Dietary Folate and Risk of Prostate Cancer in Italy

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Abstract

Folate status may affect cancer risk through its role in both methylation and nucleotide synthesis of DNA. A low dietary intake of folate has been linked to risk of several cancers, but epidemiologic studies with reference to prostate cancer are scanty. We therefore analyzed data from a case-control study of prostate cancer conducted between 1991 and 2002 in various areas of Italy. Cases were 1,294 patients with incident, histologically confirmed prostate cancer and controls were 1,451 patients admitted to the same network of hospitals of cases for acute, nonneoplastic conditions. All subjects were <75 years old. Intake of folate and other nutrients was computed from a validated food frequency questionnaire. We adjusted for energy intake using the residual method, and calculated multivariate odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression. The OR of prostate cancer was 0.66 (95% CI, 0.51-0.85) for the highest versus the lowest quintile of folate intake. The relation between dietary folate and prostate cancer was consistent across strata of age, methionine, vitamin B6, and alcohol intake, and did not vary substantially according to Gleason score of prostate cancer. The combined OR for high-folate and low-alcohol intake versus low-folate and high-alcohol intake was 0.46 (95% CI, 0.29-0.75). Therefore, this study supports a favorable role of dietary folate on prostate cancer risk. (Cancer Epidemiol Biomarkers Prev 2005;14(4):944–8)

Introduction

A deficient supply of folate, a water-soluble B vitamin, has been related to the aetiology of several diseases, including cardiovascular conditions and cancer (1). Considering the latter, folate plays an important role in both methylation and nucleotide synthesis of DNA, and is essential in the conversion of methionine to *S*-adenosylmethionine, the principal methyl donor in the body (2).

Various epidemiologic studies investigated the relationship between dietary or serum folate and cancer risk. For colorectal and breast cancers, results showed consistent inverse associations and important interactions between folate, methionine, and/or alcohol intake (3-5). Risks for subjects in the highest level of folate intake were ~40% lower in a Canadian cohort study of colorectal cancer as well as in a Chinese case-control study of breast cancer (4, 6). Other cancer sites that have been inversely associated with dietary folate in epidemiologic studies are the ovary, the oral cavity and pharynx (7, 8). However, experimental animal studies on folate and cancer found conflicting results (9).

Folate- and methyl-related nutrient status could interact with folate-related polymorphisms such as the methylenetetrahydrofolate reductase, which has been in turn related to prostate cancer risk (10-13). Nevertheless, epidemiologic data on the relation between dietary folate intake and prostate cancer are scanty (14), and a study that investigated serum folate found no association with prostate cancer risk (15).

Thus, we considered the relation between intake of folate, as well as its combination with alcohol, methionine, and vitamin B6, and risk of prostate cancer in a case-control study conducted in Italy, a population with high alcohol consumption and infrequent use of supplements and multivitamins (16, 17).

Materials and Methods

Data were derived from a case-control study of prostate cancer, conducted between 1991 and 2002 in a network of 57 teaching and general hospitals in the greater Milan area, the provinces of Pordenone and Gorizia in northern Italy, the province of Latina in central Italy, and the urban area of Naples in southern Italy (18). Cases were 1,294 men (median age 66, range 46-74 years) admitted with incident, histologically confirmed prostate cancer to a network of hospitals in the areas under investigation. Controls were 1,451 patients (median age 63, range 46-74 years) admitted to the same hospitals as cases for a wide spectrum of acute, nonmalignant conditions, unrelated to long-term modifications of diet. Among controls, 32% had nontraumatic orthopaedic disorders, 21% traumas, 17% surgical conditions, and 29% miscellaneous other illnesses, such as eye, ear, and skin disorders. Cases and controls were identified and questioned by centrally trained interviewers who regularly visited the departments of the selected hospitals and approached the patients eligible as cases or controls on the basis of the admission diagnosis reported in the clinical records. Of the subjects approached, only 3% of cases and 4% of controls refused to be interviewed.

The same questionnaire, structured in 12 sections, was used in all study centers. It included information on socio-demographic factors such as marital status, education and occupation, anthropometric variables, smoking, alcohol, coffee consumption and other lifestyle habits, physical activity, medical history, aspirin use, and history of cancer in relatives.

Information on diet was based on a food frequency questionnaire, tested for reproducibility (19, 20) and validity (21). The food frequency questionnaire was part of the comprehensive questionnaire and was interviewer administered,

Received 10/27/04; revised 12/3/04; accepted 12/15/04.

Grant support: Italian Association for Cancer Research, the Italian League against Cancer, and the Italian Ministry of Education (COFIN 2003). C. Pelucchi was supported by a fellowship from the Italian Foundation for Cancer Research.

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too. It was aimed at assessing the usual diet during the 2 years preceding diagnosis (for cases) or hospital admission (for controls), and included questions on 78 foods, food groups, or recipes, divided into six sections: (*a*) bread, cereals, and first courses; (*b*) second courses (i.e., meat, fish, and other main dishes); (*c*) side dishes (i.e., vegetables); (*d*) fruits; (*e*) sweets, desserts, and soft drinks; (*f*) milk, hot beverages, and sweeteners. For a few vegetables and fruits, consumption in season and the corresponding duration were elicited. At the end of each section, one or two open questions were used to include foods that were not in the questionnaire but were eaten at least once per week. Energy and nutrient intakes, including folate, methionine, and vitamin B6, were computed from the food frequency questionnaire using an Italian food composition database (22). No information was available on vitamin B12 intake. A separate section investigated alcohol consumption in detail. The Pearson correlation coefficients for reproducibility and validity of information on energy intake were 0.70 and 0.61, respectively (20, 21). Corresponding values for alcohol intake in men, adjusted for energy, age, and center, were 0.71 for reproducibility and 0.55 to 0.68 for validity, according to the period of interview (23). Reproducibility and validity data for folate, methionine, and vitamin B6 intake were not available.

Data Analysis. Odds ratios (OR) of prostate cancer and the corresponding 95% confidence intervals (CI) for subsequent quintiles of folate intake were derived using unconditional multiple logistic regression models (24), including terms for age (in quinquennia), study center (Pordenone, Milan, Gorizia, Latina, and Naples), education (<7, 7-11, ≥12 years), body mass index (in quintiles), tobacco smoking (never, ex, and current smokers of <15, 15-24, ≥25 cigarettes/d), alcohol drinking (in quintiles of consumption, g/d), and family history of prostate cancer in first degree relatives (no/yes). Allowance was also made for nonalcohol energy intake, using the residual method (25). The significance of the trends in risk was assessed by comparing the differences between the deviances of the models, without and with a linear term for each variable of interest, to the χ^2 distribution with 1 degree of freedom (24). To test for interactions, the differences in $-2 \times$ log(likelihood) of the models with and without interaction terms were compared with the χ^2 distribution with the same number of degrees of freedom as the interaction terms.

Results

Table 1 shows the distribution of 1,294 cases of prostate cancer and 1,451 controls according to age and selected characteristics. Compared with controls, cases were older, had a higher level of education, and reported more often than controls a family history of prostate cancer. The distribution of cases and controls according to alcohol consumption and body mass index was similar.

The relationship between dietary folate, methionine, vitamin B6, and risk of prostate cancer is shown in Table 2. Prostate cancer was inversely associated with dietary folate intake (OR, 0.66 for the highest versus the lowest quintile of intake; 95% CI, 0.51-0.85; p for trend, 0.005). The OR for an increase of folate intake equal to a standard deviation (78.56 μg/d) was 0.92 (95% CI, 0.85-0.99). When we added soluble fiber intake in the multivariate models, the OR for the highest quintile of folate was 0.73 (95% CI, 0.54-0.97). Exclusion of regular users of aspirin (90 cases and 115 controls) and diabetics (99 cases and 89 controls) from the analyses did not change the results (data not shown). For methionine, the OR for the highest quintile of intake was 1.15, and there was no trend in risk. The corresponding value for vitamin B6 was 0.95. None of these estimates was significant.

Table 1. Distribution of 1,294 cases of prostate cancer and 1,451 controls according to age and selected characteristics. Italy, 1991-2002

	Cases	Controls	OR (95% CI)*
	n (%)	*n* (%)	
Age (y)			
<60	219 (17.0)	431 (29.7)	—
60-64	310 (23.9)	359 (24.7)	—
65-69	419 (32.4)	364 (25.1)	—
≥70	346 (26.7)	297 (20.5)	—
Education (y)†			
<7	636 (49.6)	844 (58.5)	1‡
7-11	384 (29.9)	407 (28.2)	1.5 (1.2-1.8)
≥12	263 (20.5)	192 (13.3)	2.1 (1.7-2.6)
Family history of prostate cancer			
No	1,204 (93.0)	1,423 (98.1)	1‡
Yes	90 (7.0)	28 (1.9)	4.0 (2.6-6.2)
Body mass index (kg/m^2)†			
<23.67	239 (18.5)	289 (20.0)	1‡
23.67 to <25.51	299 (23.2)	289 (20.0)	1.2 (1.0-1.6)
25.51 to <27.16	262 (20.3)	290 (20.0)	1.1 (0.8-1.4)
27.16 to <29.07	248 (19.2)	289 (20.0)	1.0 (0.8-1.3)
≥29.07	242 (18.8)	289 (20.0)	1.0 (0.8-1.3)
Alcohol consumption†			
Never drinkers	71 (5.5)	72 (5.0)	1‡
Ex-drinkers	93 (7.2)	128 (8.8)	0.7 (0.4-1.0)
Current drinkers	1,130 (87.3)	1,251 (86.2)	0.9 (0.6-1.2)
<14 drinks/wk	204 (15.8)	253 (17.4)	0.8 (0.5-1.2)
14-20 drinks/wk	257 (19.9)	238 (16.4)	1.0 (0.7-1.5)
21-34 drinks/wk	336 (26.0)	383 (26.4)	0.8 (0.5-1.2)
≥35 drinks/wk	333 (25.7)	374 (25.8)	0.8 (0.5-1.2)

*Estimates from multiple logistic regression including terms for age and study center.
† The sum does not add up to the total because of some missing values.
‡ Reference category.

We analyzed the effect of folate according to the degree of differentiation of prostate cancer, measured by Gleason score (data not shown). For 372 cases, this information was not available. The OR was 0.61 (95% CI, 0.43-0.86) for the highest quintile of folate intake in subjects with Gleason score <7, and 0.47 (95% CI, 0.32-0.69) in subjects with Gleason score ≥7.

Table 3 gives the OR of prostate cancer according to dietary intake of folate in strata of age, intake of alcohol, vitamin B6, and methionine. The risk of prostate cancer for the highest quintile of consumption of folate was somewhat lower in younger men (age <65 years; OR, 0.48), and for subjects with higher alcohol (OR, 0.44), higher vitamin B6 (OR, 0.42), and higher methionine (OR, 0.47) intakes. However, the ORs were not significantly heterogeneous across strata of these selected covariates (all p values for interaction were >0.05).

Table 4 reports the combined effect of folate and alcohol on the risk of prostate cancer. The ORs of prostate cancer were 0.72 for the intermediate category and 0.46 for high-folate and low-alcohol consumption compared with low-folate and high-alcohol intake.

Discussion

Two principal mechanisms have been proposed to link low folate status to increased cancer risk. First, folate deficiency may lead to decreased levels of *S*-adenosylmethionine and cause DNA hypomethylation and proto-oncogene activation. Second, folate deficiency induces uracil misincorporation in

Table 2. Relationship between dietary folate, methionine, vitamin B6, and risk of prostate cancer among 1,294 cases and 1,451 controls in Italy, 1991-2002

	Approximated quintile* of intake					*p* for trend	Continuous OR†
	1 (low)	2	3	4	5 (high)		
Folate intake‡							
Cases/controls	291:290	244:290	277:291	265:290	217:290		
OR (95% CI)§	1	0.84 (0.66-1.08)	0.87 (0.68-1.11)	0.85 (0.66-1.08)	0.66 (0.51-0.85)	0.005	0.92 (0.85-0.99)
Methionine intake‡							
Cases/controls	245:291	240:289	240:290	282:290	287:291		
OR (95% CI)§	1	0.99 (0.77-1.27)	0.96 (0.75-1.24)	1.13 (0.88-1.44)	1.15 (0.89-1.48)	0.15	1.04 (0.97-1.12)
Vitamin B6‡							
Cases/controls	260:291	266:290	269:290	247:290	252:290		
OR (95% CI)§	1	1.00 (0.78-1.28)	0.99 (0.77-1.27)	0.85 (0.66-1.09)	0.95 (0.74-1.22)	0.34	0.95 (0.88-1.03)

*Folate, methionine, and vitamin B6 intake were energy adjusted using the residual method.
†The measurement unit was set at 1 SD of the distribution of controls, calculated on the residual values. For absolute intakes, the SD values were 78.56 μg/d for folate, 637.07 mg/d for methionine, and 0.53 mg/d for vitamin B6.
‡Cutpoints for quintiles of absolute folate intake (nonresidual) were 196, 234, 270, and 316 μg/d; corresponding values for methionine were 1580, 1882, 2174, and 2557 mg/d, and for vitamin B6 were 1.49, 1.76, 1.99, and 2.32 mg/d.
§Estimates from unconditional logistic regression models adjusted for age, study center, education, body mass index, alcohol consumption, smoking habit, family history of prostate cancer, and nonalcohol energy intake. The reference category was the first (lowest) quintile of intake.

DNA synthesis, leading to chromosome breaks in humans, which could contribute to increase cancer risk (2, 26).

There are data in support of gene-folate status interactions in the aetiology of human cancer. Dietary folate can interact with proteins encoded by variant genes, such as the methylenetetrahydrofolate reductase, and reduce the risk of colorectal cancer and other conditions (1). Common variants of the methylenetetrahydrofolate reductase gene have been studied also in relation to prostate cancer (11-13). A study analyzed the C677T variant and found a 3.5-fold increased risk of prostate cancer in men with the methylenetetrahydrofolate reductase Val/Val genotype, and cancer risk was higher among subjects with lower folate intake (11). Thus, these findings support a role of folate metabolism also in prostatic carcinogenesis, besides colorectal. In this population, however, we had no information on genetic polymorphisms.

Vitamin B6 is a coenzyme of folate in biological reactions for DNA synthesis and methylation. As folate, vitamin B6 deficiency could be associated with chromosome breakage (27). A case-control study of diet and prostate cancer found an OR of 0.70 for a high intake of vitamin B6 from foods and supplements (28).

Methionine is involved together with folate in the production of *S*-adenosylmethionine, the primary methyl donor in the body. If methionine levels are low, more folate is used as methyltetrahydrofolate to form methionine. This may lower the level of methylenetetrahydrofolate, which is necessary for DNA synthesis (26).

In this study, no association emerged between vitamin B6 or methionine and prostate cancer risk. However, folate intake had a somewhat stronger protective effect in strata of high intake of both methionine and vitamin B6. Even if heterogeneity tests were not significant and these results should therefore be considered as merely indicative, it is noteworthy that the Shanghai Breast Cancer Study found similar outcomes (4).

Alcohol was not a risk factor for prostate cancer in this study (29), nevertheless its consumption may increase folate requirements in the body and cause relative folate deficiencies (3). Alcohol is thought to interfere with folate absorption and to increase folate excretion by the kidney (30, 31). Coherently, in this investigation, the protection conferred on prostate cancer risk by dietary folate was stronger in high alcohol drinkers, although again the ORs across subgroups were not heterogeneous. Companion Italian studies have

Table 3. Relationship between dietary folate and risk of prostate cancer in strata of selected covariates. Italy, 1991-2002

	Approximated quintile* of folate intake, OR (95% CI)†					*P* for trend
	1 (low)	2	3	4	5 (high)	
Age						
<65	1	0.72 (0.49-1.04)	0.67 (0.46-0.93)	0.71 (0.49-1.01)	0.48 (0.33-0.70)	0.0005
≥65	1	0.94 (0.68-1.31)	1.11 (0.79-1.55)	0.97 (0.69-1.36)	0.86 (0.61-1.22)	0.54
Alcohol intake						
Tertile 1	1	0.85 (0.55-1.32)	1.17 (0.74-1.85)	0.82 (0.52-1.28)	0.68 (0.43-1.08)	0.13
Tertile 2	1	0.92 (0.60-1.40)	0.81 (0.53-1.21)	0.88 (0.58-1.33)	0.83 (0.55-1.28)	0.42
Tertile 3	1	0.75 (0.49-1.17)	0.72 (0.47-1.09)	0.78 (0.51-1.19)	0.44 (0.27-0.70)	0.0025
Vitamin B6						
Tertile 1	1	0.97 (0.67-1.41)	1.04 (0.70-1.54)	1.02 (0.66-1.58)	0.92 (0.54-1.56)	0.93
Tertile 2	1	0.82 (0.53-1.26)	0.85 (0.56-1.31)	0.81 (0.52-1.25)	0.61 (0.38-0.97)	0.07
Tertile 3	1	0.46 (0.25-0.85)	0.52 (0.30-0.92)	0.51 (0.29-0.88)	0.42 (0.24-0.72)	0.015
Methionine intake						
Tertile 1	1	1.18 (0.77-1.83)	1.35 (0.87-2.11)	1.11 (0.70-1.73)	0.94 (0.60-1.46)	0.76
Tertile 2	1	0.75 (0.48-1.17)	0.71 (0.46-1.11)	0.83 (0.53-1.30)	0.67 (0.42-1.08)	0.22
Tertile 3	1	0.64 (0.42-0.98)	0.66 (0.44-1.00)	0.65 (0.43-0.99)	0.47 (0.30-0.72)	0.002

*Folate, methionine, and vitamin B6 intake were energy adjusted using the residual method.
†Estimates from unconditional logistic regression models adjusted for age, study center, education, body mass index, alcohol consumption, smoking habit, family history of prostate cancer, and nonalcohol energy intake. The reference category was the first (lowest) quintile of intake.

Table 4. Relation between the combined intake of folate and alcohol and the risk of prostate cancer. Italy, 1991-2002

Level of intake†	Cases	Controls	OR (95% CI)*
Low folate, high alcohol	107	101	1‡
All other combinations	1,122	1,253	0.72 (0.52-0.99)
High folate, low alcohol	65	97	0.46 (0.29-0.75)

*Estimates from unconditional logistic regression models adjusted for age, study center, education, body mass index, smoking habit, family history of prostate cancer, and nonalcohol energy intake.
†Folate intake was divided in quintiles and alcohol intake in tertiles.
‡Reference category.

found potential folate-alcohol interactions also in relation to cancers of the breast and colorectum (5, 32). In fact, the high proportions of regular and heavy drinkers in this population make it an ideal one to study the interrelationship between folate and alcohol consumption and cancer risk.

Other components of fruit and vegetables that are correlated with folate intake may account for the inverse association observed. Soluble fiber, for example, was positively correlated with folate intake ($r = 0.56$ after controlling for energy intake) and inversely associated with prostate cancer in this study (33). Nevertheless, after further adjustment for soluble fiber, the inverse association of folate was not materially modified. In the Italian male population, vegetables and fruit account for 27.5% of folate intake and cereals for 22.1% (34). The remaining 50% comes from various other food groups. It is therefore unlikely that the protection observed for folate could be ascribed to dietary intake of a specific food group. Furthermore, bread is the food item that provides the highest proportion of folate in Italian males (16.7%; ref. 34), but bread and carbohydrates were directly related with prostate cancer risk in this study (18, 35). This supports therefore a real role of folate on prostate cancer risk.

Aspirin and nonsteroidal anti-inflammatory drugs, if taken in large therapeutic amounts, may interfere with folate metabolism (36, 37). A diagnosis of diabetes most probably implicates a modification of dietary habits. Therefore, we conducted further analyses by excluding subjects with these characteristics. Given the reassuringly similar results, we concluded that the association observed for folate could not be attributed to interferences of these factors.

Use of supplements and multivitamins is still uncommon (i.e., <3%) in Italy (16) and our findings can therefore be attributed to dietary folate only. Information on supplements, however, has not been recorded in the questionnaire.

We tried to minimize typical bias of hospital-based case-control studies (24) by excluding all control patients with diagnoses linked to long-term changes in diet or admitted for chronic conditions. On the other hand, selecting hospital controls should reduce recall bias and improve comparability of information of cases and controls (38, 39). The strengths of this investigation are its large size, the use of a validated and reproducible food frequency questionnaire (19-21, 23), allowing to adjust for total energy intake and several micro- and macronutrients, and the low percentage of refusals of the subjects contacted.

In conclusion, this study found a significant inverse association between dietary folate and prostate cancer risk. The association was confirmed after adjustment for major known risk factors of prostate cancer and for energy intake, and was consistent across age strata. Methionine and vitamin B6 were unrelated to risk of prostatic carcinogenesis. The combined effect of high-folate and low-alcohol intake further decreased prostate cancer risk, up to a 54% risk reduction.

Acknowledgments

We thank M.P. Bonifacino for editorial assistance.

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