulating levels of folate and risk for breast cancer (5). This apparent contradiction may be explained by differences in folate intake between populations. Four of the studies that reported a protective effect were conducted in non-American populations in which vitamin supplementation is unusual (22, 23, 29, 32). In our study, 72% of individuals reported inadequate folate intake (<400 μg/d) and close to 20% had a vitamin B_{12} intake of less than the recommended dietary allowance of 2.4 μg/d.

Few epidemiologic studies have evaluated vitamin B_6 and vitamin B_{12} and the risk for breast cancer. The reports are inconsistent (5, 6, 22, 23). In our study, the strongest inverse association was observed with vitamin B_{12} intake. This

Table 4. OR (95% CI) of breast cancer stratified by quartiles of vitamin B_{12} intake according to quartiles of folate intake

Quartiles of vitamin B_{12} intake	Quartiles of folate intake	Age-adjusted	Multivariable-adjusted*
1	1	1.00	1.00
	2	0.65 (0.37-1.13)	0.78 (0.43-1.41)
	3	0.60 (0.34-1.04)	0.76 (0.42-1.39)
	4	0.87 (0.53-1.44)	0.91 (0.53-1.15)
P values for trend		0.51	0.70
2	1	1.00	1.00
	2	0.86 (0.50-1.47)	0.86 (0.49-1.53)
	3	0.97 (0.55-1.72)	1.03 (0.57-1.88)
	4	0.96 (0.55-1.68)	0.75 (0.41-1.37)
P values for trend		0.99	0.45
3	1	1.00	1.00
	2	0.66 (0.37-1.15)	0.66 (0.37-1.20)
	3	0.57 (0.31-1.04)	0.58 (0.31-1.08)
	4	0.33 (0.17-0.66)	0.29 (0.14-0.61)
P values for trend		0.002	0.001
4	1	1.00	1.00
	2	0.44 (0.22-0.90)	0.52 (0.25-1.10)
	3	0.30 (0.15-0.62)	0.36 (0.17-0.77)
	4	0.35 (0.17-0.72)	0.36 (0.17-0.79)
P values for trend		0.002	0.006

*Adjusted for age, socioeconomic status, family breast cancer, menopausal status, parity, availability of BMI, total caloric intake, dietary fiber, carbohydrate intake, and polyunsaturated fat intake.

association confirms previous observations of vitamin B_{12} circulating levels (5, 6). We cannot exclude the possibility that the lack of association with vitamin B_6 which we observed might be due to reasons other than biological factors.

The main contributors to folate intake were spinach, maize porridge with milk, orange juice, beans, papaya, and mango. For vitamin B_{12}, the main contributors were maize porridge with milk, liver, red meat, milk, chicken, and sardines. We included these foods in multivariate models to confirm our results and observed that the apparent protective effects between intake of beans, liver, and sardines and breast cancer risk were particularly strong. Strong correlations between micronutrients may be an obstacle in the isolation of effects of specific nutrients, observations made on folate intake may reflect a combined effect with vitamin B_6.

The association of micronutrient intake and breast cancer seems to differ by menopausal status and levels of other micronutrients (18, 22). We observed stronger associations of folate and vitamin B_{12} with breast cancer among postmenopausal women, which are consistent with other reports on fruit and vegetable intake, micronutrient intake and circulating levels (18, 21-23). Our most striking result is the confirmation that the protection conferred by high folate intake was modified by vitamin B_{12} intake. A large population-based case-control study in Shanghai first reported a stronger inverse association of folate and breast cancer among women who consumed a high level of vitamin B_{12} (22). Our study also parallels the Chinese study in the levels of micronutrient intake; median intakes of folate, vitamin B_6, and vitamin B_{12} are very close to mean intakes reported in that study, whereas they substantially differ from those reported in American populations (5, 25). Hence, a similar variability of exposure in these two populations may explain our ability to detect these associations. Given the strength of the association observed for vitamin B_{12} in our population, and the paucity of information on this micronutrient and breast cancer risk; these results should be taken with caution.

In the fourth quartile of vitamin B_{12} intake, an increase in folate intake (from third to fourth quartiles) did not seem to further lower the risk of breast cancer substantially. This suggests the possibility of a threshold effect. Folate absorption in the jejunum, cellular uptake, as well as the metabolic pathway itself are saturable systems such that micronutrient beyond a certain level of intake may have little added effect.

Substantial experimental data have implicated folate in carcinogenesis and several mechanisms have been suggested (7). Folate is a precursor of *S*-adenosylmethionine, a methyl donor used both for common methylation reactions and for *de novo* synthesis of thymidilate. This substrate is necessary for both DNA replication and repair. In addition, DNA methylation is thought to play a role in the regulation of gene expression and gene integrity (33-35). DNA demethylation may seem to be affected by folate status: thymidilate is synthesized from deoxyuridilate and requires 5,10-methylene tetrahydrofolate as a coenzyme. Folate depletion induces an imbalance in the availability of nucleotides, uracil misincorporation into DNA, and subsequent abnormal replication and strand breaks (37, 38). Vitamin B_{12} participates in folate metabolism as a cofactor in the methyl transfer of methyltetrahydrofolate to homocysteine to form methionine. Similar to what is observed with folate depletion, low levels of vitamin B_{12} reduce methyl group exchange, thus affecting DNA methylation. Synthesis and repair are also affected because folate is ''trapped'' as methyltetrahydrofolate, preventing tetrahydrofolate from participating in thymidilate synthesis (39).

Bias and confounding must be considered as possible explanations for the observed results. The documentation of established breast cancer risk factors in this study argues against serious bias. Recall bias is always a concern in case-control studies, but lack of awareness among women in this population of possible links between micronutrient intake and the risk for breast cancer should minimize this problem. Recall of the diet before the disease onset could be biased toward current dietary intake, which may change due to the disease (40, 41). We aimed to limit this bias by recruiting incident cases before they knew their diagnosis and at an early state of their disease, thus reducing the likelihood of dietary changes resulting from the diagnosis of cancer. Alcohol intake has been associated with breast cancer risk (42), affects serum vitamin B_{12} status, and modifies the association of folate with breast cancer risk (5, 19, 24, 43). However, in our study population, alcohol intake was very low, only three individuals consumed >15 g/d; therefore, we could not fully explore its effect on the risk of breast cancer. Oral contraceptive use and hormone replacement therapy were also very low in our population (<3%) and similar among cases and controls. Most (95%) of the women in our study were involved only in housekeeping as physical activity. These variables are therefore unlikely to bias our results.

The strengths of this study include a range of micronutrient intake different from most Western populations. Although the traditional diet of Mexico is likely to provide adequate folate because of regular consumption of beans, an excellent source of this vitamin (44), these traditions are being lost rapidly in urban populations. At the same time, consumption of animal products in Mexico is low so that vitamin B_{12} intake may be marginal. In addition, food fortification with folate, zinc, iron, vitamins B_1, B_2, and B_{12} started in 1997 in Mexico, well after data collection, and Mexican women rarely consume vitamin supplements. This facilitates micronutrient intake assessment and limits misclassification of exposure. In a validation study of our questionnaire, conducted in a population similar to that of this study, we observed correlations between the intakes of folate estimated by the food frequency questionnaires and 24-hour recalls ($r = 0.22$), and vitamin B_6 ($r = 0.31$) and vitamin B_{12} ($r = 0.29$; ref. 10). Even though measurement error remains, it is likely to be nondifferential attenuating the observed associations. A source of bias that is difficult to exclude is that the cases may not be fully representative of the population from which they are derived. The minimal effect on the ORs due to control for socioeconomic status provides some reassurance that selection bias is not substantial. However, these results would require confirmation in a prospective study in Mexican women.

Vitamin deficiencies are common in the Mexican population even with national fortification programs in place (3). Abandonment of traditional foods, increasing intake of processed foods, and low intake of fruits and vegetables will probably worsen this situation. In addition, BMI has dramatically increased in Mexico so that 54% of women in reproductive age are overweight or obese (BMI $\geq$ 25 kg/m^2), a known risk factor for breast cancer (3). Also, the reproductive pattern that was protective against breast cancer is rapidly changing in Mexico as women are having later first pregnancies, fewer children and shorter lactation periods (2). Furthermore, Mexico has the highest reported prevalence of homozygous TT genotype for the 677C $\rightarrow$ T transition in the methylenetetrahydrofolate reductase (*MTHFR*) gene (32%; ref. 45). Among individuals with this genotype, low folate intake has been associated with a more substantial increased risk of breast cancer than those with other genotypes (46). All these factors are expected to increase the incidence of breast cancer in Mexican women. However, vitamin deficiency is a potentially modifiable risk factor which can be addressed by health education and expansion of fortification programs. The relation between vitamin intake and breast cancer among Mexican women deserves further evaluation, in particular, the interaction of vitamin intake and genetic polymorphisms affecting their metabolism.

Acknowledgments

We are indebted to Luisa Sánchez-Zamorano for her support in database management.

References

1. Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. Eur J Cancer 2001;37:s4–66.
2. Lopez-Rios O, Lazcano-Ponce E, Tovar V, Hernandez M. La epidemiología de cáncer de mama en México. ¿Consecuencia de la transición demográfica? Salud Publica Mex 1997;39:259–65.
3. Rivera Dommarco J, Shamah Levy T, Villalpando Hernandez S, Gonzalez de Cossio T, Hernandez Prado B, Sepulveda J. Encuesta Nacional de Nutrición 1999. Estado nutricional de niños y mujeres en México. Cuernavaca Morelos, México; Instituto Nacional de Salud Publica, 2001.
4. DRI Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline. A report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its panel on folate, other B vitamins, and choline and Subcommittee on Upper Reference Levels of Nutrients, Food and Nutrition Board Institute of Medicine. Washington, DC, National Academy Press.
5. Zhang S, Willet WC, Selhub J, et al. Plasma folate, vitamin B_6, vitamin B_{12}, homocysteine and risk of breast cancer. J Natl Cancer Inst 2003;95:373–80.
6. Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. A prospective study on folate, B12, and pyridoxal 5′-phosphate (B6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209–17.
7. Mason JB, Choi SW. Folate and carcinogenesis: developing a unifying hypothesis. Adv Enzyme Regul 2000;40:127–41.
8. Stover PJ. Physiology of folate and vitamin B_{12} in health and disease. Nutr Rev 2004;62:s3–12.
9. Romieu I, Hernandez-Avila M, Lazcano-Ponce E, Weber JP, Dewailly E. Breast cancer, lactation history, and serum organochlorines. Am J Epidemiol 2000;152:363–70.
10. Willett WC. Nutritional Epidemiology. 2nd ed. New York: Oxford University Press; 1998.
11. Hernández-Avila M, Romieu I, Parra S, Hernández-Avila J, Madrigal H, Willett W. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. Salud Publica Mex 1998;40:133–40.
12. Department of Agriculture. Composition of foods: raw, processed, prepared, 1963–1991. Agricultural handbook. Washington (DC): Government Printing Office; 1992.
13. Chavez N, Hernandez M, Roldan J, editors. Valor nutritivo de los alimentos de mayor consumo en México: Comisión Nacional de Alimentación y instituto Nacional de la Nutrición; 1992.
14. Willett W, Stampfer M. Implications of total energy intake for epidemiologic analysis. In: Willett W, editor. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998. p. 273–301.
15. Bronfman M, Guiscafre H, Castro V, et al. Estrategias para mejorar los patrones terapéuticos utilizados en diarrea aguda en unidades de atención medica primaria. II. Medición de la desigualdad: una estrategia metodologica, análisis de las características socioeconómicas de la muestra. Arch Invest Med (Mex) 1988;19:351–60.
16. Romieu I, Lazcano-Ponce E, Sanchez-Zamorano LM, Willett W, Hernandez-Avilad M. Carbohydrates and the risk of breast cancer among Mexican women. Cancer Epidemiol Biomarkers Prev 2004;13:1283–9.
17. Lahmann PH, Hoffmann K, Allen N, et al. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). Int J Cancer 2004;111:762–71.
18. Gaudet MM, Britton JA, Kabat GC, et al. Fruits, vegetables, and micronutrients in relation to breast cancer modified by menopause and hormone receptor status. Cancer Epidemiol Biomarkers Prev 2004;13:1485–94.
19. Sellers TA, Grabrick DM, Vierkant RA, et al. Does folate intake decrease risk of postmenopausal breast cancer among women with a family history. Cancer Causes Control 2004;15:113–20.
20. Sellers TA, Kushi LH, Cerhan JR, et al. Dietary folate intake, alcohol and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology 2001;12:420–8.
21. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632–7.
22. Shrubsole MJ, Jin F, Dai Q, et al. Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Res 2001;61:7136–41.
23. Levi F, Pasche C, Lucchini F, La Vecchia C. Dietary intake of selected micronutrients and breast-cancer risk. Int J Cancer 2001;91:260–3.
24. Rohan TE, Jain MG, Howe GR, Miller AB. Dietary folate consumption and breast cancer risk. J Natl Cancer Inst 2000;92:266–9.
25. Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ, Calle EE. Alcohol, folate, methionine and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. Cancer Epidemiol Biomarkers Prev 2003;12:161–4.
26. Cho E, Spiegelman D, Hunter DJ, et al. Premenopausal intakes of vitamins A C, E, folate, and carotenoids, and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2003;12:713–20.
27. Potischman N, Swanson CA, Coates RJ, et al. Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. Int J Cancer 1999;82:315–21.
28. Thorand B, Kohlmeier L, Simonsen N, Croghan C, Thamm M. Intake of fruits, vegetables, folic acid and related nutrients and risk of breast cancer in postmenopausal women. Public Health Nutr 1998;1:147–56.
29. Ronco A, De Stefani E, Bofetta P, Deneo-Pellegrini H, Mendilaharsu M, Leborgne F. Vegetables, fruits and related nutrients and risk of breast cancer: a case-control study in Uruguay. Nutr Cancer 1999;35:111–9.
30. Freudenheim JL, Marchal JR, Vena JE, et al. Premenopausal breast cancer risk and intake of vegetables, fruits and related nutrients. J Natl Cancer Inst 1996;88:340–8.
31. Graham S, Hellman R, Marshal J, et al. Nutritional epidemiology of postmenopausal breast cancer in Western New York. Am J Epidemiol 1991;134:552–66.
32. Negri E, La Vecchia C, Franceschi S. Dietary folate consumption and breast cancer risk. J Natl Cancer Inst 2000;92:1270–1.
33. Wainfan E, Dizik M, Stender M, Christman JK. Rapid appearance of hypomethylated DNA in livers of rats fed cancer promoting, methyl-deficient diets. Cancer Res 1989;49:4094–7.
34. Cheah MS, Wallace CD, Hoffman RM. Hypomethylation of DNA in human cancer cells: a site-specific change in the c-myc oncogene. J Natl Cancer Inst 1984;73:1057–65.
35. Jacob RA, Gretz DM, Taylor PC, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J Nutr 1998;128:1204–12.
36. Hussein MM, McNulty H, Armstrong N, Johnston PG, Spence RAJ, Barnett Y. Investigation of systemic folate status, impact of alcohol intake and levels of DNA damage in mononuclear cells of breast cancer patients. Br J Cancer 2005;(advanced online publication):1–7.
37. Sancar A. Mechanism of DNA excision repair. Science 1994;266:1954–6.
38. Branda RF, Blickensderfer DB. Folate deficiency increases genetic damage caused by alkylating agents and γ-irradiation in Chinese hamster ovary cells. Cancer Res 1993;53:5401–8.
39. Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by *S*-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 1992;55:131–8.
40. Giovannucci E, Stampfer MJ, Colditz GA, et al. A comparison of prospective and retrospective assessments of diet in the study of breast cancer. Am J Epidemiol 1993;137:502–11.
41. Friedenrich C, Howe G, Miller A. The effect of recall bias on the association of calorie providing nutrients and breast cancer. Epidemiology 1991;2:424–9.
42. Smith-Warner SA, Spiegelman D, Yaun SS, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA 1998;279:535–40.
43. Laufer EM, Hartman TJ, Baer DJ, et al. Effects of moderate alcohol consumption on folate and vitamin B_{12} status in postmenopausal women. Eur J Clin Nutr 2004;58:1518–24.
44. Allen LH. Folate and vitamin B_{12} status in the Americas. Nutr Rev 2004;62:S29–33.
45. Wilcken B, Bamforth F, Li Z, et al. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas world wide. J Med Genet 2003;40:619–25.
46. Shrubsole MJ, Gao YT, Cai Q, et al. MTFHR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 2004;13:190–6.