Dietary Intake of Vegetables and Fruits and the Modification Effects of *GSTM1* and *NAT2* Genotypes on Bladder Cancer Risk

Jie Lin,[1] Ashish Kamat,[2] Jian Gu,[1] Meng Chen,[1] Colin P. Dinney,[2] Michele R. Forman,[1] and Xifeng Wu[1]

Departments of [1]Epidemiology and [2]Urology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Abstract

We analyzed the association between intakes of vegetables and fruits as defined by the U.S. Department of Agriculture pyramid food groups and bladder cancer risk using data collected in a large case-control study. The study included 884 histologically confirmed bladder cancer cases and 878 healthy controls matched to cases by age (±5 years), gender, and ethnicity. Significant inverse associations were observed for intakes of total vegetables, cruciferous vegetables, orange vegetables, dark green vegetables, and bladder cancer risk. Compared with those in the lowest quartile of total vegetable intake, the odds ratios for the 2nd, 3rd, and 4th quartiles of total vegetable intake were 0.84 [95% confidence interval (95% CI), 0.64-1.10], 0.71 (95% CI, 0.54-0.95), and 0.67 (95% CI, 0.50-0.90), respectively (*P* for trend = 0.004). Compared with those in the lowest quartile, those in the highest quartile of cruciferous vegetable intake had an odds ratio of 0.69 (95% CI, 0.52-0.92; *P* for trend = 0.001) and those in the highest quartile of orange vegetable intake had an odds ratio of 0.68 (95% CI, 0.52-0.91; *P* for trend = 0.006). Furthermore, the protective effect of cruciferous vegetables was more evident in subjects carrying *GSTM1*-null (odds ratio, 0.43; 95% CI, 0.25-0.73 for the 4th quartile of intake) and *NAT2*-slow genotypes (odds ratio, 0.56; 95% CI, 0.33-0.97 for the 4th quartile of intake). No association was observed for intakes of total fruits or citrus fruits. Our data strongly support that high vegetable consumption, especially cruciferous vegetable intake, may protect against bladder cancer and that genetic variants of *GSTM1* and *NAT2* may modify the association. (Cancer Epidemiol Biomarkers Prev 2009;18(7):2090–7)

Introduction

Bladder cancer is the fourth most common cancer in U.S. men and the second most common urologic malignancy, with ~67,160 new cases and ~13,750 deaths in 2007 (1). Cigarette smoking and occupational exposure to aromatic amines are major risk factors (2).

Epidemiologic studies on diet and bladder cancer have revealed inconsistent results (3-21). A review of literature suggests that most cohort studies found no association for total vegetables and fruits. For vegetable intake, lack of an association was reported in four cohort studies (4-7) and three case-control studies (8-10) and an inverse association was found in two cohort studies (11, 12) and six case-control studies (13-18). Indeed, it is unclear from the literature which subgroup(s) of vegetables (e.g., yellow, green leafy vegetables, cruciferous vegetables) contributes to the protective effects (4, 11, 13, 14). The results are also inconsistent for the association between fruit intake and bladder cancer risk. No association was found in four cohort studies (4-6, 11) and two case-control studies for total fruits/citrus fruits (9, 16), whereas an inverse association was reported in one cohort study (12) and four case-control studies (8, 18-20).

It is increasingly recognized that genetic susceptibility contributes to bladder carcinogenesis (22, 23). However, it is less clear how genetic factors modify the association between diet and bladder cancer, with few studies reported to date (10, 14, 20). In this large bladder cancer case-control study, we investigated whether there is an association between dietary intakes of vegetables or fruits and bladder cancer risk and whether this association is modified by the two most consistent low penetrance bladder cancer susceptibility loci, *GSTM1* and *NAT2* (23).

Materials and Methods

Study Population and Epidemiologic Data Collection. Bladder cancer patients were recruited from The University of Texas M. D. Anderson Cancer Center and Baylor College of Medicine. The study started patient recruitment in 1999 and is currently ongoing. The procedures for subject recruitment and eligibility criteria were described previously (24). Briefly, cases were newly diagnosed and histologically confirmed urinary bladder cancer patients who had not received previous chemotherapy or radiotherapy before enrollment. Most cases (90%) were transitional cell carcinoma, but all other histologic subtypes of primary bladder cancer were also included. The International Classification of Diseases

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Requests for reprints: Xifeng Wu, Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Unit 1340, 1155 Hermann Pressler Boulevard, Houston, TX 77030. Phone: 713-745-2485; Fax: 713-792-4657. E-mail: xwu@mdanderson.org

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code is 188.9. There were no recruitment restrictions on age, gender, ethnicity, or cancer stage. The controls have been recruited from the Kelsey-Seybold Clinic, the largest multispecialty medical organization in Houston, Texas, which provides care to >400,000 patients at 18 clinic locations (25). Controls were recruited in a parallel timeframe as the cases. Controls were identified by reviewing short survey forms distributed to individuals visiting the clinic for the purpose of health check-ups or for addressing health concerns. The short survey forms elicit information on interest to participate in the study and basic demographic characteristics for matching. Potential control subjects were subsequently contacted by telephone to confirm their willingness to participate, and an appointment was scheduled at a Kelsey Seybold clinic site convenient to the participant. On the day of the interview, the controls visited the clinic specifically for the purpose of participating in this study but not for any treatment purposes. Control subjects were frequency matched to the cases by age (±5 years), gender, and ethnicity. Controls had no history of cancer (except nonmelanoma skin cancer). The response rates for cases and controls were 92% and 76.7%, respectively.

Written informed consent was obtained for each participant. The study has been approved by the institutional review boards of M. D. Anderson, Baylor College of Medicine, and Kelsey Seybold Clinics. Trained M. D. Anderson staff interviewers administered a 45-min risk factor questionnaire to study participants. Data were collected on demographic characteristics, occupational history, tobacco use history, medical history, and family history of cancer.

In addition to the risk factor questionnaire, a 45-min food-frequency questionnaire was administered to assess dietary intake during the year before bladder cancer diagnosis in the cases and the year before the interview in the control subjects. The food-frequency questionnaire was derived from a modified version of the National Cancer Institute Health Habits and History Questionnaire (26). The validity and reliability of the questionnaire has been documented previously (27). The questionnaire includes a semiquantitative food frequency list of 135 food and beverage items, ethnic foods commonly consumed in the Houston area, an open-ended section, and dietary behaviors such as dining at restaurant and food preparation methods. For each food item, the portion size was also queried. At the end of the interview, a blood sample of 40 mL was collected and delivered to laboratory. Blood samples were processed immediately, and DNA in peripheral blood lymphocytes was extracted and stored for molecular analysis.

Questionnaire responses were converted to the number of MyPyramid equivalents of each of the food groups of the MyPyramid Food Guidance System by use of the MyPyramid Equivalents Database corresponding to the U.S. Department of Agriculture Survey Food Codes, 1994 to 2002 (28). Briefly, the data files provide the number of MyPyramid equivalents from each MyPyramid Food Guidance System group per 100 grams of the food. Food codes used to process intakes were from the three recent national dietary surveys: What We Eat in America, National Health and Nutrition Examination Surveys 2001 to 2002 (29), National Health and Nutrition Examination Surveys 1999 to 2000 (30), and CSFII 1994 to 1996, 1998 (31). The description of what MyPyramid counts as a cup or ounce equivalent of vegetables and fruits is available from the MyPyramid Web site.[3] Total and subgroup-specific food intakes were derived by summing the daily MyPyramid equivalents across all relevant food items.

An individual who had never smoked or had smoked <100 cigarettes in his or her lifetime was defined as a never smoker. An individual who had smoked at least 100 cigarettes in his or her lifetime but had quit >12 mo before diagnosis (for cases) or before the interview (for controls) was classified as a former smoker. Current smokers were those who were currently smoking or quit <12 mo before diagnosis (for cases) or before the interview (for controls).

Genotyping for *GSTM1* and *NAT2*. DNA was isolated from peripheral blood lymphocytes by standard SDS/proteinase K method. Genotyping procedures for *GSTM1* and *NAT2* were described previously (32). In our laboratory, strict quality control procedures for genotyping are implemented to ensure high genotyping accuracy. Specifically, positive controls and negative controls were included in each plate. Five percent of the samples were randomly selected and run in duplicates to ensure accuracy of the genotyping. Laboratory staff was blind of case-control status of the samples.

Statistical Analysis. Distributions in categorical demographic variables between cases and controls were evaluated by the χ^2 test. Differences between cases and controls in continuous variables were tested using the Student's t test. Because intakes of vegetables and fruits were not normally distributed, median intakes of each food group between cases and controls were compared using the Wilcoxon rank sum test. Unconditional logistic regression was used to estimate the odds ratios and 95% confidence intervals (95% CI) while adjusting for age, gender, ethnicity, total energy, smoking status (never, former, current), smoking duration (the years between smoking initiation to present for current smokers and to the year of quitting for former smokers), number of cigarettes per day, and alcohol intake. The distribution of intake of each food group among the controls was categorized into quartiles as the cutoff points. A trend test was done to test for a linear trend in the odds ratios. Individual food items had discrete distributions, and we used a nutrient density model, in which daily intakes of foods were expressed as number of pyramid equivalents per 1,000 kcal of total energy. To account for multiple comparisons, we used the false discovery rate function in the R software (version 2.5) to estimate the false discovery rate based on the Benjamini-Hochberg method (33). We calculated the false discovery rate–adjusted p-values at 5% level to assess whether the resulting p-values were still significant after adjusting for multiple comparisons. All statistical tests were two-sided. Statistical analyses were done with the STATA software (version 8).

[3] http://www.mypyramid.gov/pyramid/index.html

Results

The study included 884 cases and 878 controls matched to cases by age (±5 years), gender, and ethnicity; therefore, there were no differences in the distribution of sex and ethnicity between cases and controls ($P = 0.61$ and $P = 0.89$, respectively; Table 1). The mean ages of the cases and controls were 64.5 and 65.0 years, respectively ($P = 0.29$). Bladder cancer cases had a significantly higher percentage of current smokers (24.89%) than the controls (8.31%; $P < 0.001$). Among ever smokers, cases reported more pack-year smoked, number of cigarettes per day, and smoking duration in years (Table 1). Compared with controls, cases had higher total energy intake (2,299.38 kcal versus 2,098.04 kcal; $P < 0.001$) and alcohol consumption (0.88 drinks per day versus 0.65 drinks per day; $P = 0.001$; Table 1).

Compared with cases, controls consumed significantly more dark-green, orange vegetables, cruciferous vegetables, and fruits other than citrus fruits, melons, and berries (Table 2). Controls also had higher total vegetable consumption than cases, but the difference was only borderline significant (Table 2). Please refer to Appendix 1 for individual food items included in each food group. When the dietary intakes among cases and controls were stratified by sex, similar patterns described above were observed for men and women; however, the differences did not reach statistical significance for women because of the small sample size of women in this study.

A significant inverse association was observed for total vegetable intake and bladder cancer risk (Table 3). Compared with the lowest quartile intake (the 1st quartile intake), the odds ratios for the 2nd, 3rd, and 4th quartiles were 0.84 (95% CI, 0.64-1.10), 0.71 (95% CI, 0.54-0.95), and 0.67 (95% CI, 0.50-0.90), respectively, with a significant does-response pattern (P for trend = 0.004). Among subgroups of vegetables, compared with the lowest quartile, subjects reporting cruciferous vegetables intake in the 3rd and 4th quartiles were at a significantly 30% (odds ratio, 0.70; 95% CI, 0.53-0.93) and 31% reduction in risk (odds ratio, 0.69; 95% CI, 0.52-0.92; P for trend = 0.001). Compared with the lowest quartile, the odds ratios for intake of orange vegetables for the 2nd, 3rd, and 4th quartiles were 0.86 (95% CI, 0.66-1.13), 0.77 (95% CI, 0.58-1.01), and 0.68 (95% CI, 0.52-0.91), respectively (P for trend = 0.006). The inverse association between intakes of dark-green vegetables and bladder cancer risk was only significant in the 3rd quartile (odds ratio, 0.74; 95% CI, 0.55-0.98), and the association for intake of tomatoes and tomato-containing products was only significant in the 2nd quartile (odds ratio, 0.72; 95% CI, 0.55-0.96; Table 3). Intakes of white potatoes and other starchy vegetables were not associated with the risk (Table 3). For the fruit groups, no associations were observed for total fruit consumption, citrus fruits only, or citrus fruits, melons, and berries combined (Table 3). However, higher intake of other fruits (see Appendix 1 for individual fruits in this group) was inversely associated with bladder cancer risk, with a dose-response trend (P trend = 0.01). Compared with the 1st quartile, the odds ratios for the highest quartile of intake was 0.69 (95% CI, 0.51-0.93).

We then did stratified analyses by smoking status. No significant associations were observed in never smokers (Supplementary Table 1). Among former smokers, inverse associations were observed in total vegetables, dark-green vegetables, cruciferous vegetables, and orange vegetables, and a dose-response relationship was significant in each of these subgroups (Supplementary Table 2). Among current smokers, intakes of dark-green vegetables and cruciferous vegetables in the 4th quartile were associated with significantly decreased risk (Supplementary Table 3).

Table 1. Selected characteristics of cases and controls

	Cases (n = 884)	Controls (n = 878)	P
Age, mean (SD)	64.47 (11.13)	64.99 (9.43)	0.29
Sex, n (%)			
Male	694 (78.51)	698 (79.50)	
Female	190 (21.49)	180 (20.50)	0.61
Ethnicity			
White	804 (90.95)	806 (91.8)	
Hispanic	37 (4.19)	29 (3.30)	
African-American	33 (3.73)	31 (3.53)	
Asian	8 (0.90)	8 (0.91)	
Other	2 (0.22)	4 (0.46)	0.89
Smoking status, n (%)			
Never	243 (27.49)	385 (43.85)	
Former	421 (47.62)	420 (47.84)	
Current	220 (24.89)	73 (8.31)	<0.001
Pack-years, median (range)	36 (0.1-176)	23 (0.05-165)	<0.001
Smoking duration, median (range)	33 (1.73)	25 (1-63)	<0.001
No. of cigarettes per day, mean (SD)	24.5 (14.3)	22.5 (15.5)	0.02
Alcohol intake (drinks per day), mean (SD)	0.88 (1.75)	0.65 (1.17)	0.001
Total energy (kcal)	2,299.38 (923.97)	2,098.04 (773.20)	<0.001

To further elucidate which individual vegetables contributed to the inverse association, the association between individual vegetable intake and bladder cancer risk was assessed. Among cruciferous vegetables, broccoli, cauliflower, green cabbage (including coleslaw and sauerkraut), and red cabbage were each individually associated with decreased bladder cancer risk in a significant dose-response pattern after false discovery rate adjustment for multiple comparisons (Supplementary Table 4). High intakes of orange, banana, and plum were each associated with decreased risk with significant dose-response trend (Supplementary Table 5). However, after adjustment for multiple comparisons, only the odds ratios for banana intake remained significant.

We next assess the modification effects of two most consistent low penetrance bladder cancer susceptibility loci, *GSTM1* and *NAT2*. *GSTM1* genotype data were available for 604 cases and 610 controls, and *NAT2* genotype data were available for 452 cases and 504 controls. We did an analysis comparing dietary differences between all subjects and subjects with genotype data available and found no differences (data not shown). The main effects of the two single-nucleotide polymorphisms were shown in Table 4. Consistent with previous report, the *NAT2*-slow genotype conferred a 1.41-fold increased risk (95% CI, 1.08-1.86; $P = 0.013$), and the association was borderline

significant for the *GSTM1*-null genotype (odds ratio, 1.22; 95% CI, 0.97-1.55; $P = 0.09$). The decreased risk associated with high consumption of cruciferous vegetables was evident in subjects who were *GSTM1*-null genotype; compared with the lowest quartile of intake, the odds ratios for the 2nd, 3rd, and 4th quartiles of intake were 0.76 (95% CI, 0.48-1.23), 0.43 (95% CI, 0.26-0.71), and 0.43 (95% CI, 0.25-0.73), respectively, and no significant associations were observed among the *GSTM1*-positive genotype (Table 5). Similarly, the decreased risk associated with cruciferous vegetable consumption was more evident in subjects carrying the *NAT2*-slow genotype, whereas no significant association was seen in *NAT2*-rapid genotype (Table 5). The interaction between cruciferous vegetable intake and *NAT2* was borderline significant ($P = 0.06$). The interaction between cruciferous vegetable intake and *GSTM1* was not significant ($P = 0.70$).

Discussion

In a large case-control study on bladder cancer, we found that total vegetable intake, intakes of cruciferous vegetables, orange vegetables, and dark-green vegetables, was associated with decreased bladder cancer risk in our study population. Moreover, the decreased risk associated with high cruciferous vegetable intake was evident in subjects carrying *GSTM1*-null or *NAT2*-slow genotypes. In contrast, total fruit intake was not associated with bladder cancer risk.

The lack of association between vegetable intake and bladder cancer risk was reported in four cohort studies (4-7) and three case-control studies (8-10), whereas two other cohort studies (11, 12) and six case-control studies (13-18) reported an inverse association. One large cohort study reported a RR of 0.49 for the highest quintile of intake of cruciferous vegetables (11). A large case-control study showed that dark-green vegetables and yellow-orange vegetables were protective with a dose-response trend (14). A few case-control studies with moderate sample sizes also support reduced bladder cancer risk with higher intakes of total vegetables or subgroups of vegetables (13, 15-18). The two cohort studies reporting inverse association (11, 12) included 33 and 21 vegetable items in the questionnaires, respectively, and the large case-control study reporting inverse association included 32 vegetable items (14), indicating a relatively high capability of these studies to capture vegetable intakes of study participants.

One class of compounds with anticarcinogenic properties present in cruciferous vegetables is isothiocyanates. We previously showed that high dietary intake of isothiocyanates was associated with decreased bladder

Table 2. Intakes of vegetables and fruits in cases and controls

	Cases	Controls	*P*
All subjects, median (range)	*n* = 884	*n* = 878	
Total vegetables	2.78 (0-37.66)	2.98 (0.24-24.99)	0.07
Dark-green vegetables	0.28 (0-21.97)	0.34 (0-8.76)	0.006
Orange vegetables	0.21 (0-5.05)	0.26 (0-7.38)	0.006
Cruciferous vegetables	0.34 (0-16.37)	0.45 (0-10.70)	0.001
White potatoes	0.85 (0-23.09)	0.75 (0-6.39)	0.002
Other starchy vegetables	0.20 (0-6.00)	0.19 (0-8.01)	NS
Tomatoes	0.56 (0-6.11)	0.55 (0-4.92)	NS
Total fruits	3.06 (0-24.35)	3.26 (0-59.61)	NS
Citrus fruits, melons, and berries	1.54 (0-20.74)	1.49 (0-47.40)	NS
Citrus fruits	0.58 (0-9.45)	0.57 (0-6.76)	NS
Other fruits	1.50 (0-15.18)	1.73 (0.01-16.91)	0.03
Men, median (range)	*n* = 694	*n* = 698	
Total vegetables	2.77 (0-37.66)	2.91 (0.24-24.99)	NS
Dark-green vegetables	0.26 (0-21.97)	0.32 (0-8.76)	0.01
Orange vegetables	0.21 (0-5.05)	0.25 (0-7.38)	0.02
Cruciferous vegetables	0.32 (0-16.37)	0.41(0-10.70)	<0.001
White potatoes	0.95 (0-9.99)	0.86 (0-6.39)	0.004
Other starchy vegetables	0.21 (0-3.14)	0.20 (0-8.01)	NS
Tomatoes	0.58 (0-6.11)	0.55 (0-4.92)	NS
Total fruits	2.96 (0-29.35)	3.12 (0-59.61)	NS
Citrus fruits, melons, and berries	1.47 (0-20.74)	1.46 (0-47.40)	NS
Citrus fruits	0.57 (0-9.45)	0.57 (0-6.76)	NS
Other fruits	1.63 (0.01-8.54)	1.49 (0-15.18)	0.02
Women, median (range)	*n* = 190	*n* = 180	
Total vegetables	2.86 (0.12-16.30)	3.56 (0.25-12.02)	NS
Dark-green vegetables	0.35 (0-6.00)	0.42 (0-4.24)	NS
Orange vegetables	0.24 (0-2.68)	0.32 (0.002-3.17)	NS
Cruciferous vegetables	0.39 (0-5.03)	0.59 (0-5.17)	NS
White potatoes	0.52 (0-23.09)	0.48 (0-5.57)	NS
Other starchy vegetables	0.18 (0-6.00)	0.16 (0-2.03)	NS
Tomatoes	0.51 (0-5.35)	0.53 (0.01-2.91)	NS
Total fruits	3.44 (0.04-22.09)	3.74 (0.04-17.72)	NS
Citrus fruits, melons, and berries	1.87 (0-16.63)	1.71 (0.01-13.52)	NS
Citrus fruits	0.78 (0-5.95)	0.58 (0-4.87)	NS
Other fruits	1.61 (0-11.66)	1.96 (0.04-10.73)	NS

NOTE: Unit is cup equivalents per day.
Abbreviation: NS, not significant.

cancer risk in the same study population (32). One mechanism for the anticarcinogenic action of isothiocyanates is through down-regulation of cytochrome *P*450 biotransformation enzyme levels and induction of phase II enzymes that detoxify residual electrophilic metabolites from the phase I enzymatic activity (34). Isothiocyanates were reported to induce apoptosis and/or arrest cell-cycle progression in two human bladder carcinoma lines (35). Storage of urine with higher level of isothiocyanates in the bladder may provide a biological explanation for the reduced risk for bladder cancer associated with higher intake of cruciferous vegetables.

In stratified analyses, the inverse association between vegetable intake and bladder cancer was more evident among smokers. Because phytochemicals found in vegetables (particularly in cruciferous vegetables) are able to activate detoxifying enzymes, smokers are expected to benefit the most from faster metabolism of carcinogens found in cigarette smoke (5, 11). Our data support this hypothesis. Alternatively, because the sample size of never smokers is much smaller than smokers in our study, the limited statistical power due to small sample size may be another explanation to the lack of association in never smokers. We also found that the inverse association among former smokers was stronger than in current smokers. One explanation is that the beneficial effect from vegetables is overwhelmed by current smoking.

We found that the association of cruciferous vegetables was more evident in subjects carrying *GSTM1*-null genotype. Similar findings were also observed in a colon cancer study (36) and in studies on lung cancer (37, 38).

Table 3. Pyramid food groups and bladder cancer risk

		Quartiles of intake				*P* for trend
		1st	2nd	3rd	4th	
Vegetables group						
Total vegetables	Cup equivalents/d	<1.88	1.88-2.98	2.99-4.54	>4.54	
	Case/control (no.)	252/219	224/220	208/219	200/220	
	OR	Ref.	0.84	0.71	0.67	0.004
	95% CI		0.64, 1.10	0.54, 0.95	0.50, 0.90	
Dark-green vegetables	Cup equivalents/d	<0.11	0.11-0.34	0.35-0.69	>0.69	
	Case/control (no.)	239/219	280/220	173/220	192/219	
	OR	Ref.	1.19	0.74	0.77	
	95% CI		0.92, 1.56	0.55, 0.98	0.58, 1.02	0.005
Cruciferous vegetables	Cup equivalents/d	<0.17	0.17-0.45	0.46-0.93	>0.93	
	Case/control (no.)	249/220	275/219	178/220	182/219	
	OR	Ref.	1.14	0.70	0.69	
	95% CI	Ref.	0.88,1.49	0.53, 0.93	0.52, 0.92	0.001
Orange vegetables	Cup equivalents/d	<0.12	0.12-0.26	0.26-0.52	>0.52	
	Case/control (no.)	274/220	223/218	199/219	188/221	
	OR	Ref.	0.86	0.77	0.68	0.006
	95% CI	Ref.	0.66,1.13	0.58,1.01	0.52, 0.91	
White potatoes	Cup equivalents/d	<0.34	0.34-0.75	0.76-1.36	>1.36	
	Case/control (no.)	195/220	209/219	207/219	273/220	
	OR	Ref.	0.99	0.89	1.02	0.96
	95% CI	Ref.	0.75, 1.32	0.67, 1.20	0.75, 1.39	
Other starchy vegetables	Cup equivalents/d	<0.08	0.09-0.19	0.20-0.35	>0.35	
	Case/control (no.)	206/222	214/217	234/221	230/218	
	OR	Ref.	1.01	1.07	0.98	1.00
	95% CI	Ref.	0.76, 1.33	0.81, 1.41	0.73, 1.30	
Tomatoes	Cup equivalents/d	<0.30	0.30-0.55	0.56-0.92	>0.92	
	Case/control (no.)	245/220	187/219	201/219	251/220	
	OR	Ref.	0.72	0.75	0.89	0.50
	95% CI	Ref.	0.55, 0.96	0.56, 1.00	0.67, 1.18	
Fruits group						
Total fruits	Cup equivalents/d	<1.74	1.74-3.26	3.27-5.29	>5.29	
	Case/control (no.)	249/220	212/219	200/219	223/220	
	OR	Ref.	0.92	0.89	0.9	0.49
	95% CI	Ref.	0.69, 1.21	0.67, 1.19	0.67, 1.20	
Citrus fruits, melons, and berries	Cup equivalents/d	<0.63	0.63-1.49	1.50-2.73	>2.72	
	Case/control (no.)	214/220	220/219	219/219	231/220	
	OR	Ref.	1.05	1.07	1.07	0.60
	95% CI	Ref.	0.80,1.39	0.81, 1.42	0.81,1.44	
Citrus fruits	Cup equivalents/d	<0.13	0.13-0.57	0.58-1.37	>1.37	
	Case/control (no.)	223/218	213/221	219/238	220/210	
	OR	Ref.	1.02	1.16	0.97	0.89
	95% CI	Ref.	0.77, 1.35	0.88, 1.52	0.73, 1.30	
Other fruits	Cup equivalents/d	<0.86	0.86-1.73	1.74-2.97	>2.97	
	Case/control (no.)	284/220	213/219	198/219	189/219	
	OR	Ref.	0.9	0.8	0.69	0.01
	95% CI	Ref.	0.69, 1.19	0.60, 1.06	0.52, 0.93	

NOTE: Odds ratio adjusted for age, sex, ethnicity, smoking status, smoking duration, number of cigarettes per day, total energy intake, and alcohol consumption.
Abbreviations: Ref., reference; OR, odds ratio.

Table 4. *GSTM1* and *NAT2* genotypes and bladder cancer risk

	Cases	Controls	OR* (95% CI)	*P*
GSTM1				
Positive	292 (48.34)	324 (53.11)	Ref.	
Null	312 (51.66)	286 (46.89)	1.22 (0.97-1.55)	0.09
NAT2				
Rapid	171 (37.83)	240 (47.62)	Ref.	
Slow	281 (62.17)	264 (52.38)	1.42 (1.08-1.86)	0.01

*Adjusted by age, gender, ethnicity, and smoking status.

Because isothiocyanate itself is a substrate for GSTM1, in *GSTM1*-positive genotype, the ability of isothiocyanate to inhibit phase I activation and induce phase II detoxification enzymes is compromised because of the rapid isothiocyanates metabolism by GSTM1. In contrast, in *GSTM1*-null genotype, the protective effect of isothiocyanate is used to the maximum. Our recent result (32) showed that the decreased risk for bladder cancer associated with high isothiocyanate intakes was significant, regardless of *GSTM1* genotype. The reason for the inconsistent results is unknown. One possibility is that there may be compounds other than isothiocyanates that could potentially contribute to the protective effects on bladder cancer risk, which may be modified by the *GSTM1* genotype.

We also found that the association between cruciferous vegetables and bladder cancer was more evident in subjects carrying *NAT2*-slow genotype. The major function of NAT2 is inactivating aromatic amines through *N*-acetylation; other tasks include activating certain arylamine metabolites through *N*- and *O*-acetylation (39). Increased risk for bladder cancer has been consistently reported to be associated with *NAT2*-slow acetylator genotypes in large studies and in meta-analysis (23). Our results indicated that subjects carrying *NAT2*-slow acetylator genotypes may benefit more from high cruciferous vegetable intake.

The protective effects of orange vegetables in the current study were in agreement with findings from a large case-control study (14). We previously reported the protective role of dietary total and individual carotenoids that are concentrated in red, yellow, and orange vegetables and fruits (40). Carotenoids have a wide range of anticancer properties (41), including the ability to quench free radicals and reactive oxygen species and enhance cell-cell communications (42).

An interesting finding of our study is that only tomato juice but not raw tomato intake was associated with the reduced risk. The risk reduction may be attributed to lycopene or to other nutrients found in tomatoes, such as vitamin C (43). Lycopene is thought to inhibit proliferation of cancer cells at the G_0-G_1 cell cycle phase (44) and protect cellular DNA from oxidative damage (45). Lycopene concentrations from tomato products are increased when the foods are processed at high temperatures, thus, canned pasteurized tomato juice and source contain more bioavailable lycopene than fresh tomatoes (46). However, most previous studies did not differentiate raw versus cooked tomatoes (12, 14, 19). Only one study reported on raw and cooked tomatoes but did not find an association for either with bladder cancer risk (8). Comparison of the frequencies of cooked tomato consumption between our study and the study by Kellen et al. (8) revealed that the range of cooked tomato consumption was larger in our study. For example, the tertile cutoff points (in grams per day) in our study were 8.0 and 38.2, whereas in their study, the cutoff points (in grams per day) were 12.3 and 30.8. The greater variation of consumption in our study population may increase the statistical power to detect an association.

Another interesting finding of this study is the significant decreased risk associated with high intake of fresh garlic. Garcia-Closas et al. (10) found a borderline protective effect of Liliaceae vegetables. Compelling evidence has suggested that garlic and its organic allyl sulfur components are effective inhibitors of carcinogenesis (47). Garlic and its allyl sulfur constituents have been reported to exert their protective effects on carcinogenesis in animal and *in vitro* studies by several mechanisms, including inhibition of carcinogen-induced DNA adduct formation (48), blockage of cell growth and cell proliferation (49), induction of differentiation and/or apoptosis (50), and inhibition of lipid peroxidation (51). However, in the Netherland Cohort Study, intake of allium vegetables was not associated with bladder cancer risk (12). Given the scanty published data, the role of allium vegetables, especially garlic intake, in bladder cancer etiology warrants further study.

Our data did not support an association of total and/or citrus fruits on bladder cancer risk. Our results are consistent with several cohort studies (4-6, 11) and case-control studies (9, 16). However, a number of case-control studies reported an inverse association between bladder cancer and intakes of total fruits (8, 12, 18-20) and citrus fruits (12, 14, 21). An early meta-analysis revealed an odds ratio of 1.4 (95% CI, 1.08-1.83) for low fruit intake (3). According to the U.S. Department of Agriculture MyPyramid Food Guidance System, the recommended fruit intake for women is 1.5 cups for 31 to 50 years old and 2.0 cups for ages 30 or younger. The

Table 5. Cruciferous vegetable intake and bladder cancer risk stratified by *GSTM1* and *NAT2* genotypes

Cases/controls (no.)	OR*	95% CI	*P*
GSTM1 positive			
80/78	Ref.		
99/87	1.12	0.71, 1.76	0.63
55/75	0.67	0.40, 1.10	0.12
58/84	0.66	0.40, 1.08	0.10
GSTM1 null			
101/60	Ref.		
98/77	0.76	0.48, 1.23	0.27
58/81	0.43	0.26, 0.71	0.001
55/68	0.43	0.25, 0.73	0.002
NAT2 rapid			
41/58	Ref.		
63/61	1.67	0.93, 2.98	0.9
37/60	0.91	0.48, 1.70	0.76
30/61	0.74	0.39, 1.42	0.37
NAT1 slow			
95/56	Ref.		
81/76	0.65	0.40, 1.06	0.09
49/69	0.39	0.23, 0.66	0.001
56/63	0.51	0.30, 0.88	0.02

*Adjusted for age, sex, ethnicity, smoking status, smoking duration, number of cigarettes per day, total energy intake, and alcohol consumption.

recommended daily amount is 2.0 cups for men at all ages.[4] We showed that the median fruit intake was about 3 cup equivalents in men and more than three cups in women (See Table 3). Specifically, in our study population, 67.9% male controls and 65.8% male cases reported consuming at least two cups of fruits daily; and 76.0% of female controls and 72.4% of female cases consumed two cups or more fruits daily, suggesting that a high percentage of our study subjects (cases and controls) meet the U.S. Department of Agriculture recommended daily fruit intake. Thus, the high fruit intake in the study population may explain the lack of association.

The various units used to quantify fruits intake in different studies make it difficult to compare fruit intake across study populations. For example, fruits intake was reported in units as grams per day (4, 12), times per day per week (19), or servings per year (14). A few studies did not report the units used to quantify fruit intake (18, 20, 21). Future studies in this regard should have a consistent measure to quantify fruit intake. It should be noted that, in our study, although there was no association for total fruit intake or total citrus fruit intake, individual fruits orange, banana, apple, and plum were each associated with reduced risk. However, most previous studies reported odds ratios of fruits and/or citrus fruits as a group without separate analysis of individual fruits. The findings from this study suggest that examining total fruit intake or total citrus fruits intake may obscure potential protective effects of individual fruits.

It is well known that individuals who eat healthy diets generally have relatively healthy lifestyles, lower incidences of smoking, and higher levels of physical activities. Although our analyses were well controlled for smoking because smoking duration, number of cigarettes per day, and smoking status were adjusted in the multivariate model, residual confounding by smoking cannot be completely ruled out. Another limitation in case-control studies is recall bias, in which healthy controls are more likely to recall ''healthy food'' intake (such as vegetable and fruits intakes) than patients, leading to biased estimates of relative risk. However, in our study, the odds ratio was not lower for all vegetables or fruits groups studied, suggesting that recall bias was not of a major concern. Moreover, the case-control study design may subject to selection bias, in which the foods intake of participants and nonparticipants differ. Thus, prospective cohort studies are needed to better unveil the causal relationship between diet and cancer.

In conclusion, our data strongly support that high vegetable consumption, in particular, cruciferous vegetables and orange vegetables, may protect against bladder cancer and that genetic variants in *GSTM1* and *NAT2* may modify the association. The results add to the few publications in the literature to stress that genetic variants could modify the association between diet and bladder cancer. This study has important implication in bladder cancer prevention.

[4] http://www.mypyramid.gov/pyramid/fruits_amount.aspx#

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Appendix A. Specific Food Items in Each Food Group

Dark-green vegetables: broccoli, green turnip, raw spinach, cooked spinach, green mustard, collards, kale.

Cruciferous vegetables: broccoli, cauliflower, green mustard, collards, green cabbage, coleslaw, sauerkraut, red cabbage, green turnip, kale, bokchoy.

Orange vegetables: green cabbage, coleslaw, sauerkraut, winter squash, carrots, sweet potatoes.

White potatoes: salty snacks (potato chips); French fries and fried potatoes, including hash browns and in breakfast tacos; other potatoes, including boiled, baked, mashed, and in potato salads; beef stew or potpie made with beef, carrots, or other vegetables; vegetable soup, vegetable beef, chicken vegetable, or tomato soup.

Other starchy vegetables: green peas; corn; vegetable soup; vegetable beef, chicken vegetable, or tomato soup.

Tomatoes: catsup; raw tomatoes, including in salad; salsa, picante, and taco sauce; packaged or canned meatless vegetarian chili; tomato juice; canned chili; pizza; spaghetti, lasagna, and other pasta with tomato-based sauces; beef stew or potpie made with beef, carrots, or other vegetables; salad dressing (low fat); barbecue; chicken or turkey in mixed dishes such as stews, potpies, or casseroles; salad dressing.

Citrus fruits, melons, and berries: cantaloupe; watermelon; blueberry; strawberry; orange; orange juice; grapefruit juice; drinks with some juice in them, such as Sunny Delight or Start; Hi-C, Kool-Aid, or other fruit drinks with added vitamin C; green cabbage, coleslaw, and sauerkraut; yogurt; low-fat yogurt; salad dressing; kiwi; orange juice with calcium; cranberry juice; other melons such as honeydew and casaba; grapefruit; other berries such as raspberries, blackberries, boysenberries; real mayonnaise or mayonnaise substitutes.

Citrus fruits: orange, orange juice, grapefruit juice, orange juice with calcium, grapefruit.

Other fruits: apple; banana; peach; apricot; prune; plum; high-fiber cereals like All Bran, Raisin Bran, and Fruit-n-Fiber; other pies or cobblers; breakfast bars, granola bars, and power bars; pear; avocado; pineapple; grape; apple juice or grape juice; raisin; cherry.

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