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Folate Intake and Prostate Cancer Risk: A Case-Control Study

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Folate deficiency has been implicated in the carcinogenesis of several tumor types. The role of folate in prostate cancer remains indeterminate. We investigated folate as a risk factor for prostate cancer among 140 biopsy-confirmed prostate cancer patients, 230 age-matched clinic controls, and 250 negative prostate biopsy controls. Dietary folate intake was inversely associated with overall risk of prostate cancer as compared to clinic controls (*P* for a linear trend = 0.003). When stratified by disease severity, dietary folate and folate from natural sources were associated with reduced risk of high-grade cancer as compared to both clinic controls (*P* for a linear trend = 0.0009 and 0.02, respectively) and biopsy negative controls (*P* for a linear trend = 0.03 and 0.05, respectively). There was no interaction between alcohol consumption and folate intake. These analyses support an inverse association between dietary folate intake and prostate cancer risk and primarily risk of high-grade prostate cancer.

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INTRODUCTION

Prostate cancer is a highly prevalent and deadly cancer among older men. As the most commonly diagnosed new cancer and the second most common cause of cancer deaths in men in the United States (1), it is important to consider modifiable lifestyle factors that can reduce the risk of prostate cancer. Although many major risk factors such as age and ethnicity are not modifiable, recent data suggests that risk may be decreased by diet (2).

Folate is a micronutrient that is involved in one-carbon metabolism, a process that regulates many important biologic functions including nucleotide synthesis and deoxyribonucleic acid (DNA) methylation (3). DNA methylation is an important form of transcriptional control; both hypomethylation and hypermethylation alter gene expression (4). Folate nutrition has been associated with changes in methylation status in a number of important genes involved in carcinogenesis (5). Additionally, folate deficiencies are suspected to be responsible for increased misincorporation of uracil into DNA, which can result in double-stranded DNA breaks (6). Proper folate nutrition helps promote DNA integrity, and as a result, it is suggested that folate deficiency could result in increased risk of several cancers such as colon cancer (7,8) and breast cancer (9–11).

The association between prostate cancer risk and folate intake remains under study. In a cohort study of 964 men conducted

in Western Australia, researchers found an inverse association between folate status and prostate cancer risk (10). This association has not been universally demonstrated. In a cohort of U.S. men, Stevens et al. (12) found no significant association between folate and the risk of prostate cancer, nor did they find an association when folate status was modified by dietary supplements or antagonists such as alcohol. Similarly, in a cohort of Finnish male smokers, Weinstein et al. (13) found no strong evidence of an association between folate status and prostate cancer risk.

Ethanol has been described as both a primary and cocarcinogen (14). Ethanol can be converted to the carcinogen acetaldehyde in an intermediate reductive step in the gastrointestinal tract. However, ethanol is most well characterized in carcinogenesis for its role in exacerbating nutritional deficiencies, specifically folate deficiency (15,16). Although ethanol has been shown to be involved in the carcinogenesis of several organ systems, it does not appear to be an independent risk factor for prostate cancer (17–21). However, a recent case-control study conducted in Italy found that the combined effect of high folate intake and low alcohol consumption was associated with a 54% reduction in prostate cancer risk (22).

In the present study we determine the independent and joint associations between folate, alcohol, and prostate cancer risk among a population of veterans receiving health care at a VA hospital. In contrast to previous studies, we obtained information from three groups: men with biopsy-confirmed prostate cancer, men with elevated PSA levels but no cancer upon biopsy, and men with a normal PSA level (<4 ng/ml) not being treated for a prostate condition (screen negative clinic controls). Thus, we are able to examine the impact of folate and alcohol upon risk of cancer as compared to men with no suggestion of disease and as compared to men at higher risk of disease (screen positive but biopsy negative). Results from comparisons of both biopsy positive vs. clinic controls and biopsy positive vs. biopsy negative are reported.

MATERIALS AND METHODS

Subjects in this study were recruited between November of 2001 to June 30, 2006, through the Portland Veterans Affairs Medical Center (PVAMC) as a part of the Diet and Prostate Cancer Risk study. The primary aim of this case-control study was to determine the effects of diet on prostate cancer risk.

Biopsy-negative controls and prostate cancer cases were identified from among men referred to the PVAMC urology clinic for a prostate biopsy. Eligibility criteria included no previous diagnosis of cancer or dementia, no participation in another research study, and no medical conditions that in the view of the urologist would make study participation an undue burden. As shown in Fig. 1, following exclusions and refusals, 490 men (76.1% of those eligible for contact or 58.9% of all eligible) agreed to participate in the study and completed the Diet History Questionnaire (DHQ) (23). For the current analyses, we excluded subjects who did not undergo biopsy and those with a prostatic intraepithelial neoplasia (PIN) diagnosis. An additional 4 prostate cancer cases and 5 biopsy negative controls failed to fully complete the dietary questionnaire (greater than 10% missing items), resulting in a total of 140 incident prostate cancer cases and 250 biopsy negative controls for analyses (Fig. 1).

To identify screen negative clinic controls, information was obtained biannually on all men seen at the PVAMC primary care clinic who had a PSA test result <4 ng/ml within the past 12 mo and who met the eligibility criteria as described for the biopsy subjects. From this list, 1 potential control subject, frequency age-matched by 5-yr age group to each biopsy subject, was randomly selected. If this control was deemed ineligible, a second frequency age-matched control was randomly selected. If the second selected subject was deemed ineligible, he was not replaced in the data set. As shown in Fig. 1, following exclusions and refusals, a total of 235 (66.4% of those contacted or 54.9% of total eligible) subjects agreed to participate and completed the DHQ. Finally, 3 participants were excluded from the final analyses, as it was determined that they were being treated for a prostate condition; and 2 were missing information for greater than 10% of the items on the DHQ. Thus our final analyses include 140 men with biopsy confirmed prostate cancer, 250 men with a negative biopsy result, and 230 age-matched, PSA normal, clinic controls.

Information on diet history was obtained using an adapted version of the National Cancer Institute Diet History Questionnaire (DHQ). Participants were asked to recall their usual dietary intake of 124 foods, nutrient supplement use (including folate), beverage consumption, and use of herbal remedies for the previous 12 mo. A separate risk factor questionnaire requested information on prostate cancer risk factors including age, race, ethnic origin, body mass index (BMI), family history of cancer, use of nonsteroidal anti-inflammatory drugs (NSAIDs), comorbid conditions, occupational history, alcohol consumption, and smoking. Frequency and amount of supplemental folate intake from nonfood dietary supplements and multivitamins (in the form of folic acid) was also assessed. DHQ data was analyzed using the NCI Diet*Calc software described by Subar et al (24). Folate intake from foods and supplements was calculated within the Diet*Calc software as folate (mcg), dietary folate equivalents (DFE, mcg), natural folate (mcg, food folate) and synthetic folate (mcg, supplemental folic acid). Because all data collection for our study occurred after the introduction of folate fortification (1998) of cereal grains, the NCI Diet*Calc variable of dietary folate equivalents (DFE); a calculation of synthetic folate in foods multiplied by a factor 1.7 (as synthetic folate is more readily absorbed) plus natural folate, was used for analyses of food folate. Total folate intake was calculated as dietary folate (DFE) intake plus synthetic folate derived from nonfood supplements. Alcohol consumption is presented as grams of alcohol per day. We used a conversion factor for each type of alcohol to calculate the total alcohol intake in g/day (for beer,

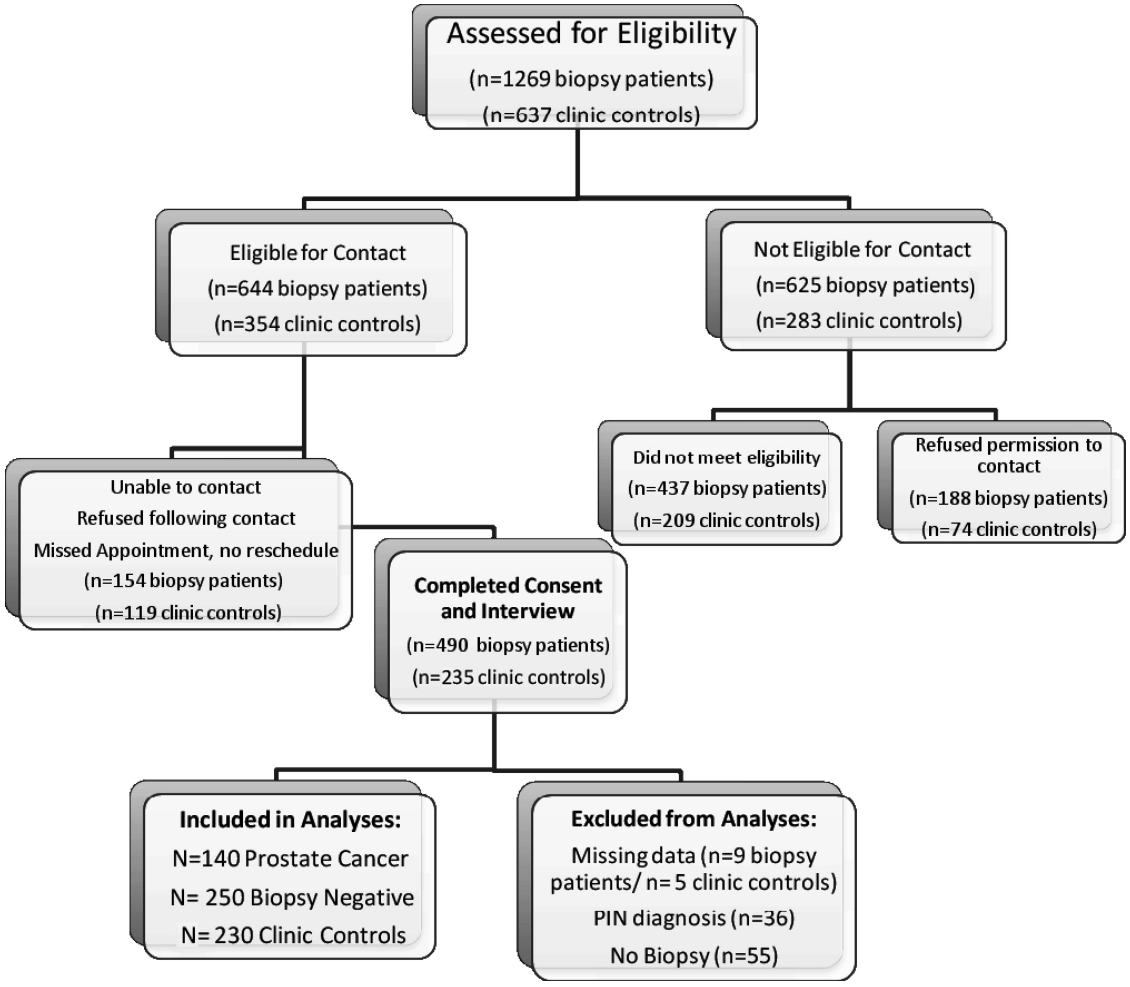

FIG. 1. Recruitment and eligibility for biopsy subjects and PSA normal clinic controls.

12.8 g/12 oz drink; for wine, 11 g/5oz drink; for liquor, 14g/1.5 oz drink).

We interviewed cases and biopsy negative controls prior to their scheduled prostate biopsy procedure with the intent of collecting answers from patients prior to a potential cancer diagnosis. All patients provided written informed consent according to both the PVAMC and Oregon Health & Science University (OHSU) Institutional Review Boards' requirements. This protocol, consent forms, and Health Insurance Portability and Accountability Act (HIPAA) authorization forms were reviewed and approved by the PVAMC and OHSU Institutional Review Boards.

All statistical analyses were performed using the Statistical Analysis System (SAS)/PC program, version 9.1 (SAS Institute, Inc., Cary, NC). Differences in covariate distribution among cases and controls were determined using a chi-square test for categorical variables and a Wilcoxon rank sum test for continuous variables, as they were not normally distributed. Tests were considered statistically significant at a P value (2-sided) of less than 0.05. Correlations between folate and other B vitamins and methionine were assessed with Pearson's correlation coefficient. Odds ratios (OR) as estimates of the relative risk for prostate cancer associated with each level of folate or alcohol intake and 95% confidence intervals (CI) were obtained using unconditional logistic regression. Risk of prostate cancer as compared to PSA normal clinic controls and risk of prostate cancer as compared to biopsy negative controls were modeled separately. All models were adjusted for age. Potential confounders—including body mass index (BMI), total calorie intake, smoking, use of NSAIDs, race, marital status, family history of cancer and family history of prostate cancer, fruit and vegetable intake and PSA level (biopsy-negative model only)—were considered for inclusion in the multivariate model. Confounders were entered into the logistic regression model independently and were maintained in the final adjusted model if they 1) changed the OR of the primary predictor variable by more than 10%, 2) were considered established risk factors for prostate cancer, or 3) were independent predictors of prostate cancer in this study population. Age, race, and BMI were maintained in the final adjusted model for comparison of cases to PSA normal clinic controls.

PSA (entered as categorical variables) was also included in the final adjusted model for comparison of cases to biopsy-negative controls. Total caloric intake was included in both models to account for consistent overreporting or underreporting on the DHQ. Alcohol intake was considered as both an independent risk factor and a modifier of the folate effect. Dietary intake variables and alcohol consumption were entered into the models as quartiles of intake based on the distribution of intake among the PSA normal clinic control subjects. After careful construction of the multivariate models, we also considered the potential confounding effect of supplemental and dietary intake of other nutrients involved in one-carbon metabolism (methionine or vitamins B_6/B_{12}). Of these, only vitamin B6 altered the OR of folate by greater than 10%. We considered the potential for collinearity between these variables and folate and determined that despite being strongly correlated, addition of B6 to both models did not attenuate the relationship between folate and prostate cancer risk and was maintained in the fully adjusted models. The significance of a trend in risk across levels of folate and alcohol intake was evaluated by entering categorical folate variables into the logistic model as different values of a single ordinal variable. Tests were considered statistically significant at a P value for the trend OR of less than 0.05.

To assess the potential for effect modification by alcohol, we modeled the multiplicative interaction between dietary and total folate intake and quartile of alcohol intake. Significance of the interaction term was determined using the chi-square score statistic. To determine if the association between folate, alcohol, and prostate cancer varied by disease severity, we stratified our analyses by disease severity as determined using the Gleason score. Gleason score, and particularly Gleason with a cut point of 7, is supported in the literature as a primary predictor of mortality (25,26). Cases were characterized as having less aggressive (Gleason score of <7) or more aggressive (Gleason score of ≥ 7) disease.

RESULTS

Select demographic characteristics of study participants are presented in Table 1. PSA normal, clinic controls had a significantly greater BMI than prostate cancer cases and biopsy-negative controls, whereas biopsy-negative controls were significantly younger than both prostate cancer cases and clinic controls and more highly educated and more likely to have never smoked than prostate cancer cases.

In Table 2, we present means and ranges of intake of selected nutrients. Reported lycopene intake was significantly greater among both clinic and biopsy-negative controls as compared to prostate cancer cases. Calcium, vitamin E, methionine, and dietary and synthetic folate intake were significantly greater among clinic controls as compared to prostate cancer cases; and vitamin B12 was significantly greater among clinic controls as compared to biopsy-negative controls. Vitamin B6 (mg) and vitamin B12 (μg) were both determined to be moderately positively correlated with dietary folate equivalents (μg; $r^2 = 0.86$ and 0.48, respectively).

In Table 3, we show the adjusted model for prostate cancer vs. PSA normal controls and the adjusted model plus vitamin B6. Although folate and vitamin B6 are strongly correlated, when we adjusted the model for vitamin B6, the association between folate and prostate cancer risk is strengthened, counter to what we would expect should there be concerns with collinearity. Hence, we will discuss primarily results from the fully adjusted model including vitamin B6. When compared to PSA normal controls, men in the highest quartile of dietary folate consumption (DFE) had significantly lower risk of any prostate cancer (OR for DFE = 0.19, 95% CI = 0.06–0.56) and specifically high-grade prostate cancer (OR for DFE = 0.08, 95% CI = 0.02–0.39). Further, this inverse association with high-grade prostate cancer risk may be explained primarily by intake of natural folate (OR for natural folate only for highest vs. lowest consumption = 0.15, 95% CI = 0.03–0.68).

These results were attenuated for total folate: the combination of dietary and supplemental folate. Intake of folate from synthetic sources in foods was associated with a significant reduction in risk of all prostate cancer (OR = 0.45, 95% CI = 0.21–0.97), although intake of synthetic folate from non-food supplements only was not independently associated with prostate cancer risk overall or as stratified by severity of disease. Alcohol was not significantly associated with overall risk of prostate cancer, although the linear trend for risk of low-grade prostate cancer was significant (OR = 1.29, 95% CI = 1.00–1.66). While this association was statistically significant as determined by a P value for trend of 0.05, the confidence intervals for all categories of comparison included 1.0. We report similar although slightly weaker associations for the comparison of prostate cancer cases to biopsy negative controls (see Table 4).

There was no statistically significant interaction between alcohol and dietary and total folate intake when it was evaluated in all models (Wald chi-square P value for interaction was greater than 0.05 in all models considered). We did observe that one prostate cancer case had exceedingly high alcohol intake (325.0 g/day). Excluding this individual from all alcohol analyses did not materially change our results.

DISCUSSION

Overall, as compared to PSA normal clinic controls and biopsy-negative controls, we found a significant decrease in risk of high-grade prostate cancer with increasing total and dietary folate intake. We found no significant association between supplemental folate intake and prostate cancer risk. Alcohol was associated with an increased risk of low-grade prostate cancer as compared to clinic controls, although no association was seen for all or high-grade prostate cancer. No significant associations were seen between alcohol consumption and risk of prostate cancer as compared to biopsy-negative controls. Further, there

TABLE 1
Frequencies of selected characteristics of men in the Diet and Prostate Cancer Risk study, 2001–2006[a]

Characteristic	Prostate Cancer Cases ($n = 140$)		Biopsy Negative Controls ($n = 250$)		PSA Normal Controls ($n = 230$)	
Age, yr						
Mean age	65.6	62.9**	64.3†			
Range	52–86	46–83	50–85			
	N	%	*N*	%	*N*	%
Ethnic origin						
White	125	90.7	215	91.2	211	94.3
Black	9	6.4	8	3.2	6	2.6
Missing/other	6	4.3	27	10.8	13	5.7
BMI						
Less than 25	28	20.0	37	14.8	36	15.7*†
25–29	50	35.7	90	36.0	73	31.7
30–34	45	32.1	89	35.6	65	28.3
≥35	17	12.1	34	13.6	56	24.4
Family history of prostate cancer						
Yes	18	12.9	27	10.8	16	7.0
Smoking						
Never	21	15.0	63	25.2*	42	18.3
Former	80	57.1	132	52.8	127	55.2
Current	38	27.1	47	18.8	59	25.7
Missing	1	0.7	8	3.2	2	0.9
Education						
≤ 12 yr	58	41.4	57	22.8**	70	30.4
Some college/technical	47	33.6	106	42.4	90	39.1
≥College graduate	34	24.3	81	32.4	68	29.6
Missing/other	1	0.7	6	2.4	2	0.9
Marital status						
Single/divorced/widowed	41	29.3	81	32.4	75	32.6
Married/partner	97	69.3	158	63.2	152	66.1
Missing	2	1.4	11	4.4	3	1.3
NSAID use						
Yes	48	34.3	92	36.8	91	39.6
No	91	65.0	149	59.6	137	59.6
Missing	1	0.7	9	3.6	2	0.9

[a] Abbreviations are as follows: PSA, prostate-specific antigen; BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drug. P value for chi-square difference between cases and respective control groups, $^{*}P < 0.05$, $^{**}P < 0.001$, and between control groups, $^{\dagger}P < 0.05$.

appeared to be no significant interaction between alcohol and folate consumption in either comparison, although due to small sample size, power for this analysis is limited.

Folate is involved in the process of one-carbon metabolism, and dietary folate deficiencies have been linked to gene-specific changes in DNA methylation and reduced thymine synthesis; both scenarios that can result in carcinogenesis (27). Further, others have shown that both hypomethylation and hypermethylation can result from folate deficiency (5). Hypomethylation can promote carcinogenesis by decreasing regulatory controls on proto-oncogenes and other genes whose expression is closely regulated. Conversely, hypermethylation can lead to cancer by blocking the expression of tumor suppressor genes (4). Glutathione S-transferase pi1 (*Gstp1*) is one such protective gene that has been well characterized for its effects in the detoxification of potential carcinogens. Hypermethylation of the *Gstp1* promoter and the resultant loss of function are associated with an increased risk of prostate cancer (28).

Our results largely support an inverse association between folate intake and prostate cancer risk. However, the lack of an association between supplemental folate intake and prostate cancer risk was unexpected given the strength of the association between dietary folate intake and risk. A possible explanation for these discrepant results may be the different forms of folate

TABLE 2
Mean (range) intake of selected nutrients among participants[a]

Nutrient	Prostate Cancer ($n = 140$)	Biopsy Negative ($n = 250$)	PSA Normal ($n = 230$)
Alcohol (g/day)	14.3 (0.0–325.9)	11.3 (0.0–198.2)	8.1 (0.0–148.7)
Lycopene (mg/day)	5.5 (0.4–44.3)	6.3 (0.04–89.8)*	6.9 (0.8–111.6)*
Calcium (mg/day)	895.7 (192.8–2,466.8)	1,014.3 (167.4–5,467.1)	1028.2 (191.8–3,040.7)*
Vitamin E (mg/day)	9.7 (2.3–30.4)	10.0 (1.9–33.3)	10.5 (3.2–29.0)*
Fruit (servings/day)	2.4 (0.2–9.1)	2.5 (0.0–25.9)	2.5 (0.18–14.1)
Vegetable (servings/day)	3.8 (0.7–14.1)	3.8 (0.5–12.1)	3.8 (0.6–9.6)
Food energy (kcal/day)	2,360.4 (664.2–6,865.7)	2,445.7 (411.6–6,810.5)	2,493.4 (610.5–5699.3)
Methionine (g/day)	2.0 (0.6–6.5)	2.1 (0.3–6.8)	2.2 (0.7–5.6)*
Vitamin B6 (μg/day)	2.3 (0.7–4.7)	2.3 (0.6–5.5)	2.4 (0.7–5.4)
Supplemental vitamin B6 (μg/day)	3.5 (0.0–66.3)	4.3 (0.0–66.3)	3.5 (0.0–66.3)
Vitamin B12 (μg/day)	7.7 (1.7–19.5)	7.7 (0.7–24.8)	8.6 (1.9–29.1)†
Supplemental vitamin B12 (μg/day)	3.1 (0.0–6.0)	3.3 (0.0–6.0)	3.1 (0.0–6.0)
Total folate (μg/day)	703.4 (165.9–1,374.1)	739.5 (121.2–1,647.8)	760.8 (174.4–1,761.4)
Dietary folate equivalents (μg/day)	488.7 (141.7–1,207.6)	517.2 (121.2–1,247.8)	538.9 (156.3–1,237.8)**
Natural folate (μg/day)	270.3 (60.3–664.9)	280.4 (64.1–909.3)	284.6 (70.8–704.0)
Synthetic folate from foods (μg/day)	128.9 (16.0–411.9)	139.5 (3.5–472.0)	149.8 (7.8–521.7)**
Supplemental folate (μg/day)	214.7 (0.0–571.4)	222.3 (0.0–571.4)	221.9 (0.0–571.4)

[a]Abbreviation is as follows: PSA, prostate-specific antigen. Wilcoxon 2-sample test for difference between means of cases and respective control groups, $^{*}P < 0.05$, $^{**}P < 0.01$, and between control groups $^{\dagger}P < 0.05$.

found in foods vs. supplements. Supplemental or synthetic folate, including that used in food fortification, is in the form of folic acid. This compound is in the oxidized state and contains only one conjugated glutamate residue, whereas naturally occurring folates are mainly polyglutamated and in the reduced form (tetrahydrofolates) (29). Although folic acid is more easily absorbed, it must be reduced in the liver by dihydrofolate reductase before entering the folate cycle. In populations with folate fortification of foods, unmetabolized folic acid is found in circulating blood and may compete with the metabolism, cellular transport, and regulatory functions of natural folates. How this interference may impact folate-dependent enzymes and pathways is not yet clear, but it may play a role in the relationship between folate status and cancer risk (29). To further clarify in our data set the potential differential effect of natural folate vs. synthetic, we analyzed our data using solely intake of naturally occurring folate as the primary exposure variable. The inverse association between more severe (Gleason 7+) disease and natural folate intake remained significant in both the PSA normal clinic control (P for trend = 0.02, OR for high vs. low quartile = 0.15, 95% CI = 0.03–0.68) and the biopsy negative control (P for trend = 0.05, OR for high vs. low quartile = 0.17, 95% CI = 0.03–0.90) models. In the models of synthetic folate from foods, there remained a significant inverse association with overall prostate cancer risk; but the association with more severe disease was attenuated for both the PSA normal clinic control and the biopsy-negative control comparisons.

Our results suggest that adequate dietary folate intake may be involved in reducing the risk of prostate cancer, and specifically, naturally occurring folate may reduce risk of high-grade or clinically relevant prostate cancer. Findings from previous epidemiologic studies of folate and alcohol intake and prostate cancer risk have been inconsistent. Pelucchi et al. (22), in their Italian case-control study, found a 54% risk reduction among men with high folate and low alcohol diets. Their results, however, did not vary based on disease severity. Results from the Cancer Prevention II (CPS-II) Nutrition Cohort study, showed a weak, nonsignificant, inverse association of increased folate with advanced prostate cancer incidence only. Alcohol consumption did not significantly alter the association. Another cohort study conducted in Finland found no association between folate or alcohol intake and prostate cancer risk (13).

To our knowledge, none of the studies conducted postfortification separated intake of natural vs. synthetic folate. However, the CPS study did stratify the analyses prefortification and postfortification (12). This stratification did not alter their results. Mean alcohol consumption in our population was somewhat lower than that reported in previous studies (18,20–22), although our range of intake was quite wide (0 g/day to 325.9 g/day). The low mean alcohol intake in our population may be the result of reporting bias, as subjects were interviewed in a clinic setting and may have been biased toward underreporting. Further, 70% of the men that reported no current alcohol consumption reported that in the past they had consumed at least one drink per day for a period of 6 mo or more, with an average of 22 yr of consumption. It is unclear if there is any lasting effect of alcohol consumption on current nondrinkers.

TABLE 3
Dietary intake of folate and alcohol and risk of total, low- and high-grade prostate cancer as compared to PSA normal (<4 ug/ml) controls [odds ratios (OR) and 95% confidence interval (95% CI)]a

	Prostate Cancer Cases vs. PSA Normal Controls					Low-Grade Prostate Cancer (Gleason <7) vs. PSA Normal Controls					High-Grade Prostate Cancer (Gleason ≥ 7) vs. PSA Normal Controls				
	No. 140/230	OR	95% CI	OR[b]	95% CI[a]	No. 69/230	OR	95% CI	OR[b]	95% CI	No. 68/230	OR	95% CI	OR[b]	95% CI
Total folate (food + supplement; μg/day)															
≤ 551.9	51/57	1.00	Referent	1.00	Referent	25/57	1.00	Referent	1.00		26/57	1.00	Referent	1.00	Referent
> 551.9 ≤ 758.7	29/58	0.67	0.36–1.24	0.65	0.35–1.21	15/58	0.63	0.29–1.34	0.73	0.33–1.60	13/58	0.61	0.27–1.99	0.55	0.24–1.29
> 758.7 ≤ 934.1	29/58	0.52	0.28–0.98	0.54	0.29–1.03	11/54	0.43	0.19–0.98	0.56	0.24–1.32	16/58	0.52	0.23–1.15	0.46	0.20–1.06
>934.1	31/57	0.62	0.32–1.21	0.58	0.29–1.19	18/57	0.69	0.43–1.44	1.13	0.45–2.81	13/57	0.46	0.19–1.10	0.32	0.12–0.82
P trend			0.17		0.12			0.57		0.84			0.08		0.02
Dietary folate equivalents (DFE) (μg/day)															
≤ 411.1	51/58	1.00	Referent	1.00	Referent	27/58	1.00	Referent	1.00	Referent	22/58	1.00	Referent	1.00	Referent
> 411.1 ≤ 520.2	29/57	0.66	0.35–1.23	0.65	0.32–1.29	17/57	0.68	0.31–1.48	0.90	0.39–2.10	22/57	0.65	0.29–1.50	0.45	0.18–1.13
> 520.2 ≤ 669.0	59/57	0.46	0.22–0.95	0.39	0.16–0.89	13/57	0.53	0.22–1.28	0.79	0.27–2.37	14/57	0.47	0.19–1.20	0.22	0.07–0.67
>669.0	31/58	0.35	0.15–0.83	0.19	0.06–0.56	12/58	0.40	0.14–1.16	0.41	0.10–1.65	10/58	0.32	0.10–1.03	0.09	0.02–0.39
P trend			0.02		0.003			0.13		0.30			0.08		0.0009
Natural (food) folate (μg/day)															
≤ 207.8	44/59	1.00	Referent	1.00	Referent	24/59	1.00	Referent	1.00		18/59	1.00	Referent	1.00	Referent
> 207.8 ≤ 280.7	44/56	0.89	0.44–1.77	0.95	0.45–2.01	18/56	0.96	0.40–2.27	1.25	0.50–3.12	25/56	0.89	0.36–1.21	0.66	0.23–1.86
> 280.7 ≤ 345.4	25/57	0.59	0.27–1.27	0.58	0.24–1.41	9/57	0.51	0.18–1.44	0.83	0.27–2.60	16/57	0.73	0.27–1.96	0.38	0.11–1.25
>345.5	27/58	0.67	0.29–1.56	0.57	0.21–1.56	18/58	1.14	0.42–3.17	1.92	0.57–6.48	9/58	0.38	0.11–1.30	0.15	0.03–0.68
P trend			0.31		0.18			0.91		0.57			0.24		0.02

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TABLE 3
Dietary intake of folate and alcohol and risk of total, low- and high-grade prostate cancer as compared to PSA normal (<4 ug/ml) controls [odds ratios (OR) and 95% confidence interval (95% CI)]a (*Continued*)

	Prostate Cancer Cases vs. PSA Normal Controls					Low-Grade Prostate Cancer (Gleason <7) vs. PSA Normal Controls					High-Grade Prostate Cancer (Gleason ≥ 7) vs. PSA Normal Controls				
	No. 140/230	OR	95% CI	OR[b]	95% CI[a]	No. 69/230	OR	95% CI	OR[b]	95% CI	No. 68/230	OR	95% CI	OR[b]	95% CI
Synthetic folate (folic acid; μg/day)															
≤97.9	55/58	1.00	Referent	1.00	Referent	28/58	1.00	Referent	1.00	Referent	27/58	1.00	Referent	1.00	Referent
>97.9 ≤ 139.9	30/57	0.55	0.30–1.03	0.57	0.31–1.07	15/57	0.57	0.26–1.24	0.64	0.29–1.41	12/57	0.41	0.17–0.96	0.41	0.17–0.96
>139.9 ≤ 189.7	27/57	0.50	0.26–0.95	0.51	0.26–1.01	15/57	0.58	0.26–1.26	0.77	0.32–1.81	12/57	0.43	0.18–1.02	0.33	0.13–0.84
>189.8	28/58	0.52	0.26–1.04	0.45	0.21–0.97	11/58	0.38	0.15–0.96	0.42	0.15–1.20	17/58	0.63	0.26–1.55	0.43	0.16–1.17
P trend			0.07		0.04			0.07		0.14			0.33		0.06
Supplemental folate (μg/day)															
None	53/91	1.00	Referent	1.00	Referent	25/91	1.00	Referent	1.00	Referent	28/91	1.00	Referent	1.00	Referent
≤285.7	16/20	1.74	0.80–3.81	1.86	0.84–4.12	39711	1.87	0.72–4.89	1.97	0.73–5.31	7/20	1.97	0.69–5.62	2.06	0.72–5.91
>285.7	71/119	0.97	0.60–1.56	0.98	0.61–1.58	35/119	1.06	0.57–1.96	1.10	0.59–2.05	33/119	0.80	0.43–1.50	0.78	0.41–1.47
P trend			0.86		0.85			0.85		0.84			0.46		0.38
Alcohol intake (g/day)															
≤ 0.02	30/62	1.00	Referent	1.00	Referent	13/62	1.00		1.00	Referent	17/62	1.00	Referent	1.00	Referent
>0.02 ≤ 0.88	33/52	1.13	0.58–2.19	1.11	0.57–2.17	11/52	1.02	0.40–2.56	1.08	0.42–2.80	19/52	0.94	4137–2.04	0.87	0.37–2.04
>0.88 ≤ 9.05	37/58	1.10	0.58–2.09	1.13	0.59–2.16	21/58	1.66	0.72–3.82	1.93	0.82,4.53	16/58	0.68	0.29–1.53	0.64	0.27–1.53
>9.05	40/58	1.13	0.60–2.13	1.08	0.57–2.06	24/58	1.71	0.76–3.85	1.69	0.73–3.90	16/58	0.71	0.31–1.53	0.66	0.28–1.53
P trend			0.55		0.56			0.06		0.05			0.44		0.38

[a]Abbbreviation is as follows: PSA, prostate-specific antigen. Unconditional logistic regression models adjusted for age, total caloric intake, race, body mass index (BMI), and family history of prostate cancer.

[b]Unconditional logistic regression models adjusted for age, total caloric intake, race, BMI, family history of prostate cancer, and vitamin B6 intake.

TABLE 4
Dietary intake of folate and alcohol and risk of total, low-, and high-grade prostate cancer as compared to biopsy negative controls [odds ratios (OR) and 95% confidence interval (95% CI)][a]

	Prostate Cancer Cases vs. Biopsy Negative Controls					Low-Grade Prostate Cancer (Gleason < 7) vs. Biopsy Negative Controls					High-Grade Prostate Cancer (Gleason ≥ 7) vs. Biopsy Negative Controls				
	No. 140/230	OR	95% CI	OR[b]	95% CI	No. 69/230	OR	95% CI	OR[b]	95% CI	No. 68/230	OR	95% CI	OR[b]	95% CI
Total folate (food + supplement; μg/day)															
≤551.9	51/79	1.00	Referent	1.00	Referent	25/79	1.00	Referent	1.00	Referent	26/79	1.00	Referent	1.00	Referent
>551.9 ≤ 758.7	29/46	1.01	0.55–1.87	1.01	0.54–1.87	15/46	1.04	0.48–2.23	1.11	0.51–2.43	13/46	0.99	0.43–2.27	0.91	0.39–2.13
>758.7 ≤ 934.1	29/61	0.57	0.30–1.05	0.55	0.29–1.03	11/61	0.55	0.24–1.29	0.53	0.23–1.24	16/61	0.45	0.20–1.05	0.40	0.17–0.95
>934.1	31/64	0.68	0.35–1.31	0.68	0.33–1.40	18/64	0.84	0.36–1.93	1.13	0.44–2.92	13/64	0.51	0.21–1.25	0.38	0.14–1.06
*P*trend			0.14		0.15			0.44		0.76			0.09		0.04
Dietary folate equivalents (DFE; (μg/day)															
≤411.1	51/77	1.00	Referent	1.00	Referent	27/77	1.00	Referent	1.00	Referent	22/77	1.00	Referent	1.00	Referent
>411.1 ≤ 520.2	40/69	0.68	0.37–1.25	0.64	0.33–1.24	17/69	0.71	0.32–1.55	0.82	0.34–1.95	22/69	0.59	0.25–1.38	0.41	0.16–1.06
>520.2 ≤ 669.0	27/53	0.67	0.33–1.35	0.60	0.27–1.35	13/53	0.74	0.30–1.83	1.03	0.35–3.06	14/53	0.61	0.23–1.63	0.37	0.12–1.12
>669.0	22/51	0.54	0.22–1.29	0.45	0.16–1.32	12/51	0.58	0.19–1.74	0.90	0.22–3.65	10/51	0.45	0.13–1.57	0.22	0.05–0.98
P trend			0.16		0.14			0.32		0.93			0.21		0.03
Natural (food) folate (μg/day)															
≤207.8	44/71	1.00	Referent	1.00	Referent	24/71	1.00	Referent	1.00	Referent	18/71	1.00	Referent	1.00	Referent
>207.8 ≤ 280.7	44/73	0.75	0.39–1.42	0.70	0.35–1.43	18/73	0.86	0.38–1.92	0.97	0.41–2.32	25/73	0.74	0.31–1.80	0.44	0.15–1.29
>280.7 ≤ 345.4	25/52	0.59	0.27–1.31	0.59	0.23–1.48	9/52	0.53	0.19–1.50	0.90	0.28–2.89	16/52	0.64	0.22–1.89	0.29	0.07–1.11
>345.5	27/54	0.75	0.31–1.83	0.74	0.25–2.26	18/54	1.20	0.40–3.57	3.22	0.75–13.74	9/54	0.46	0.12–1.79	0.17	0.03–0.90
P trend			0.49		0.54			0.93		0.18			0.35		0.05

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TABLE 4
Dietary intake of folate and alcohol and risk of total, low-, and high-grade prostate cancer as compared to biopsy negative controls [odds ratios (OR) and 95% confidence interval (95% CI)][a] (*Continued*)

	Prostate Cancer Cases vs. Biopsy Negative Controls					Low-Grade Prostate Cancer (Gleason < 7) vs. Biopsy Negative Controls					High-Grade Prostate Cancer (Gleason ≥ 7) vs. Biopsy Negative Controls				
	No. 140/230	OR	95% CI	OR[b]	95% CI	No. 69/230	OR	95% CI	OR[b]	95% CI	No. 68/230	OR	95% CI	OR[b]	95% CI
Synthetic folate (folic acid; μg/day)															
≤97.9	51/77	1.00	Referent	1.00	Referent	28/77	1.00	Referent	1.00	Referent	27/77	1.00	Referent	1.00	Referent
>97.9 ≤ 139.9	40/62	0.60	0.33–1.11	0.60	0.32–1.10	15/62	0.64	0.30–1.39	0.69	0.32–1.53	12/62	0.43	0.18–1.02	0.39	0.16–0.95
>139.9 ≤ 189.7	27/59	0.58	0.31–1.09	0.56	0.29–1.08	15/59	0.67	0.30–1.49	0.76	0.33–1.76	12/59	0.48	0.20–1.16	0.40	0.16–1.00
>189.8	22/52	0.72	0.37–1.43	0.73	0.35–1.52	11/52	0.58	0.23–1.43	0.72	0.27–1.97	17/52	0.84	0.34–2.06	0.73	0.28–1.92
P trend			0.19		0.20			0.17		0.38			0.43		0.22
Supplemental folate (μg/day)															
None	53/103	1.00	Referent	1.00	Referent	25/103	1.00	Referent	1.00	Referent	28/103	1.00	Referent	1.00	Referent
≤285.7	16/11	3.02	1.25–7.31	3.00	1.24–7.29	9/11	4.33	1.49–12.60	4.10	1.39–12.11	7/11	2.57	0.80–8.28	2.46	0.76–7.96
>285.7	71/136	0.88	0.55–1.40	0.86	0.54–1.38	35/136	0.96	0.52–1.74	0.96	0.52–1.75	33/136	0.70	0.37–1.32	0.65	0.34–1.27
P trend			0.64		0.65			0.91		0.91			0.40		0.35
Alcohol intake (g/day)															
≤0.02	30/67	1.00	Referent	1.00	Referent	13/67	1.00	Referent	1.00	Referent	17/67	1.00	Referent	1.00	Referent
>0.02 ≤ 0.88	33/52	1.40	0.73–2.69	1.41	0.73–2.72	11/52	1.03	0.41–2.60	1.03	0.40–2.62	19/52	1.50	0.65–3.51	1.51	0.65–3.54
> 0.88 ≤ 9.05	37/59	1.29	0.68–2.44	1.28	0.68–2.44	21/59	1.60	0.70–3.64	1.59	0.70–3.64	16/59	1.02	0.42–2.47	1.04	0.43–2.53
>9.05	40/72	1.20	0.64–2.25	1.21	0.65–2.27	24/72	1.59	0.70–3.60	1.58	0.69–3.62	16/72	1.00	0.43–2.31	0.96	0.41–2.25
P trend			0.63		0.63			0.10		0.10			0.63		0.63

[a]Abbreviations are as follows: PSA, prostate-specific antigen; BMI, body mass index. Unconditional logistic regression models adjusted for age, total caloric intake, race, BMI, PSA, and family history of prostate cancer.

[b]Unconditional logistic regression models adjusted for age–total caloric intake–race–BMI–PSA–family history of prostate cancer and vitamin B6 intake.

Of note, in our analyses, we considered for inclusion in our models other nutrients involved in one-carbon metabolism including methionine, vitamin B12, and vitamin B6. Although this approach falls short of the mathematical modeling of the folate-mediated one-carbon metabolism developed by some (30,31), we do attempt to consider the potential for the influence of other nutrients on the role of folate in this pathway. Unexpectedly, we found that although vitamin B6 and folate were highly correlated, this did not attenuate the risk estimate for folate, suggesting both folate and vitamin B6 are independent risk factors. In fact, inclusion of vitamin B6 strengthened the associations with folate. This would suggest that vitamin B6 and folate may be functioning jointly, an observation supported by the biology of one-carbon metabolism.

Limitations of this study are similar to those inherent in all case-control studies. Because the questionnaire asked participants to estimate consumption of a variety of foods over the past 12 mo, some measurement error in estimates of consumption could occur. Despite this, techniques to reduce measurement error were employed including use of a validated assessment tool (NCI–Diet History Questionnaire) asking about portion size and accounting for seasonality of some foods. Further, a referral for prostate biopsy may result in men overestimating or underestimating their intake of folate-containing foods such as fruits and vegetables. However, because we interviewed the biopsy negative controls and prostate cancer cases prior to diagnosis, we would expect any impact of referral on reporting to be equivalent, particularly in these two groups.

Selection bias is a common concern in case-control studies and may be a particular concern in the current analyses as a result of the low response rates among both clinic control and biopsy subjects. Selection bias may result when both cases and controls are not representative of the same target population, or as a result of "self-selection," such that subjects volunteering to participate are not representative of the entire target population. The potential for selection bias due to cases and controls not representing the target population is small in our study, as all subjects were recruited from a population of PSA screened veterans accessing health care through the VA system. However, our low response rates among both clinic controls (54.9% of all eligible) and biopsy subjects (58.9% or all eligible) may result in bias due to self-selection if consented individuals were systematically different from nonresponders with regards to the exposure of interest. In an attempt to address this potential source of bias, we evaluated differences in age and race/ethnicity among those who consented to participate and those who did not. There were no significant differences in age or in race/ethnicity between responders and nonresponders (data not shown). Suggesting that with regards to at least these two important risk factors for prostate cancer, there was no apparent difference between the men who did and did not participate. In sum, the availability of two control groups and recruitment of subjects through VA clinics ensures that our controls are representative of the same target population of interest and that all subjects were regularly receiving annual PSA screenings. Thus the potential impact of selection bias is minimized, although still a concern due to our small sample size and low response rates.

Finally, there is the potential for misclassification of controls in both of our control groups. It has been previously noted that 20% to 25% of men with normal PSAs, and a similar percent of those with a negative prostate biopsy, actually do have cancer. However, as we have no reason to believe that this misclassification would be differential with regard to folate intake, the primary impact of this misclassification is likely to be an attenuation of the OR to the null, suggesting that the present findings may be a more conservative estimate of the real association.

Overall, our findings contribute additional support to previous reports suggesting an inverse association between dietary folate and particularly natural folate intake and prostate cancer risk. Further, there is some suggestion that this protective effect of folate may be of greatest importance in the prevention of clinically significant, more severe disease.

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