A Prospective Study of Folate Intake and the Risk of Breast Cancer

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LOW FOLATE INTAKE HAS INCREASED TUMOR OCCURRENCE IN ANIMAL MODELS AND HAS BEEN ASSOCIATED WITH HIGHER RISK OF COLORECTAL CANCER IN EPIDEMIOLOGIC STUDIES, PARTICULARLY IN THE PRESENCE OF ALCOHOL CONSUMPTION. DEFICIENT FOLATE STATUS CAN REDUCE THE AVAILABILITY OF S-ADENOSYLMETHIONINE FOR DNA METHYLATION AND MAY THEREBY INFLUENCE GENE EXPRESSION. DIMINISHED FOLATE STATUS MAY ALSO RESULT IN ABNORMAL DNA SYNTHESIS DUE TO MISINCORPORATION OF URACIL INTO DNA LEADING TO CHROMOSOME BREAKS AND DISRUPTION OF DNA REPAIR. ALCOHOL IS A KNOWN FOLATE ANTAGONIST AND THUS COULD PLUSSIBLY INCREASE THE REQUIREMENT FOR FOLATE INTAKE.

We hypothesized that higher folate intake might reduce risk of breast cancer, particularly among women with greater alcohol consumption, which itself moderately increases breast cancer risk. Because no prospective data are available, we examined these hypotheses in the Nurses’ Health Study, a large cohort study among US women.

METHODS

Study Cohort

In 1976, 121,700 female registered nurses aged 30 to 55 years living in 11 states completed a mailed questionnaire about their medical history and lifestyle. Biennially, a mailed questionnaire has been sent to update information on potential risk factors and to ascertain newly diagnosed diseases. In 1980, a 61-item food frequency questionnaire was included to obtain dietary information. In 1984, the dietary questionnaire was expanded to 126 items. Similar questionnaires were used to update diet in 1986 and 1990. Through May 31, 1996, the follow-up rate was 95% complete as percentage of potential person-years.

A total of 3483 cases of breast cancer were documented. Total folate intake was not associated with overall risk of breast cancer. However, among women who consumed at least 15 g/d of alcohol, the risk of breast cancer was highest among those with low folate intake. For total folate intake of at least 600 µg/d compared with 150 to 299 µg/d, the multivariate relative risk (RR) was 0.55 (95% confidence interval [CI], 0.39-0.76; $P$ for trend = .001). This association was only slightly attenuated after additional adjustment for intake of beta carotene, lutein/zeaxanthin, preformed vitamin A, and total vitamins C and E. The risk of breast cancer associated with alcohol intake was strongest among women with total folate intake of less than 300 µg/d (for alcohol intake ≥15 g/d vs <15 g/d, multivariate RR, 1.32; 95% CI, 1.15-1.50). For women who consumed at least 300 µg/d of total folate, the multivariate RR for intake of at least 15 g/d of alcohol vs less than 15 g/d was 1.05 (95% CI, 0.92-1.20). Current use of multivitamin supplements, the major source of folate, was associated with lower breast cancer risk among women who consumed at least 15 g/d of alcohol (for current users of supplements vs never users, RR, 0.74; 95% CI, 0.59-0.93).

Conclusions

Our findings suggest that the excess risk of breast cancer associated with alcohol consumption may be reduced by adequate folate intake.

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kcal/d), left 10 or more items blank, or had a previous diagnosis of cancer (other than nonmelanoma skin cancer); 88,818 participants remained. Among the women included, 80% also completed the 1984 dietary questionnaire, and 76% completed the 1986 and 1990 dietary questionnaires (TABLE 1).

**Semiquantitative Food Frequency Questionnaires**

The validity and reliability of the food frequency questionnaires used in the Nurses’ Health Study have been described elsewhere.12-14 For each food in the questionnaires, a commonly used unit or portion size (eg, 1 orange) was specified, and women were asked how often on average over the previous year they had consumed that amount of each food. There were 9 responses, ranging from “never” to “6 or more times per day.” Nutrient intake was computed by multiplying the frequency response by the nutrient content of the specified portion sizes. Total alcohol intake was the sum of the alcohol content contributed from beer, wine, and liquor, assuming 12.8 g of ethanol for 360 mL (12 oz) of beer, 11.0 g for 120 mL (4 oz) of wine, and 14.0 g for a shot of liquor (45 mL [1.5 oz], 80 proof as a standard). We also asked questions on duration, brand, and type of multivitamin supplement use, which were updated biennially. A comprehensive database on the folate content of the multivitamin preparations was developed.

In a sample of 173 participants, the correlation coefficient between alcohol intake as assessed by the 1980 dietary questionnaire and by four 1-week food diaries was 0.90.8 In a sample of 188 participants, the correlation coefficients between folate intake calculated from the 1980 dietary questionnaire and erythrocyte folate concentrations in 1987 were 0.55 for total folate (including supplements) and 0.38 for folate from foods only.2 In this sample, the mean (±SEM) erythrocyte folate concentrations for increasing quintiles of total folate intake were 682 ± 34, 773 ± 23, 804 ± 25, 804 ± 25, and 920 ± 48 nmol/L.2 All were within the normal range (>340 nmol/L).

**Ascertainment of Breast Cancer Cases**

Incident cases of invasive breast cancer were identified by self-report on each biennial questionnaire from 1982 to 1996. Deaths in the cohort were identified by reports from family members, the postal service, and a search of the National Death Index15; we estimated that 98% of all deaths were identified. Women who reported breast cancer (or their next of kin if they had died) were asked for permission to obtain hospital records and pathology reports. We excluded noninvasive breast cancer cases in this analysis, but we included 217 invasive breast cancer cases for which no medical records could be obtained because the accuracy of self-reporting was extremely high among those for whom we were able to obtain medical records (>99%). Physicians without knowledge of di-

### Table 1. Age-Standardized Baseline Characteristics by Total Folate Intake in 1980 in the Nurses’ Health Study*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>&lt;150 (n = 7072)</th>
<th>150-299 (n = 42,785)</th>
<th>300-449 (n = 16,089)</th>
<th>450-599 (n = 8,126)</th>
<th>≥600 (n = 14,746)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45</td>
<td>46</td>
<td>47</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>Total folate, µg/d†</td>
<td>124</td>
<td>223</td>
<td>357</td>
<td>527</td>
<td>814</td>
</tr>
<tr>
<td>Dietary folate, µg/d†</td>
<td>126</td>
<td>223</td>
<td>329</td>
<td>290</td>
<td>326</td>
</tr>
<tr>
<td>Multivitamin supplement use, %</td>
<td>7</td>
<td>11</td>
<td>28</td>
<td>79</td>
<td>95</td>
</tr>
<tr>
<td>Alcohol consumption ≥15 g/d, %</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Parity (births)</td>
<td>2.9</td>
<td>3.0</td>
<td>2.9</td>
<td>2.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Age at first birth, y</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Age at menarche, y</td>
<td>12.6</td>
<td>12.5</td>
<td>12.5</td>
<td>12.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Postmenopausal, %</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Mother or sister with breast cancer, %</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>History of benign breast disease, %</td>
<td>23</td>
<td>24</td>
<td>25</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163</td>
<td>164</td>
<td>164</td>
<td>164</td>
<td>164</td>
</tr>
<tr>
<td>Weight change since age 18 y, kg</td>
<td>8.5</td>
<td>8.6</td>
<td>8.0</td>
<td>7.9</td>
<td>7.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.4</td>
<td>24.6</td>
<td>24.4</td>
<td>24.1</td>
<td>24.1</td>
</tr>
<tr>
<td>Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy, kJ/d (kcal/d)</td>
<td>6170 (1469)</td>
<td>6577 (1566)</td>
<td>6791 (1617)</td>
<td>7409 (1764)</td>
<td>6094 (1451)</td>
</tr>
<tr>
<td>Beta carotene, µg/d†</td>
<td>1969</td>
<td>3705</td>
<td>6054</td>
<td>5131</td>
<td>5530</td>
</tr>
<tr>
<td>Lutein/zeazanthin, µg/d†</td>
<td>1714</td>
<td>3992</td>
<td>7473</td>
<td>6209</td>
<td>6648</td>
</tr>
<tr>
<td>Preformed vitamin A (including supplements), IU/d†</td>
<td>2212</td>
<td>3099</td>
<td>4385</td>
<td>6972</td>
<td>11,095</td>
</tr>
<tr>
<td>Total vitamin C (including supplements), mg/d†</td>
<td>137</td>
<td>217</td>
<td>312</td>
<td>391</td>
<td>586</td>
</tr>
<tr>
<td>Total vitamin E (including supplements), IU/d†</td>
<td>33</td>
<td>39</td>
<td>56</td>
<td>92</td>
<td>171</td>
</tr>
</tbody>
</table>

*All factors except age are directly standardized. Values presented are means unless indicated otherwise. †Nutrients are adjusted for total energy intake.
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etary information of each study participant reviewed the records. During 16 years of follow-up, 3483 breast cancer cases were documented (827 premenopausal cases, 2345 postmenopausal cases, and 311 cases with uncertain menopausal status).

Statistical Analysis

Person-years for each participant were calculated from the date of returning the 1980 questionnaire to the date of diagnosis of breast cancer, death, or May 31, 1996, whichever came first. Nutrient intakes were adjusted for total energy by the residual method. We also classified women according to their duration of multivitamin use. Because the US Food and Drug Administration regulations limited the maximum folate content in supplements to 100 µg before 1973, we considered 1973 as the starting point for calculation of multivitamin use relevant to folate intake.

We calculated incidence rate by dividing the number of cases by person-years of follow-up in each category. The relative risk (RR) was calculated as the rate in a specific category of total folate divided by that in the reference category (150-299 µg/d), with adjustment for 5-year age categories by the Mantel-Haenszel method. In multivariate analysis using pooled logistic regression with 2-year time increments, we simultaneously adjusted for 5-year age categories and other covariates listed in the footnotes to Table 2. We did not adjust for use of oral contraceptives, lactation, or smoking status, as they were not associated with breast cancer risk in multivariate analyses.

The incidence of breast cancer was related to the cumulative average of dietary intake from all available dietary questionnaires, using methods for repeated measures described elsewhere. Nondietary covariates were updated biennially. Analyses were further stratified by menopausal status, alcohol intake (<15 g/d, ≥15 g/d, about 1 drink), and methionine intake (quintiles). For all RRs, we calculated 95% confidence intervals (CIs). All P values were 2-tailed. The test for trend was conducted using the median value of the cumulative average nutrient intake for each quintile analyzed as a continuous variable. Log likelihood ratio tests were used to compare models with or without interaction terms between total folate intake and alcohol consumption.

RESULTS

In the 1980 baseline population, the first 3 categories of total folate intake mainly represented folate from food sources (cold breakfast cereal, orange juice, and leafy vegetables were the major sources);
79% of women in the fourth category and 95% of women in the fifth category were multivitamin supplement users (Table 1). Women who consumed more folate were more likely to have greater intakes of beta carotene; lutein/zeaxanthin; preformed vitamin A, including supplements; and total vitamins C and E, including supplements. We did not observe any important differences in established breast cancer risk factors at baseline across the 5 categories of total folate intake.

Total folate intake was not associated with risk of breast cancer among the whole cohort (Table 2) or among premenopausal women (multivariate RRs for increasing categories of total folate = 1.23, 1.00 [referent], 0.99, 1.15, and 0.99 [95% CI, 0.79-1.23]; P for trend = .98). Total folate intake was weakly inversely associated with risk of postmenopausal breast cancer; the comparable multivariate RRs were 1.10, 1.00 [referent], 0.93, 0.94, and 0.86 (95% CI, 0.76-0.98, P for trend = .02). Folate intake derived from foods alone was not significantly related to overall risk of breast cancer (multivariate RRs = 1.10, 1.00 [referent], 0.96, 1.10, and 1.22 [95% CI, 0.81-1.83]; P for trend = .82).

Higher intake of alcohol increased breast cancer risk; the multivariate RRs were 1.07 (95% CI, 0.98-1.17) for women consuming 0.1 to 4.9 g/d, 1.11 (95% CI, 1.00-1.22) for women consuming 5 to 14.9 g/d, and 1.24 (95% CI, 1.11-1.39) for women consuming at least 15 g/d compared with nondrinkers. Because a benefit of folate may be particularly important among women regularly consuming alcohol, we further examined the association between total folate intake and breast cancer risk by level of alcohol consumption (Table 2). Among women consuming at least 15 g/d of alcohol, for total folate intake of at least 600 µg/d compared with 150 to 299 µg/d, the multivariate RR was 0.55 (95% CI, 0.39-0.76, P for trend = .001) (Table 2). The RR was essentially the same after further adjustment for beta carotene, including supplements (RR = 0.56; 95% CI, 0.41-0.79) (Table 2), and did not appreciably change after controlling one at a time for lutein/zeaxanthin (RR = 0.57; 95% CI, 0.41-0.79); preformed vitamin A, including supplements (RR = 0.62; 95% CI, 0.48-0.80).

Table 3. Relative Risk (RR) and 95% Confidence Interval (CI) of Breast Cancer According to the Categories for Cumulative Average Intake of Total Folate and Dietary Methionine Intake in a Cohort of 88,818 Women From 1980 to 1996

<table>
<thead>
<tr>
<th>Methionine</th>
<th>Folate Intake, µg/d</th>
<th>&lt;150</th>
<th>150-299</th>
<th>300-449</th>
<th>450-599</th>
<th>≥600</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quintile 1 (≤1.50 g/d)</td>
<td>Cases, No.</td>
<td>49</td>
<td>319</td>
<td>136</td>
<td>82</td>
<td>61</td>
<td>.03</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)*</td>
<td>1.26 (0.93-1.72)</td>
<td>1.00</td>
<td>0.81 (0.66-0.99)</td>
<td>0.95 (0.74-1.21)</td>
<td>0.79 (0.60-1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quintile 2 (1.51-1.69 g/d)</td>
<td>Cases, No.</td>
<td>21</td>
<td>350</td>
<td>192</td>
<td>89</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Multivariate RR (95% CI)*</td>
<td>0.85 (0.54-1.32)</td>
<td>1.00</td>
<td>0.86 (0.72-1.03)</td>
<td>0.81 (0.64-1.03)</td>
<td>0.74 (0.57-0.96)</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Quintile 3 (1.70-1.85 g/d)</td>
<td>Cases, No.</td>
<td>14</td>
<td>307</td>
<td>193</td>
<td>109</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Multivariate RR (95% CI)*</td>
<td>0.76 (0.44-1.31)</td>
<td>1.00</td>
<td>0.87 (0.72-1.04)</td>
<td>0.96 (0.77-1.20)</td>
<td>0.95 (0.76-1.20)</td>
<td>.88</td>
<td></td>
</tr>
<tr>
<td>Quintile 4 (1.86-2.07 g/d)</td>
<td>Cases, No.</td>
<td>17</td>
<td>217</td>
<td>191</td>
<td>132</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Multivariate RR (95% CI)*</td>
<td>1.54 (0.93-2.56)</td>
<td>1.00</td>
<td>1.03 (0.85-1.26)</td>
<td>1.29 (1.03-1.61)</td>
<td>1.11 (0.87-1.41)</td>
<td>.23</td>
<td></td>
</tr>
<tr>
<td>Quintile 5 (≥2.08 g/d)</td>
<td>Cases, No.</td>
<td>11</td>
<td>218</td>
<td>241</td>
<td>123</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Multivariate RR (95% CI)*</td>
<td>0.96 (0.52-1.77)</td>
<td>1.00</td>
<td>1.03 (0.85-1.24)</td>
<td>0.96 (0.77-1.21)</td>
<td>0.96 (0.78-1.19)</td>
<td>.63</td>
<td></td>
</tr>
</tbody>
</table>

*The model included the same indicator variables as in Table 2.
We observed no association between total folate intake and breast cancer risk among women who consumed less than 15 g/d of alcohol. The test for interaction between total folate and alcohol consumption and breast cancer risk was statistically significant (P = .02). When total folate and alcohol intakes were examined in combination among the whole cohort, higher total folate intake appeared to mitigate the excess risk of breast cancer because of higher consumption of alcohol (FIGURE). The risk of breast cancer associated with alcohol intake was strongest among women with total folate intake less than 300 µg/d (for alcohol ≥15 g/d vs <15 g/d, multivariate RR = 1.32; 95% CI, 1.15-1.50). For women consuming at least 300 µg/d of total folate, the multivariate RR for at least 15 g/d of alcohol vs less than 15 g/d was 1.05 (95% CI, 0.92-1.20).

Because intake of methionine, mainly from meats, may influence the availability of methyl groups and thus affect the requirement for folate intake, 1,3 we also evaluated the association between total folate intake and breast cancer risk by methionine intake (TABLE 3). We observed a 21% to 26% lower risk of breast cancer with intake of total folate of at least 600 µg/d compared with intake of 150 to 299 µg/d among women in the lowest 2 quintiles of methionine intake. Among women who consumed at least 15 g/d of alcohol and were in the lowest 2 quintiles of methionine intake, the multivariate RRs for increasing categories of total folate were 1.01, 1.00 (referred), 0.85, 0.93, and 0.45 (95% CI, 0.27-0.74).

Because multivitamin supplements were the major source of folate intake, especially among women with intake of at least 450 µg/d of total folate, we examined multivitamin use in relation to risk of breast cancer according to alcohol consumption (TABLE 4). Among women who consumed at least 15 g/d of alcohol, the multivariate RRs were 0.77 (95% CI, 0.57-1.03) for past multivitamin users and 0.74 (95% CI, 0.59-0.93) for current multivitamin users compared with never users. These associations existed among both premenopausal (for current users, RR = 0.60; 95% CI, 0.37-0.98) and postmenopausal (for current users, RR = 0.76; 95% CI, 0.58-1.01) women. We observed no association with multivitamin use among women who consumed less than 15 g/d of alcohol.

To evaluate possible distortion of the findings by differences in mammography rates among women with higher intake of total folate, we compared the cumulative average intake of total folate of women who had mammography during 1993 or 1994 with the intake of those who had not. The mean of cumulative average total folate intake (385 µg/d) of women who had mammography was only slightly higher than that of women who had not (369 µg/d).

**COMMENT**

In this large prospective cohort, total folate intake was not associated with overall risk of breast cancer. However, higher total folate intake or multivitamin use was associated with a lower risk of breast cancer among women who regularly consumed alcohol.

Epidemiological data relating folate intake to breast cancer risk are limited. In 1 case-control study with 297 cases and 311 controls, premenopausal women consuming at least 460 µg/d of dietary...
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Folates had a statistically significant 50% lower risk compared with women who consumed 304 µg/d or less. In another case-control study with 439 breast cancer cases and 494 controls, a statistically nonsignificant 30% lower risk was found among postmenopausal women consuming at least 451 µg/d of folate compared with women who consumed 289 µg/d or less.

A direct association between moderate alcohol consumption and breast cancer incidence has been consistently observed in epidemiological studies across study designs, including in this cohort. In a pooled analysis from 6 prospective cohorts, an increment of 10 g/d of alcohol was associated with a 9% increase in breast cancer risk. Our data suggest that alcohol consumption modified the association of folate intake with breast cancer risk, similar to the interaction between folate and alcohol observed for colon cancer and coronary heart disease.

Alcohol interferes with several aspects of normal folate transport and metabolism including dietary intake, intestinal absorption, transport to tissues, storage, and release by liver, thereby disrupting folate supply to tissues.

The prospective design and high follow-up rates in this study reduce the concern that methodological biases explain these findings. Although the folate intake calculated from our dietary questionnaire reasonably reflects long-term intake of study subjects, some misclassification still exists, which would tend to weaken any true associations. Residual confounding by nondietary factors is unlikely to explain our findings because controlling for recognized risk factors of breast cancer had minimal effect on the RR. Our findings for folate were also independent of beta carotene and vitamins A, C, and E. Although we cannot completely exclude the possibility that other constituents of multivitamins contribute to lower breast cancer among regular alcohol consumers, our findings strongly suggest that folate is the primary factor.

Folate intake above the amounts consumed by many persons appears necessary for maximal reduction of neural tube defects, cardiovascular disease, and colorectal cancer as well as breast cancer in this study. In recognition of these relationships, the Recommended Dietary Allowance (RDA) for folate was recently increased from 180 µg/d to 400 µg/d. The basis for the previous RDA was to protect against development of clinically folate deficiency. The Food and Drug Administration now requires the fortification of grain products with folic acid, which is estimated to increase average folate intake by about 100 µg/d.

Our findings suggest that this may have an unplanned positive effect on breast cancer incidence.

In summary, our findings suggest that the excess risk of breast cancer associated with alcohol consumption may be reduced by ensuring adequate folate intake. Because of the potential practical importance of these findings, the relation of folate intake to risk of breast cancer among women who consume alcohol needs to be examined in other studies.

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REFERENCES


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