Original Contribution

Folate Nutrition and Prostate Cancer Incidence in a Large Cohort of US Men

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Folate has important roles in DNA synthesis, repair, and methylation and is inversely associated with the risk of some cancers. The authors examined this association among 65,836 men in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. During 9 years of follow-up, 5,158 men were diagnosed with prostate cancer. Folate intakes were estimated from the questionnaire administered at enrollment in 1992–1993, and Cox proportional hazards models were used to calculate hazard rate ratios adjusted for potential confounders. Neither dietary nor total folate intake was associated with prostate cancer overall. However, higher folate levels were associated with a nonsignificant decreased risk of advanced prostate cancer (multivariate rate ratio = 0.78, 95% confidence interval: 0.53, 1.15 for the highest vs. lowest quintiles of dietary folate and rate ratio = 0.79, 95% confidence interval: 0.54, 1.17 for the highest vs. lowest quintile of total folate). The association was similar for quintiles 2–5, suggesting that only a small increase in folate intake was needed to alter the risk of advanced prostate cancer. Because the statistical power of the analysis with advanced prostate cancer was limited by the low number of cases, further study is needed to establish this association.

cohort studies; folic acid; men; prostatic neoplasms

Abbreviations: CI, confidence interval; CPS-II, Cancer Prevention Study II; FFQ, food frequency questionnaire; PSA, prostate-specific antigen.

Prostate cancer is the most common incident cancer and the second most common fatal cancer among men in the United States. The American Cancer Society predicted that 232,090 new cases and 30,350 deaths would occur in 2005 (1). Efforts to reduce mortality have focused primarily on early detection and treatment rather than on prevention because the established risk factors (age, ethnicity, and family history) are not modifiable. However, some evidence suggests that nutritional factors may influence prostate cancer risk (2–4). Diets rich in meat and animal fat have been thought to increase risk (5), while those high in certain vegetables and other nutrients may decrease risk (6).

One dietary component that has been implicated as important in several cancers is the B vitamin folate. Both insufficient dietary consumption and low circulating levels of folate have been associated with higher risk of colorectal, cervical, and breast cancer in some studies (reviewed by Sanjoaquin et al. (7), Zhang (8), Strohle et al. (9), and Garcia-Closas et al. (10)). An essential vitamin, folate is needed for DNA synthesis, repair, and methylation. Insufficient levels of folate can compromise nucleotide synthesis, leading to an imbalance in the levels of deoxuryridine monophosphate and deoxythymidylic acid and the subsequent misincorporation of uracil instead of thymidine in DNA (11). This misincorporated uracil is poorly repaired and can lead to mutations and chromosomal breaks (12). Folate deficiency also affects the availability of S-adenosylmethionine, the donor of the methyl group for DNA methylation. The resultant DNA hypomethylation (13, 14) can alter gene expression and decrease chromosomal stability (15). Cumulatively,
these events facilitate carcinogenesis. Thus, better folate nutrition is expected to reduce the risk of cancer.

The results of epidemiologic studies of folate in relation to prostate cancer have been inconsistent. Two nested case-control studies examining blood folate in relation to prostate cancer incidence showed a small, nonsignificant increase in risk associated with the highest versus lowest folate levels (16, 17). In contrast, dietary folate was associated with a 34 percent lower risk of prostate cancer in one large case-control study in Italy (18), whereas a smaller European case-control study found a nonsignificant increased risk associated with higher folate intake (19).

In this study, we assessed the association between folate and prostate cancer incidence in a prospective cohort of more than 65,000 US men over 9 years of follow-up (1992–2001). We considered both dietary folate, which consists of primarily 5-methyltetrahydrofolate, and total folate, which includes folate from supplements in the form of folic acid, because of the different bioavailability of these forms of the vitamin (20) and to study a broader range of intake. The influence of alcohol consumption, which has been found to interact with folate intake in colon (21) and breast (8) cancer, was also investigated.

MATERIALS AND METHODS

Study cohort

Men in this study were drawn from the male participants in the Cancer Prevention II (CPS-II) Nutrition Cohort, a prospective study of cancer incidence and mortality among 86,404 men and 97,788 women. The Nutrition Cohort, described in detail elsewhere (22), was initiated in 1992 as a subgroup of CPS-II, a prospective study of cancer mortality involving approximately 1.2 million Americans begun in 1982. Participants in the Nutrition Cohort were recruited from CPS-II members who resided in 21 US states and were between the ages of 50 and 74 years. At enrollment in 1992–1993, participants completed a self-administered questionnaire that included demographic, medical, dietary, and lifestyle information. Follow-up questionnaires were sent to all living Nutrition Cohort members in 1997, 1999, and 2001 to update exposure information and to ascertain newly diagnosed cancers. The response rate on all of the follow-up questionnaires, after multiple mailings, was at least 90 percent. For the present study, the follow-up period ended on August 31, 2001. All aspects of the CPS-II Nutrition Cohort study have been approved by the Emory University Institutional Review Board (Atlanta, Georgia).

Of the 86,404 men enrolled in 1992, we excluded those lost to follow-up (n = 3,468) and those who reported any prevalent cancer other than nonmelanoma skin cancer prior to enrollment (n = 9,001). Also excluded were men whose self-reported prostate cancer was not verified (n = 200), men who reported a diagnosis date more than 6 months after the interview date (n = 11), men with stage 1 prostate cancer (n = 52), men for whom dietary data were missing or uninterpretable (n = 7,824), and men whose primary cause of death was not prostate cancer (n = 12). After these exclusions, the analytic cohort consisted of 65,836 men.

Identification of prostate cancer cases

We identified 5,158 incident prostate cancer cases between enrollment in 1992–1993 and August 31, 2001. We identified 4,992 through self-report on the follow-up questionnaires and subsequently verified the prostate cancer diagnosis through either medical records (n = 4,029) or linkage with state cancer registries (n = 963). An additional 137 cases not self-reported were identified and verified by state cancer registries during the process of verifying another cancer. Finally, 29 cases for whom prostate cancer was the underlying cause of death were identified through linkage with the National Death Index (23).

Some analyses were limited to men who had advanced prostate cancer. For these analyses, we included cases whose prostate cancer was verified by medical records as stage IV disease, those with a Gleason score of 8 or higher or grade 3–4, cases whose prostate cancer was verified by the cancer state registry and was classified as regional or distant, and prostate cancer deaths. For all except the prostate cancer deaths, the staging and classification information refers to the status of the tumor at diagnosis. A total of 278 advanced prostate cancer cases were included in this analysis.

Dietary assessment

Intakes of folate, ethanol, and other nutrients were assessed at enrollment by using a semiquantitative, 68-item food frequency questionnaire (FFQ), which was a modification of the brief Health Habits and History Questionnaire developed by Block et al. (22, 24). The FFQ inquired about portion size and frequency of consumption of a variety of foods. Use of vitamin supplements was also assessed through questions on frequency of multivitamin use. Daily nutrient intakes from diet and supplements were estimated by using the Diet Analysis System, version 3.8a (25). Dietary folate was derived primarily from leafy green vegetables, fruit juice, bran and granola cereals, and fortified cereals. Total folate was estimated by combining dietary and supplemental folate intake, assuming that each multivitamin contained 400 μg of folic acid. The total folate estimates were not adjusted in any way to accommodate the difference in the bioavailability of dietary and supplemental folate.

The FFQ was validated among 441 Nutrition Cohort participants who completed four 24-hour dietary recall interviews and a repeat FFQ. For men, the correlation coefficients for dietary folate were 0.51 between the FFQ and dietary recall interviews and 0.73 between the baseline FFQ and repeat FFQ (26), indicating that the FFQ provided a valid and reliable assessment of dietary folate.

Statistical analysis

Cox proportional hazards modeling (27) was used to calculate hazard rate ratios and corresponding 95 percent confidence intervals for the relation between dietary and total folate intake and prostate cancer incidence. Dietary and total folate (micrograms/day) intakes were categorized into quintiles or tertiles for the analyses, as indicated. We estimated
p values for linear trend by modeling folate as a categorical variable, with the median value assigned for each quintile.

All Cox models were stratified on the exact year of age of the men at enrollment and were adjusted for race (White, Black, other) and other potential confounders. Other covariates that were included were education, family history of prostate cancer, alcohol consumption (grams/day), total energy intake (quintiles of calories), vitamin B12 (quintiles of milligrams/day), prostate-specific antigen (PSA) screening (yes, no, missing), and history of diabetes (yes, no, missing). All covariates except age, PSA screening, and history of diabetes were modeled as dummy variables by using the categories shown in table 1. PSA screening and history of diabetes were modeled as time-dependent variables.

No information on PSA screening was obtained at enrollment. Therefore, the screening status of men in the cohort for the period between 1992 and 1997 was determined from their responses to the follow-up questionnaire administered in 1997, on which men were asked whether they had ever had a PSA blood test for prostate cancer screening. Those who answered yes were then asked when they were first tested (before 1992, 1992–1993, 1994–1995, 1996–1997, or 1998). Men who developed prostate cancer between 1992 and 1997 were categorized as screened if they reported...
having a PSA test 1 year or more before their diagnosis. Noncases were defined as screened if they reported on the 1997 questionnaire ever having a PSA test. For the intervals between 1997 and 1999 and between 1999 and 2001, men were classified as screened, regardless of case status, if they reported on the questionnaire administered at the beginning of the interval ever having a PSA test. Data for only those men defined as screened within each interval (approximately 80.4 percent of the cohort) were used in the sensitivity analysis.

The potential that alcohol consumption might modify the association between folate intake and prostate cancer incidence was assessed by modeling multiplicative interaction terms between folate intake and three strata of alcohol intake. The statistical significance of the interaction terms was assessed by using the likelihood ratio test (28). All reported p values are two sided.

RESULTS

Intake of folate from dietary sources ranged from less than 204 µg/day in the lowest quintile to more than 347 µg/day in the top quintile. About half of the men (57 percent) in the fourth quintile of total folate (370–640 µg/day) used supplemental vitamins (table 1).

Men who had higher intakes of dietary and total folate were more likely to have other behaviors associated with a healthy lifestyle. Folate consumption was higher among men who were leaner; were more educated; consumed fewer calories, fat, red meat, and alcohol; and consumed more lycopene, vitamin B12, methionine, calcium, and multivitamins; and these men were more likely than men in the lowest quintile of folate intake to have been screened for prostate cancer with PSA testing. Overall, PSA testing was reported by 80.4 percent of the men included in the analytic cohort.

The association of dietary and total folate intakes with prostate cancer incidence is shown in table 2. When we compared men in the lowest quintile with men whose intake of dietary folate was higher, we found no significant association with overall risk of prostate cancer (multivariate rate ratio $= 1.03, 95$ percent confidence interval (CI): 0.94, 1.13 for the highest vs. lowest quintile). No significant association was found when only advanced prostate cancers were considered, although the point estimates for quintiles 2–5 of dietary folate were 19–27 percent below the incidence rate in the lowest quintile of dietary folate (<204 µg/day), without evidence of a trend with increasing dose. The results for total folate consumption are shown in the lower part

| TABLE 2. Rate ratios and 95% confidence intervals for prostate cancer incidence associated with dietary and total folate intakes for all and for advanced cases, CPS-II* Nutrition Cohort, United States, 1992–2001 |
|---|---|---|---|---|---|---|
| | Dietary folate (µg/day) | | | Total folate (µg/day) | | |
| Folate | No. | RR† | 95% CI* | No. | RR† | 95% CI* |
|<204 | 13,039 | 913 | 1.00 (ref***) | 66 | 1.00 (ref) |
|204–<250 | 13,211 | 997 | 1.02 | 0.93, 1.11 | 55 | 0.81 | 0.56, 1.16 |
|250–<293 | 13,191 | 1,052 | 1.05 | 0.96, 1.15 | 52 | 0.75 | 0.52, 1.10 |
|293–<347 | 13,188 | 1,136 | 1.11 | 1.01, 1.21 | 51 | 0.73 | 0.50, 1.07 |
|≥347 | 13,207 | 1,060 | 1.03 | 0.94, 1.13 | 54 | 0.78 | 0.53, 1.15 |
|p-trend | | | | 0.29 | | 0.21 |
|<223 | 13,143 | 902 | 1.00 (ref) | 68 | 1.00 (ref) |
|223–<286 | 13,246 | 1,070 | 1.12 | 1.02, 1.22 | 56 | 0.81 | 0.56, 1.16 |
|286–<370 | 13,201 | 1,085 | 1.11 | 1.01, 1.22 | 47 | 0.67 | 0.46, 0.99 |
|370–<640 | 13,153 | 1,009 | 1.06 | 0.96, 1.16 | 54 | 0.81 | 0.55, 1.18 |
|≥640 | 13,093 | 1,092 | 1.11 | 1.01, 1.22 | 53 | 0.79 | 0.54, 1.17 |
|p-trend | | | | 0.35 | | 0.58 |

* CPS-II, Cancer Prevention Study II; CI, confidence interval; ref, reference.
† All deaths from prostate cancer or Surveillance, Epidemiology, and End Results Program extent of disease codes 50–85, lymph node involvement 1–8, Gleason’s score 8–10, or missing extent of disease and general summary stages 3–8.
‡ Rate ratios (RRs) were adjusted for age, race, education, total calories, total calcium, ethanol, family history of prostate cancer, vitamin B12, prostate-specific antigen screening, and history of diabetes.

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of the nonadvanced cases in whom prostate cancer was detected by PSA screening have a less clinically important form of cancer than the rest of the cases. However, it must be noted that the statistical power of these analyses was limited by the low number of advanced cases.

We examined both dietary and total folate in this analysis because of the potential influence of the difference in bioavailability between food folate and folic acid found in multivitamins (20) and to capture a greater range of exposure. The bioavailability of folic acid, which is a synthetic folate, is expected to be superior to that of food folates (20). We found similar results for dietary and total folate intakes despite the fact that quintiles 4 and 5 of total folate included more cases than the rest of the cases. However, it must be noted that the statistical power of these analyses was limited by the low number of advanced cases.

We found some suggestion of a dose response in the analyses restricted to PSA-screened men. If real, the idea that increased folate is associated with only advanced prostate cancer could indicate that this vitamin plays a role in progression of this malignancy. Alternatively, it could be that the inverse association with folate intake applies to all prostate cancer cases but was not seen in our analysis because some of the nonadvanced cases in whom prostate cancer was detected by PSA screening have a less clinically important form of cancer than the rest of the cases. However, it must be noted that the statistical power of these analyses was limited by the low number of advanced cases.

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Furthermore, the similarity in the associations for higher levels of dietary and total folate suggests that any difference in bioavailability of the different forms of this vitamin is not important for this association.

Our findings differ from those of the four previous known epidemiologic studies (16–19) of the association of folate with prostate cancer. Three of these studies found a small, but nonsignificant increased risk of prostate cancer associated with higher levels of folate. However, the results from the two nested case-control studies (16, 17) are not directly comparable to our findings because these studies used blood folate levels rather than dietary information to estimate folate exposure. The Serbian case-control study (19) used dietary folate to assess exposure, but the mean intake of folate exposure. The fact that we still observed moderately higher levels of folate. The fact that we still observed moderately higher levels in the second half of follow-up. If fortification did increase folate intake equivalently among the men in the cohort, then the expected 100-µg/day increase in dietary folate should shift most of the men in the lowest quintile to levels seen in quintiles 3 and 4. Since the associations in the last three quintiles were very similar, we would expect that moving the lowest quintile into this range would eliminate any association between this reference group and higher levels of folate. The fact that we still observed a weak, inverse association of increased folate with advanced prostate cancer supports this possibility.

Splitting our follow-up into pre- and postfortification years revealed that increased dietary folate was somewhat more protective for advanced prostate cancer in the second half than in the first half of follow-up. If fortification did increase folate intake equivalently among the men in the cohort, then the expected 100-µg/day increase in dietary folate should shift most of the men in the lowest quintile to levels seen in quintiles 3 and 4. Since the associations in the last three quintiles were very similar, we would expect that moving the lowest quintile into this range would eliminate any association between this reference group and higher levels of folate. The fact that we still observed a weak, inverse association of increased folate with advanced prostate cancer supports this possibility.


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<td>&lt;204</td>
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* CPS-II, Cancer Prevention Study II; CI, confidence interval; ref, reference.
† Rate ratios (RRs) were adjusted for age, race, education, total calories, total calcium, ethanol, family history of prostate cancer, vitamin B12, prostate-specific antigen screening, and history of diabetes.
‡ All deaths from prostate cancer or Surveillance, Epidemiology, and End Results Program extent of disease codes 50–85, lymph node involvement 1–8, Gleason’s score 8–10, or missing extent of disease and general summary stages 3–8.

We found that alcohol consumption did not significantly alter the weak, inverse association of increased folate with advanced prostate cancer incidence. This finding differs from those regarding this interaction in breast cancer, where risk is increased by increased alcohol consumption only for those women with low levels of folate (31–35). We may not have been able to detect an effect of alcohol because there were too few men with advanced prostate cancer or the level of alcohol consumption was too low. Alternatively, it may be that the effect of alcohol is different in prostate cancer than it is in breast cancer, and no effect modification of the folate association should be expected. The fact that moderate levels of alcohol consumption (up to three drinks or 45 g/day) are not associated with increased risk of prostate cancer supports this possibility.
effect of folate fortification on dietary intake among the men in this cohort is different than what we expect.

Strengths of this study include its prospective design and large size, which enabled us to examine risk among subpopulations. Information on known covariates made it possible to control for several potential confounders, including race, PSA screening, and diabetes (36).

An important limitation of this study is the low number of advanced prostate cancer cases, which reduced the statistical power of analyses of this subgroup. Additionally, we were unable to correct for the likely misclassification in our estimates of folate intake because folate fortification was implemented during the later years of follow-up. Finally, no information was collected at baseline on use of B-complex vitamin supplements, which may have resulted in underestimation of the total folate intakes by some men.

In summary, we found that neither dietary intake nor total folate intake was associated with prostate cancer in general. For men with advanced prostate cancer, small increases in dietary and total folate were associated with a nonsignificant decrease in risk. These findings require replication with a larger group of men with advanced prostate cancer.

ACKNOWLEDGMENTS
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REFERENCES
12. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromo-