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High-Folate Diets and Breast Cancer Survival in a Prospective Cohort Study

Thomas A. Sellers, Steven R. Alberts, Robert A. Vierkant, Dawn M. Grabrick, James R. Cerhan, Celine M. Vachon, Janet E. Olson, Lawrence H. Kushi, and John D. Potter

Abstract: *Recent evidence suggests that adequate dietary folate may attenuate the risk of breast cancer associated with intake of alcohol. However, patients with breast cancer have been commonly treated with antifolate chemotherapies. The present analysis was performed to test the hypothesis that high folate intake may diminish the effectiveness of chemotherapy and, therefore, adversely influence survival. Women at risk of postmenopausal breast cancer (*n = *37,105) participated in the Iowa Women's Health Study. Total folate intake (diet + supplements) was estimated from a food frequency questionnaire administered at baseline in 1986 and categorized into tertiles. From all incident breast cancer cases ascertained in the cohort, we selected those with a diagnosis between 1986 and 1994, chemotherapy as first course of treatment, and adequate diet assessment. Mortality was determined through the State Health Registry of Iowa and the National Death Index. Cox regression was used to estimate survival while adjusting for important covariates. Through 14 yr of follow-up, 80 deaths occurred among the 177 breast cancer cases treated with chemotherapy. Among these patients, high folate intake was not associated with worse survival. After adjustment for age, extent of disease, total calories, alcohol, and estrogen receptor status, women with total folate intake in the highest tertile had a mortality risk ratio of 0.88 (95% confidence interval = 0.44–1.76) compared with cases in the lowest tertile of folate. These findings, although preliminary, afford some reassurance that folate supplementation is unlikely to have a significant adverse effect on survival after chemotherapy for breast cancer.*

Introduction

Several recent studies have generated considerable interest in dietary folate as a nutrient to decrease risk of breast cancer. Data from the Nurses Health Study suggested that high intakes of folate attenuated the increased risk of breast cancer associated with intake of alcohol (1). This observation was quickly replicated with analyses of the Canadian National Breast Cancer Screening Trial (2), a large case-control study from Italy (3), and the Iowa Women's Health Study (IWHS) (4). Although the exact mechanism remains unknown, there are several biologically plausible mechanisms, including alterations in DNA methylation, maintenance of DNA integrity, and DNA repair (5).

Because folate is a water-soluble vitamin, there should be minimal safety concerns about supplementation. The Food and Drug Administration has proposed that the diets of all Americans be fortified with 140 μg of folic acid per 100 g of cereal-grain product (6) as a means to reduce the incidence of neural tube birth defects. Moreover, cancer patients often take megadoses of vitamins in the belief it may improve their ability to fight the disease (7). A possible consequence of folate supplementation that merits careful examination is the influence on the efficacy and toxicity of cancer chemotherapy. In particular, drugs that inhibit folic acid function and metabolism have been a mainstay of cancer chemotherapy for the last four decades (8). Given the prior success of drugs such as methotrexate, a new generation of antifolate chemotherapy agents is being developed, including drugs such as LY-231514 (MTA, Alimta).

The rationale for the use of antifolates is based on the observation that mammalian cells are unable to produce folate, and tumor cells have greater folate requirements than normal cells. Depriving tumor cells of folate leads to a deficiency of bases for DNA synthesis (particularly thymidine) and consequent incorporation of uracil and the disintegration of DNA strands through induction of double-strand breaks. Although a number of antifolate drugs have been developed, each functions through inhibition of one or more of the three key enzymes in folate metabolism. Thus it is conceivable that folate supplementation would have negative consequences on therapeutic efficacy of antifolates. This report seeks to provide observational data on the topic through analysis of the association between folate intake and breast cancer survival in a prospective cohort study of postmenopausal women.

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Methods

Detailed methods of the IWHS have been published elsewhere (9). Briefly, in January 1986, a 16-page questionnaire was mailed to 98,029 women aged 55–69 yr randomly selected from the state of Iowa drivers' license list. The 41,836 respondents (42.7% response rate) form the cohort under study. Rates of breast cancer among responders and nonresponders were similar (10).

The questionnaire solicited information on factors known or suspected to be relevant to breast cancer risk, including body mass index, reproductive history, family history, and alcohol use. It also included a 127-item semiquantitative food frequency questionnaire that was virtually identical to that used in the 1984 dietary assessment in the Nurses Health Study (11). The questionnaire also assessed regular use of multivitamins and supplements containing only individual B vitamins. Because there are numerous multivitamin preparations, we asked respondents to provide the brand name of the multivitamin and the frequency of intake. These brands were individually coded for estimation of the intake of vitamins from supplements. No information was obtained on the duration of vitamin supplement use.

Follow-up questionnaires were mailed in 1987, 1989, 1992, and 1997 to establish vital status and change of address. Cancer incidence was ascertained through the Iowa Cancer Registry, a part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program (12). Field representatives routinely visited hospitals and clinics in and around Iowa. For cancer patients who were Iowa residents at the time of diagnosis, information including personal identifiers, tumor size and grade, estrogen and progesterone receptor status, first course of therapy, and extent of disease was abstracted according to SEER protocols. Annually, we matched by computer a list of cohort members and the records of Iowans with incident cancer in the Cancer Registry using combinations of first, last, and maiden name, ZIP code, birth date, and Social Security number. Deaths were ascertained from the Iowa Cancer Registry or the National Death Index, and the underlying cause of death was obtained.

Before analysis, the following exclusions were applied: premenopausal at baseline (n = 569), partial or total mastectomy (n = 1,870), or any cancer other than skin cancer at baseline (n = 2,293). From this at-risk cohort of 37,105 women, we also excluded women who left ≥30 items blank on the food frequency questionnaire (n = 2,432) or whose responses resulted in extreme energy intake (<600 or >5,000 kcal/day, n = 280). All analyses focus on first incident primary breast cancers within this cohort of 34,393 women. Because the use of antifolate therapy (primarily methotrexate) for the treatment of breast cancer in the United States has decreased since 1995 (13), we considered only breast cancer cases diagnosed between 1986 and 1994. Analyses were limited to breast cancer cases who received chemotherapy (single or multiple agent) as part of their first course of treatment. Finally, we excluded women if their date of cancer death was within 30 days of diagnosis.

We calculated the length of follow-up for each woman as the time from diagnosis of breast cancer until date of death or 31 December 1999. Survival curves were generated using the Kaplan–Meier method (14). Comparisons of cancer survival across levels of folate in 1986 were made using log-rank tests (15). Analyses considered dietary folate alone and total folate intake, including dietary and supplement-derived folate. Intake was categorized into tertiles on the basis of the approximate distribution of the entire IWHS cohort. To further examine the association of folate consumption with survival, we calculated relative risks and 95% confidence intervals using Cox proportional hazards regression (16). Two sets of regression models were fit to the data: one unadjusted and one accounting for potential confounding variables. The following variables are factors known or believed to be associated with cancer mortality and were evaluated as potential confounders: year of cancer diagnosis, age at cancer diagnosis, estrogen and progesterone receptor status, and extent of disease. Body mass index, caloric intake, and alcohol consumption were also considered potential confounders because of their known confounding or modifying effects on the association between folate and breast cancer incidence. The relatively small number of eligible subjects did not allow us to account simultaneously for the effects of all variables described above. Thus a subset of variables was included in the final Cox regression model on the basis of whether their inclusion in the model altered the risk ratios of any of the folate variables by >10%.

Primary analyses included all eligible subjects, but secondary analyses were also run that included only subjects with regional tumor stage. All statistical tests are two sided, and all analyses were carried out using the SAS (SAS Institute, Cary, NC) and Splus (Mathsoft, Seattle, WA) software systems.

Results

Through 1994, 1,158 women in the cohort at risk developed primary breast cancer. Of these, we excluded 137 women with in situ tumors and 3 who died within 30 days of diagnosis. Thus the total number of incident breast cancers eligible for analysis was 1,018. Because the primary hypothesis to be tested was that dietary folate might influence response to chemotherapy, we further selected those women for whom SEER data indicated use of any chemotherapy during the first course of treatment. This resulted in the selection of 177 cases: 49 with localized disease, 110 with regional disease, and 18 with distant metastases. Through 1999, a total of 80 of these 177 women had died. Seventy-two of these deaths (90%) were a result of cancer. Most of these cancer deaths (n = 67, 93.1%) were from breast cancer; the remainder were from uterine and brain cancer and not specified, and two deaths from leukemia. The eight noncancer deaths were attributed to diabetes mellitus, acute myocardial infarction, coronary atherosclerosis, pulmonary embolism and infarct,

primary pulmonary hypertension, chronic airway obstruction, crushing injury, and unspecified septicemia.

There were no differences between living and deceased cases with regard to mean age at diagnosis, hormone receptor status, baseline caloric intake in 1986, use of alcohol, or body mass index (Table 1). Deceased subjects tended to have greater extent of disease and to be diagnosed with the primary cancer in the earliest years of follow-up. However, deceased cases tended to contribute fewer person-years of observation than cases alive at the censoring date. There were no striking differences between the two groups in the consumption of folate at baseline in 1986, whether or not supplements were included.

If high folate intake limits the efficacy of antifolate chemotherapy, women with the highest intakes would be expected to have the highest total mortality. Table 2 presents mortality rate ratios by tertile of folate intake. We considered separately folate intakes from diet alone or diet plus supplements. There was no strong evidence that women in the highest tertile of intake had higher mortality than breast cancer patients in the lowest tertile, with multivariate-adjusted rate ratios of 0.88 and 0.85 for folate including and not including supplements, respectively. Figure 1 also provides very little evidence that folate intake is associated with cancer survival (P = 0.72, log-rank test). Given the strong association of stage with survival, additional analyses were performed among the 110 women with regional disease. The results were not materially changed (Fig. 2). The multivariate results, including adjustment for alcohol intake, differed little from the age-adjusted results.

To address the possible concern that diet at baseline in 1986 does not adequately reflect diet at the time of breast cancer diagnosis, we performed additional analyses in which ascertainment of breast cancer cases was further limited to 1986–1992. These results, which were based on only 73 deaths, were similar (data not shown). We also performed analyses that considered only deaths from cancer. These results were also similar to those presented here for total mortality (data not shown).

Table 1. Comparison of All-Cause Mortality With Demographic and Clinical Variables[a]

	Living Subjects (n = 97)	Deceased Subjects (n = 80)
Year of cancer diagnosis		
1986–1988	31 (32)	39 (49)
1989–1991	29 (30)	26 (32)
1992–1994	37 (38)	15 (19)
Mean age at cancer diagnosis,[b] yr	65.7 ± 4.2	64.8 ± 4.4
Mean years of follow-up[b]	9.4 ± 2.6	4.3 ± 3.1
Estrogen receptor status		
Negative	61 (63)	44 (55)
Positive	23 (24)	23 (29)
Missing	13 (13)	13 (16)
Progesterone receptor status		
Negative	47 (48)	38 (48)
Positive	36 (37)	26 (32)
Missing	14 (14)	16 (20)
Extent of disease		
Local	42 (43)	7 (9)
Regional	54 (56)	56 (70)
Distant	1 (1)	17 (21)
Folate consumption (diet + supplements), μg		
≤280	36 (37)	28 (35)
281–460	31 (32)	29 (36)
>460	30 (31)	23 (29)
Folate consumption (diet only), μg		
≤250	33 (34)	27 (34)
251–340	30 (31)	26 (32)
>340	34 (35)	27 (34)
Daily alcohol consumption, g		
0	49 (51)	51 (64)
≤4	31 (32)	15 (19)
>4	17 (18)	14 (17)
Baseline caloric intake		
≤1,500	30 (31)	22 (27)
1,501–2,000	34 (35)	27 (34)
>2,000	33 (34)	31 (39)
Body mass index		
≤24.30	29 (30)	18 (23)
24.31–28.30	32 (33)	26 (32)
>28.30	36 (37)	36 (45)

a: Values are numbers of subjects, with percentages in parentheses, unless otherwise noted.

b: Values are means ± SD.

Discussion

Folate supplementation is beneficial for the population as a means to reduce neural tube birth defects and possibly attenuate the risk of breast cancer associated with alcohol use. Because cancer patients have been reported to take supplemental doses of vitamins (7), it is important to evaluate potential negative consequences of folate supplementation on response to chemotherapy drugs, the primary action of which is to deplete folate. To our knowledge, this is the first study to examine this issue in humans. The results provide no evidence that survival is worse among breast cancer patients in the highest tertile of folate intake (460 μg/day) than in patients in the lowest tertile (≤280 μg/day).

Methotrexate is the classical antifolate drug that has been used against a variety of malignancies. It works by targeting dihydrofolate reductase, an enzyme that is involved in pyrimidine synthesis. The combination of cytoxan, methotrexate, and 5-fluorouracil (CMF) was the standard of care for breast cancer patients with node-positive disease for many years. Because the regimen requires administration every 2 wk for 6 mo, its use was gradually replaced in the 1980s by other drugs, such as adriamycin (AC), that can be given on a less-frequent basis and over a shorter period of time. However, CMF is still used for treatment of metastatic disease (17) as well as first-line therapy of local disease in selected situations. Because some patients do not respond to CMF or develop resistance, other antifolates have been developed that target the key enzymes involved in folate metabolism [thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransase (GARFT)]. For example, 5-fluorouracil tar-

Table 2. Association of Folate Consumption With All-Cause Mortality[a]

	Cancer Deaths	Person Years	Univariate RR (95% CI)	Multivariate RR (95% CI)[b]
All eligible breast cancer cases				
Folate consumptio, (diet plus supplements), µg				
≤280	28	466	1.00 (ref)	1.00 (ref)
281–460	29	406	1.21 (0.72–2.04)	1.34 (0.67–2.67)
>460	23	380	1.01 (0.58–1.75)	0.88 (0.44–1.76)
Folate consumption (diet only), µg				
≤250	27	433	1.00 (ref)	1.00 (ref)
251–340	26	394	1.07 (0.63–1.84)	0.92 (0.42–1.99)
>340	27	425	1.03 (0.60–1.76)	0.85 (0.38–1.91)
Subset to cases with regional disease				
Folate consumption (diet plus supplements), µg				
≤280	22	289	1.00 (ref)	1.00 (ref)
281–460	22	262	1.12 (0.62–2.03)	1.19 (0.57–2.50)
>460	12	249	0.63 (0.31–1.27)	0.64 (0.29–1.44)
Folate consumption (diet only), µg				
≤250	19	277	1.00 (ref)	1.00 (ref)
251–340	18	240	1.09 (0.57–2.08)	0.92 (0.35–2.42)
>340	19	284	0.99 (0.53–1.88)	1.01 (0.38–2.71)

a: Abbreviations are as follows: RR, relative risk; CI, confidence interval.

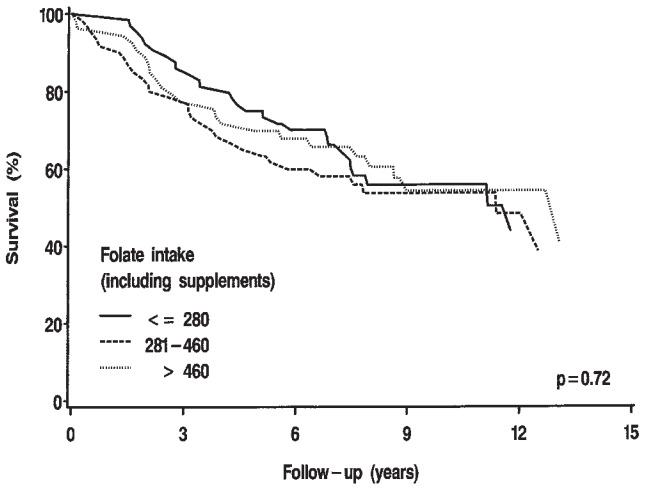

Figure 1. Kaplan–Meier survival estimates by folate intake among all breast cancer cases treated with chemotherapy.

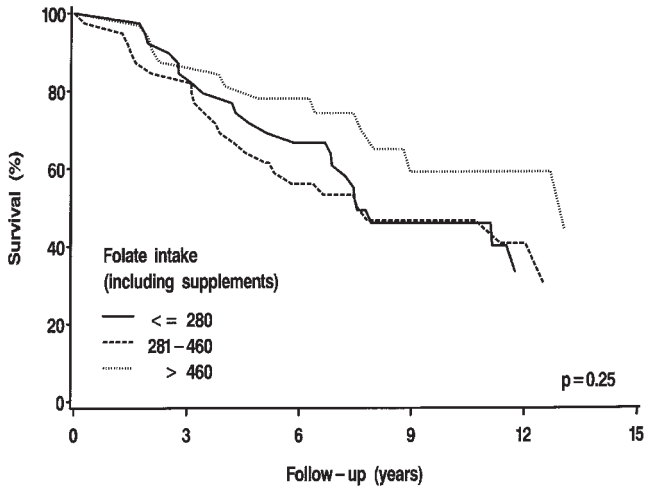

Figure 2. Kaplan–Meier survival estimates by folate intake among regional disease breast cancer cases treated with chemotherapy.

gets TS (18) and lometrexol targets GARFT (19). The new-generation antifolate agent LY-231514 is designed to inhibit TS, DHFR, and GARFT (20). Clinical trials are evaluating the efficacy of LY-231514 alone or in combination with chemotherapy in patients with breast cancer. Thus there is a critical need for data on the significance of folate intakes on breast cancer survival.

Phase I studies of lometrexol revealed far greater toxicities than would have been predicted (21). Similar observations have been made with LY-231514. These observations led to a series of investigations designed to explore whether supplementation with exogenous folate can minimize unwanted effects without minimizing drug efficacy. In the mouse, low folate stores lead to a prolonged effect of the drug (22). Addition of folate enhances the therapeutic index up to a certain range, after which a negative impact on the antitumor effect of the drug is observed (23). In the rat model, nutritional folate status is associated with the efficacy and toxicity of chemotherapy, with some variation noted by type of agent used (24). The potential adverse effect on tumor response of folate supplementation in patients receiving antifolate therapy has not been well defined. However, several on-going studies are using folate supplementation to decrease the toxicities associated with these agents.

Several limitations must be considered in the interpretation of our results. One pertains to the reliance on data from the SEER registry to identify the subset of patients who received chemotherapy. The available data were limited to first

course of therapy. Thus it is conceivable that some women who were excluded from the survival analysis were given chemotherapy at a later time. However, our approach was unlikely to result in the inclusion of women who did not receive chemotherapy and is unlikely to be differential with regard to folate intake. Another limitation of the SEER data is that the type of chemotherapeutic agent used was not recorded. This precluded the ability to distinguish which patients received CMF and which patients received AC. Our decision to include only breast cancer patients diagnosed before 1995 was designed partially to address this issue. The effect of misclassification of chemotherapy exposure on the results is unclear, although it is reasonable to presume that prescription of drug by the oncologist was independent of the patients' folate intake, which was almost certainly unknown. It was of some concern that only 17.4% (177 of 1,018) of breast cancer patients were reported to have received chemotherapy. This appears to primarily reflect the fact that most of the breast cancers diagnosed were localized (68.7%), for whom standard care does not necessarily include chemotherapy.

Another limitation is the relatively small number of eligible cases for analysis, potentially leading to limited statistical power to detect differences in survival across levels of folate or within strata defined by use of alcohol. To address this issue, we conducted post hoc power analyses on the basis of the available sample size, the observed number of deaths, a two-sided test of hypothesis, and a type I error rate of 0.05. Results indicated an 80% power to detect a 2.2-fold difference in mortality for subjects in the third tertile of folate consumption compared with those in the first tertile. Although it is difficult to interpret a null result, this power analysis suggests that the available sample size was sufficient to rule out a large effect of folate on mortality. However, it should be acknowledged that this effect is large relative to the expected effect of chemotherapy on survival in the adjuvant setting, which is <20%.

The assessment of usual diet in our study has strengths and limitations. For studies of etiology, it is usually considered advantageous to measure diet before disease onset to avoid possible recall bias. Because diet was measured at baseline in 1986, before onset of breast cancer, the measurement of usual folate intake is probably free of bias. The careful measurement of supplemental vitamin use is another notable strength. However, it is not entirely clear whether the estimate of usual diet is most appropriate for correlations with outcomes after cancer diagnosis, especially if diet changes as a result of the diagnosis. If the folate status at the time of chemotherapy is the relevant exposure, then assessment of diet before diagnosis, as we have done, is appropriate. Our post hoc analyses restricted to the first 6 yr of follow-up yielded virtually identical results, supporting this assertion. Because chemotherapy is associated with nausea, vomiting, and cachexia, measurement of diet during or immediately after active chemotherapy would be a far less appropriate strategy, although folate status could be measured through biochemical assays of serum. Jacques et al. (25) compared plasma levels of folate intake estimated from an earlier version of the Willett semiquantitative food frequency questionnaire and noted a correlation of 0.60, which has greater than for any other estimated nutrient.

In summary, results of this study provide no compelling evidence that high folate intakes before diagnosis adversely affect breast cancer survival among women treated with chemotherapy. The fact that recent evidence suggests that adequate folate may minimize toxicity and side effects of antifolate therapies (26) offers additional assurances that the practice of folate supplementation may not portend a worse survival from breast cancer. However, because this is an observational study, replication of these findings is warranted, especially because the number of women with very high intakes of folate is limited. Finally, it seems possible that, on the basis of specific genetic differences in folate metabolism, there may be differences in therapy-related outcomes in breast cancer patients, as has already been shown in leukemia patients (27).

Acknowledgments and Notes

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