Consumption of Raw Cruciferous Vegetables is Inversely Associated with Bladder Cancer Risk

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Abstract

Cruciferous vegetables contain isothiocyanates, which show potent chemopreventive activity against bladder cancer in both *in vitro* and *in vivo* studies. However, previous epidemiologic studies investigating cruciferous vegetable intake and bladder cancer risk have been inconsistent. Cooking can substantially reduce or destroy isothiocyanates, and could account for study inconsistencies. In this hospital-based case-control study involving 275 individuals with incident, primary bladder cancer and 825 individuals without cancer, we examined the usual prediagnostic intake of raw and cooked cruciferous vegetables in relation to bladder cancer risk. Odds ratios (OR) and 95% confidence intervals (CI) were estimated with unconditional logistic regression, adjusting for smoking and other bladder cancer risk factors. We observed a strong and statistically significant inverse association between bladder cancer risk and raw cruciferous vegetable intake (adjusted OR for highest versus lowest category = 0.64; 95% CI, 0.42-0.97), with a significant trend (*P* = 0.003); there were no significant associations for fruit, total vegetables, or total cruciferous vegetables. The associations observed for total raw crucifers were also observed for individual raw crucifers. The inverse association remained significant among current and heavy smokers with three or more servings per month of raw cruciferous vegetables (adjusted ORs, 0.46 and 0.60; 95% CI, 0.23-0.93 and 0.38-0.93, respectively). These data suggest that cruciferous vegetables, when consumed raw, may reduce the risk of bladder cancer, an effect consistent with the role of dietary isothiocyanates as chemopreventive agents against bladder cancer. (Cancer Epidemiol Biomarkers Prev 2008; 17(4):938–44)

Introduction

Some studies suggest that cruciferous vegetable consumption may reduce the risk of various cancers (1-3). Bladder cancer is particularly interesting because dietary isothiocyanates, a group of key anticarcinogens occurring in cruciferous vegetables, show potent chemopreventive effects against bladder cancer in both *in vitro* and *in vivo* models (4-9). Moreover, the unique *in vivo* pharmacokinetics and disposition of orally ingested isothiocyanates in humans renders the bladder the best targeted organ for the anticancer effect of dietary isothiocyanates (5). Isothiocyanates are metabolized primarily via the mercapturic acid pathway *in vivo*, and are rapidly and almost exclusively excreted and concentrated in the urine in the form of *N*-acetylcysteine conjugates (NAC-ITCs; refs. 10-12). NAC-ITCs are unstable and dissociate to release isothiocyanates (4, 13), and storage of urine containing NAC-ITCs in the bladder likely further enhances the exposure of bladder cells to these compounds. As a result, the bladder epithelium that gives rise to the majority of bladder cancers (90-95% in the United States; ref. 14), is likely the tissue most exposed to isothiocyanates and their metabolites *in vivo*. Therefore, as the richest source of isothiocyanates in the human diet, cruciferous vegetables may be especially promising for bladder cancer chemoprevention.

Only a few epidemiologic studies have investigated the relationship between bladder cancer risk and intake of cruciferous vegetables, with inconsistent findings (15-20). Not accounting for whether cruciferous vegetables are eaten raw or cooked may partly explain this inconsistency, because cooking may substantially destroy and/or reduce isothiocyanates obtained from cruciferous vegetables, subsequently attenuating the cancer-preventive potential of these vegetables. Isothiocyanates are stored in plants as glucosinolates (β-thioglucoside *N*-hydroxysulfates), and are released by myrosinase (thioglucoside glucohydrolase), an enzyme that coexists with, but is physically separated from glucosinolates in the plant under normal conditions (21). When plant cells are damaged, such as during food processing or chewing, myrosinase is released and catalyzes the conversion (21, 22).

It is noteworthy that the yield of isothiocyanates may be largely reduced by normal cooking procedures due to heat-inactivating myrosinase, destroying heat-labile isothiocyanates and losing glucosinolates (23-25). Although human intestinal microflora also possess myrosinase activity and is able to partially hydrolyze ingested glucosinolates, studies have shown that isothiocyanate exposure after the consumption of cooked cruciferous vegetables is 60% to 90% less than that after the ingestion of raw cruciferous vegetables (23-26). Under certain

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Table 1. Descriptive characteristics of bladder cancer cases and hospital controls

	Bladder cancer cases		Controls		P (t test)
	No.*	Means ± SD	No.	Means ± SD	
Age (y)	275	64.8 ± 10.2	825	64.8 ± 10.5	0.9894
Vegetables (servings/mo)	252	73.8 ± 41.9	764	80.7 ± 43.1	0.0257
Fruits (servings/mo)	265	42.3 ± 29.2	795	49.6 ± 32.3	0.0007
Meats (servings/mo)	261	33.9 ± 26.5	789	30.4 ± 21.5	0.0568
Cruciferous (servings/mo) †	262	13.5 ± 14.5	782	15.8 ± 15.3	0.0353
Raw cruciferous (servings/mo) ‡	267	4.5 ± 6.5	800	5.9 ± 7.4	0.0037
Pack-years	272	41.9 ± 35.0	804	23.0 ± 29.8	<0.0001

	Bladder cancer cases, no. (%)	Controls, no. (%)	P (χ^2 test)
Education			
<High school	100 (36.6)	203 (24.7)	
>High school	173 (63.4)	619 (75.3)	0.0001
Smoking status			
Never	55 (20.0)	315 (38.2)	
Quit	152 (55.5)	410 (49.8)	
Current	67 (24.5)	99 (12.0)	<0.0001

*Numbers vary due to missing data.
†Cruciferous vegetables: broccoli (raw and cooked), cabbage (raw and cooked), cauliflower (raw and cooked), Brussels sprouts, kale, turnip, collard, mustard greens.
‡Raw cruciferous vegetables: raw broccoli, raw cabbage, raw cauliflower.

circumstances, such as antibiotic treatment which reduces bowel microflora, the production of isothiocyanates in the gastrointestinal tract is almost negligible after the consumption of cooked cruciferous vegetables (26). Therefore, raw cruciferous vegetables may be the form more appropriate for assessing the association with bladder cancer risk. To evaluate this hypothesis, we examined the role of cruciferous vegetables in relation to bladder cancer risk with the consideration of raw versus cooked consumption in a hospital-based case-control study.

Materials and Methods

The study population included individuals who received medical services at Roswell Park Cancer Institute between 1982 and 1998, and agreed to complete a comprehensive epidemiologic questionnaire. The case group consisted of 275 individuals with bladder cancer (International Classification of Diseases-9 codes 188 and 233.7), identified from the Roswell Park Cancer Institute tumor registry and diagnostic index. Approximately 95% of cases were transitional cell papilloma or carcinoma, and squamous cell carcinoma, adenoma, and others accounted for the other 5% of cases. Patients in the case group were predominantly Caucasian (98%) and ranged in age from 25 to 86 years old. Controls were frequency matched to cases on age, gender, and decade in which they completed the questionnaire, with a control to case ratio of 3:1. Controls included in the analysis (n = 825) were individuals with conditions other than cancer, including infectious and parasitic diseases (20%), diseases of the circulatory system (12%), ill-defined signs and symptoms (17%), disease of the genitourinary system (13%), benign neoplasms (8%), and other various conditions (28%) treated at Roswell Park Cancer Institute during the same time period as were cases. Controls were randomly selected from a pool of 6,302 eligible patients. Like the cases, participants in the control group were predominantly Caucasian and ranged in age from 21 to 92 years old. The Roswell Park Cancer Institute Institutional Review Board approved the conduct of the study.

All participants completed the Patient Epidemiology Data System questionnaire, which was offered to all new patients as part of the admission process. The overall response rate for both controls and cases was ~50%. Median time between diagnosis and participation in the Patient Epidemiology Data System was 21 days; 68% of cases participated within 2 months of diagnosis. The Patient Epidemiology Data System questionnaire requested detailed information on demographic background, occupational and environmental exposures, tobacco and alcohol consumption, reproductive experiences, medical history, and family history of cancer, as well as a 44-item food frequency questionnaire assessing usual diet several years before diagnosis. The 44-item food frequency questionnaire was designed to provide an assessment of intakes of fruits and vegetables, cruciferous vegetables, and foods providing good sources of vitamins A, C, and E, fat, and fiber (27).

For each food category (fruits, vegetables, cruciferous vegetables, raw cruciferous vegetables), monthly frequency of use was calculated. Intake for each food category was divided into quartiles based on the distribution of intake in the controls. For individual cruciferous vegetables, intake was divided into two categories (<1 or ≥1 servings per month) for the purpose of comparison among vegetables. The cruciferous vegetable category consisted of broccoli (raw and cooked), cabbage (raw and cooked), cauliflower (raw and cooked), Brussels sprouts, and greens including kale, turnip, collard, and mustard greens. Raw cruciferous vegetables were defined as raw broccoli, cabbage, and cauliflower to better represent dietary intake of isothiocyanates.

For continuous variables, differences between case and control groups were evaluated with two-tailed t tests.

Table 2. ORs and 95% CIs for the association of bladder cancer with fruit, vegetable, and cruciferous vegetable intake

	Number		Adjusted OR* (95% CI)
	Cases	Controls	
Vegetables (servings/mo)			
<51	80	191	1.00
51-74.5	61	198	0.80 (0.53-1.22)
75-105	61	187	0.88 (0.58-1.36)
>105	50	188	0.77 (0.49-1.21)
			P for trend = 0.35
Fruits (servings/mo)			
<27.5	87	200	1.00
27.5-46	71	201	1.04 (0.70-1.56)
46.5-64.5	61	196	0.92 (0.60-1.40)
>64.5	46	198	0.71 (0.45-1.11)
			P for trend = 0.15
Cruciferous (servings/mo)			
<6	96	213	1.00
6-10.5	56	179	0.81 (0.53-1.22)
11-22	57	201	0.65 (0.43-0.98)
>22	53	189	0.81 (0.53-1.24)
			P for trend = 0.17
Raw cruciferous (servings/mo)			
<2	111	244	1.00
2-3	76	204	0.82 (0.56-1.20)
4-6	30	161	0.44 (0.27-0.72)
>6	50	191	0.64 (0.42-0.97)
			P for trend = 0.003

*ORs and 95% CIs were calculated with unconditional logistic regression adjusted for age (continuous), education level (<high school or >high school), gender (male or female), total meat intake (continuous), smoking status (never, quit, or current), and pack-years (continuous).

Pearson's χ^2 was used to compare cases and controls for the categorical variables of education level and smoking status. Odds ratios (OR) and 95% confidence intervals (95% CI) of food type and bladder cancer risk were computed by unconditional logistic regression in separate models using the lowest quartile as the referent category. The adjusted logistic models included age (continuous), education level (<high school or >high school), gender (male or female), total meat intake (continuous), smoking status (never, quit, or current), and pack-years (continuous). Total meat intake was included in the model to partially adjust for differences in dietary patterns associated with cruciferous vegetable intake. To control for smoking in ever smokers, pack-years were computed as the number of packs of cigarettes smoked per day multiplied by the number of years smoking to incorporate all past smoking experience. Tests for trend were conducted by using the median values for each quartile as continuous variables in logistic regression. To test for multiplicative interactions, the cross-product terms were included in a logistic regression model. All statistical tests were two-sided with a type I error rate of 0.05. Statistical analyses were done using SAS for Windows, version 9.1.

Results

Descriptive characteristics of cases and controls are presented in Table 1. The same mean ages for cases and controls (64.8 years, $P = 0.99$) indicated their well-matched status. On average, cases consumed fewer fruits and vegetables, including both raw and total crucifers, and had lower levels of education than controls. As anticipated, there was a significant difference in the distribution of smoking status between cases and controls with more current smokers (24.5%) and fewer never smokers (20%) among the cases than the controls (12.0% current smokers and 38.2% never smokers; $P < 0.0001$). Among both former and current smokers, the cases had more pack-years on average than the controls (41.9 versus 23.0; $P < 0.0001$). Heavy smokers had a substantially higher risk of bladder cancer, with those smoking 33 or more pack-years having more than three times the risk of bladder cancer compared with never smokers (OR, 3.6; 95% CI, 2.5-5.1).

ORs and 95% CIs for the association of bladder cancer with monthly intake of fruits, vegetables, crucifers, and raw crucifers are presented in Table 2. In unadjusted analyses, bladder cancer risk was negatively associated with increasing consumption of each of these categories (results not shown). After adjustment for gender, education level, age, total meat intake, smoking status, and pack-years, however, only associations with total raw cruciferous vegetable intake remained statistically significant (*P* for trend = 0.003), with those in the higher quartiles of consumption of total raw cruciferous vegetable intake (4-6 and >6 servings/mo) having a 36% to 56% lower risk of bladder cancer compared with those in the lowest quartile (<2 servings/mo; ORs, 0.44 and 0.64; 95% CI, 0.27-0.72 and 0.42-0.97, respectively).

Associations between intake of individual cruciferous vegetables and bladder cancer risk are shown in Table 3.

Table 3. ORs and 95% CIs for the association of bladder cancer with intakes of individual cruciferous vegetables

	Number		Adjusted OR* (95% CI)
	Cases	Controls	
Broccoli raw (servings/mo)			
<1	213	525	1.00
≥1	59	282	0.57 (0.40-0.81)
Broccoli cooked (servings/mo)			
<1	113	276	1.00
≥1	159	537	0.88 (0.65-1.20)
Cabbage raw (servings/mo)			
<1	145	347	1.00
≥1	126	470	0.68 (0.50-0.91)
Cabbage cooked (servings/mo)			
<1	176	492	1.00
≥1	96	323	0.88 (0.64-1.20)
Cauliflower raw (servings/mo)			
<1	209	550	1.00
≥1	59	260	0.68 (0.48-0.97)
Cauliflower cooked (servings/mo)			
<1	151	365	1.00
≥1	122	450	0.72 (0.54-0.98)
Brussels sprouts (servings/mo)			
<1	195	578	1.00
≥1	76	234	1.02 (0.73-1.42)
Kale, turnip, collard, mustard greens (servings/mo)			
<1	213	633	1.00
≥1	53	168	0.88 (0.60-1.28)

*ORs and 95% CIs were calculated with unconditional logistic regression adjusted for age (continuous), education level (<high school or >high school), gender (male or female), total meat intake (continuous), smoking status (never, quit, or current), and pack-years (continuous).

Table 4. Adjusted ORs and 95% CIs for the association of bladder cancer with intakes of raw broccoli, cabbage, and cauliflower

Cabbage raw (servings/mo)	Broccoli raw (servings/mo)	
	<1	≥1
<1	1.00*	0.74 (0.43-1.31)
≥1	0.81 (0.57-1.16)	0.44 (0.28-0.68)
	P for interaction = 0.36	
Cauliflower raw (servings/mo)	**Broccoli raw (servings/mo)**	
	<1	≥1
<1	1.00*	0.62 (0.38-1.03)
≥1	0.95 (0.58-1.55)	0.48 (0.30-0.76)
	P for interaction = 0.60	
Cauliflower raw (servings/mo)	**Cabbage raw (servings/mo)**	
	<1	≥1
<1	1.00*	0.71 (0.50-1.02)
>1	0.69 (0.38-1.25)	0.55 (0.36-0.85)
	P for interaction = 0.75	

NOTE: ORs and 95% CIs were calculated with unconditional logistic regression adjusted for age (continuous), education level (<high school or >high school), gender (male or female), total meat intake (continuous), smoking status (never, quit, or current), and pack-years (continuous).
*Referent.

In unadjusted analyses, intakes of broccoli, cabbage, and cauliflower (cooked and/or raw) were significantly and inversely associated with bladder cancer risk (results not shown). After adjustment for gender, education level, age, total meat intake, smoking status, and pack-years, however, the inverse associations for most cooked cruciferous vegetables were no longer statistically significant. Other cruciferous vegetables including Brussels sprouts, kale, turnips, collard and mustard greens were not associated with risk of bladder cancer. In contrast to the associations observed for cooked cruciferous vegetables, all three raw cruciferous vegetables (broccoli, cabbage, cauliflower) remained significantly inversely associated with the risk of bladder cancer after adjustment for potential confounders. The risk estimates for high versus low intake of the raw cruciferous vegetables ranged from 0.57 to 0.68. To further examine the association between individual raw cruciferous vegetables and bladder cancer risk, joint classifications of broccoli and cabbage, broccoli and cauliflower, and cabbage and cauliflower were created (Table 4). The adjusted ORs showed negative associations with increasing intake of any of those three raw cruciferous vegetables. The likelihood ratio test did not indicate any significant interaction between these three raw cruciferous vegetables.

Using median raw cruciferous vegetable intake in controls as the cutoff point, analyses of raw cruciferous vegetable intake and bladder cancer risk were stratified by various known confounders (Table 5). The results were similar between males and females, and between younger (<66 years old) and older subjects, and regardless of gender and age, patients with a high consumption of raw cruciferous vegetables had a lower risk of bladder cancer (results not shown). Smoking is a major risk factor for bladder cancer. When the data were stratified by smoking status, although all groups experienced decreased risk of bladder cancer, a significant inverse association between raw cruciferous vegetable intake and bladder cancer risk was observed in current smokers (OR, 0.46; 95% CI, 0.23-0.93) only. When dichotomizing smoking exposure at 33 pack-years, the inverse association was significant among heavy smokers (≥33 pack-years; OR, 0.60; 95% CI, 0.38-0.93), but not among lighter smokers (<33 pack-years; OR, 0.69; 95% CI, 0.39-1.23).

Discussion

Before statistical adjustment for potential confounders, consumption of total fruit, vegetables, cruciferous vegetables, and raw cruciferous vegetables were inversely associated with risk of bladder cancer in this hospital-based case-control study. However, only associations with raw cruciferous vegetable intake remained statistically significant after adjustment for smoking and other risk factors of bladder cancer. Moreover, each individual raw cruciferous vegetable was significantly inversely associated with risk of bladder cancer, in contrast to their cooked counterparts, for which there were weak or nonsignificant associations. These results are consistent

Table 5. ORs and 95% CIs for the association of bladder cancer risk with raw cruciferous vegetable intake (servings per month) by smoking status and pack-years

	Number		OR (95% CI)	Adjusted OR (95% CI)
	Cases	Controls		
Overall				
<3	158	359	1.00	1.00
≥3	109	441	0.56 (0.42-0.74)	0.63 (0.46-0.86)*
Stratified by smoking status				
Never				
<3	31	133	1.00	1.00
≥3	22	170	0.56 (0.31-1.00)	0.63 (0.33-1.18)†
Quit				
<3	81	179	1.00	1.00
≥3	68	222	0.68 (0.46-0.99)	0.73 (0.48-1.11)†
Current				
<3	45	47	1.00	1.00
≥3	19	48	0.41 (0.21-0.81)	0.46 (0.23-0.93)†
Stratified by pack-years for ever smokers				
<33				
<3	34	101	1.00	1.00
≥3	30	139	0.64 (0.37-1.12)	0.69 (0.39-1.23)‡
≥33				
<3	91	117	1.00	1.00
≥3	56	120	0.60 (0.40-0.91)	0.60 (0.38-0.93)‡

*ORs and 95% CIs were calculated with unconditional logistic regression adjusted for age (continuous), education level (<high school or >high school), gender (male or female), total meat intake (continuous), smoking status (never, quit, or current), and pack-years (continuous).
† Adjusted for age (continuous), education level (<high school or >high school), gender (male or female), total meat intake (continuous), and pack-years (continuous).
‡ Adjusted for age (continuous), education level (<high school or >high school), gender (male or female), total meat intake (continuous), and smoking status (never, quit, or current).

with the fact that intake of raw cruciferous vegetables provides two to nine times the amount of isothiocyanates in humans compared with similar intakes of their cooked counterparts (23-26).

Using a cell culture system, we previously showed that dietary isothiocyanates and their urinary metabolites (NAC-ITCs) exerted potent antiproliferative effects on human bladder cancer cells at *in vivo*–achievable concentrations (4-7). The induction of carcinogen-detoxifying phase 2 enzymes and the inhibition of carcinogen-induced carcinogenesis in the bladder of animals has also been reported (8, 9). It is highly possible that intake of isothiocyanate-rich cruciferous vegetables has a preventive effect on human bladder cancer, and such protective effects may, to a certain extent, correspond to the amount of isothiocyanates provided by these vegetables. This may also explain why, in our data, the combinations of broccoli, cabbage, and cauliflower showed enhanced associations. The lack of association between bladder cancer risk and intake of Brussels sprouts, kale, turnips, and collard and mustard greens could relate to the common overcooking of these vegetables, although other mechanisms might also apply. In addition to these vegetables being consumed less frequently, they are seldom consumed uncooked. It is noteworthy that cruciferous vegetables also contain many other compounds with chemopreventive potentials such as indoles, carotenoids, vitamin C, folic acid, dietary fiber, and selenium. Thus, the observed inverse associations may also result from other compounds or their combined effects with isothiocyanates.

Cigarette smoking is the leading risk factor for bladder cancer, and is estimated to be responsible for 50% of the bladder cancers in men, and 25% in women (28). We found a 2-fold to 4-fold increase in risk of bladder cancer in smokers compared with never smokers, which is consistent with other studies (29-31). Interestingly, in a stratified analysis, the strong and significant inverse associations between intake of raw cruciferous vegetables and risk of bladder cancer were only observed among current smokers, especially among heavy smokers. It is known that isothiocyanates, in addition to inhibiting the proliferation of premalignant and malignant cells via the induction of apoptosis and arrest of cell cycle progression, also modulate carcinogen metabolism by inhibiting carcinogen-activating phase 1 enzymes and inducing carcinogen-detoxifying phase 2 enzymes (5).

As smoking constantly exposes the bladder to carcinogens (32), smokers may benefit from the metabolism, detoxification, and excretion of carcinogens. In fact, our preliminary study showed that certain dietary isothiocyanates could inhibit the formation of 4-aminobiphenyl-DNA adducts in human bladder cancer cells. 4-Aminobiphenyl, a major carcinogen of human bladder cancer derived from cigarette smoking as well as occupational exposure, causes DNA damage and tumorigenic transformation in the bladder (33-36), and indeed, 4-aminobiphenyl-DNA adducts were detected in a large percentage of human bladder cancer biopsies and were closely correlated to the amount of cigarettes smoked (37-39). Thus, it is possible that dietary isothiocyanates derived from cruciferous vegetables may be particularly effective in modifying smoking-related bladder carcinogenesis.

Several studies have previously examined the association between cruciferous vegetable intake and bladder cancer risk (15-20). However, to our knowledge, only one study (16), published in 1999, reported a statistically significant inverse association (relative risk = 0.49 for the highest versus the lowest cruciferous vegetable consumption; 95% CI, 0.32-0.75, *P* for trend = 0.008). In another study, Zeegers et al. (20) reported that only cauliflower, but not other cruciferous vegetables, was significantly and inversely associated with bladder cancer risk. An inverse, but not statistically significant, association was also reported in another study (relative risk = 0.75 for highest versus lowest consumption; ref. 18). The lack of statistical significance in those studies might be due to several reasons; however, differences in isothiocyanate content between the consumption of raw versus cooked cruciferous vegetables could contribute to inconsistent results.

Recently, Zhao et al. (40) estimated the amount of dietary isothiocyanates consumed in a study in Singapore based on food frequency questionnaires, and found that high isothiocyanate intake was inversely associated with bladder cancer risk (OR, 0.71; 95% CI, 0.57-0.89). Furthermore, this association between the intake of isothiocyanates and bladder cancer risk was more evident in ever smokers and heavy smokers in stratified analyses, which is consistent with our findings. However, the estimation for isothiocyanate intake in that study may not be representative of intake in the United States because the isothiocyanate content profiles of cruciferous vegetables in Singapore seem to be different from those obtained from the United States (26, 41).

Moreover, it is difficult to accurately estimate isothiocyanate intake from a food frequency questionnaire because the content of glucosinolate (isothiocyanate precursor) is markedly variable even among the same kind of vegetables (21, 42), and also depends on whether cruciferous vegetables are consumed raw or cooked. In the future, using the concentration of urinary isothiocyanates and their metabolites as an internal dose to address the relationship between intake of cruciferous vegetables and bladder cancer risk may provide more complete information.

In our study, no statistically significant associations were found between total fruit intake or total vegetable intake and risk of bladder cancer. The small range of exposure may result in such null associations in epidemiologic studies. However, in our study, the contrast between high and low consumption was far greater for total fruit or vegetable intake than for raw cruciferous vegetable intake as shown in Table 2. Therefore, it is unlikely that the null associations for groupings other than raw cruciferous vegetables were a result of a limited range of intake. Given that all individual raw cruciferous vegetables showed strong and significant associations with bladder cancer risk and that there is plausible biological evidence to support this finding, it is also unlikely that our results were chance findings.

However, there were several limitations in this study. First, the sample size was relatively small, which led to small cell sizes in some strata, producing risk estimates with wide CIs, increasing the potential to miss a true association. The lower number of female patients may be one reason why the association between intake of raw cruciferous vegetables and bladder cancer risk among women was not statistically significant (results not shown). Furthermore, the food frequency questionnaire was completed after disease diagnosis, which may have biased the assessment of exposures of interest. This may be the most problematic for older patients because exposures to both raw cruciferous vegetable and smoking at early ages might be more important than at late ages. Third, this study was a hospital-based case-control study which might have been subject to selection bias, especially given a response rate of ~50% for both cases and controls. Selection bias could also occur due to a healthy volunteer effect in which controls tend to be healthier than the general population. However, in this study, controls as well as cases came to the hospital with a suspicion of cancer, but the controls were diagnosed with other diseases instead of cancer. As all participants in this study were patients, it is less likely that the healthy volunteer effect presents an important source of bias in these analyses.

In summary, this study is the first epidemiologic study considering raw versus cooked consumption in the evaluation of the relationship between cruciferous vegetable intake and bladder cancer risk. We found that only intake of raw cruciferous vegetables, but not cooked, fruit or other vegetables, showed a strong and statistically significant inverse association with bladder cancer risk. This result is consistent with the experimental data, showing in both *in vitro* and *in vivo* models that exposure to dietary isothiocyanates, a family of chemopreventive agents abundant in cruciferous vegetables, has great potential against bladder cancer carcinogenesis. Our data supports the possibility that intake of raw cruciferous vegetables may reduce the risk of bladder cancer.

References

1. Verhoeven DT, Goldbohm RA, van Poppel G, Verhagen H, van den Brandt PA. Epidemiological studies on brassica vegetables and cancer risk. Cancer Epidemiol Biomarkers Prev 1996;5:733–48.
2. Murillo G, Mehta RG. Cruciferous vegetables and cancer prevention. Nutr Cancer 2001;41:17–28.
3. Kirsh VA, Peters U, Mayne ST, et al. Prospective study of fruit and vegetable intake and risk of prostate cancer. J Natl Cancer Inst 2007; 99:1200–9.
4. Tang L, Li G, Song L, Zhang Y. The principal urinary metabolites of dietary isothiocyanates, *N*-acetylcysteine conjugates, elicit the same anti-proliferative response as their parent compounds in human bladder cancer cells. Anticancer Drugs 2006;17:297–305.
5. Tang L, Zhang Y. Isothiocyanates in the chemoprevention of bladder cancer. Curr Drug Metab 2004;5:193–201.
6. Tang L, Zhang Y. Dietary isothiocyanates inhibit the growth of human bladder carcinoma cells. J Nutr 2004;134:2004–10.
7. Tang L, Zhang Y. Mitochondria are the primary target in isothiocyanate-induced apoptosis in human bladder cancer cells. Mol Cancer Ther 2005;4:1250–9.
8. Munday R, Munday CM. Selective induction of phase II enzymes in the urinary bladder of rats by allyl isothiocyanate, a compound derived from brassica vegetables. Nutr Cancer 2002;44:52–9.
9. Okazaki K, Yamagishi M, Son HY, et al. Simultaneous treatment with benzyl isothiocyanate, a strong bladder promoter, inhibits rat urinary bladder carcinogenesis by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Nutr Cancer 2002;42:211–6.
10. Mennicke WH, Gorler K, Krumbiegel G, Lorenz D, Rittmann N. Studies on the metabolism and excretion of benzyl isothiocyanate in man. Xenobiotica 1988;18:441–7.
11. Jiao D, Ho CT, Foiles P, Chung FL. Identification and quantification of the *N*-acetylcysteine conjugate of allyl isothiocyanate in human urine after ingestion of mustard. Cancer Epidemiol Biomarkers Prev 1994;3:487–92.
12. Ye L, Dinkova-Kostova AT, Wade KL, Zhang Y, Shapiro TA, Talalay P. Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. Clin Chim Acta 2002; 316:43–53.
13. Conaway CC, Krzeminski J, Amin S, Chung FL. Decomposition rates of isothiocyanate conjugates determine their activity as inhibitors of cytochrome p450 enzymes. Chem Res Toxicol 2001;14:1170–6.
14. Juffs HG, Moore MJ, Tannock IF. The role of systemic chemotherapy in the management of muscle-invasive bladder cancer. Lancet Oncol 2002;3:738–47.
15. Garcia-Closas R, Garcia-Closas M, Kogevinas M, et al. Food, nutrient and heterocyclic amine intake and the risk of bladder cancer. Eur J Cancer 2007;43:1731–40.
16. Michaud DS, Spiegelman D, Clinton SK, Rimm EB, Willett WC, Giovannucci EL. Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. J Natl Cancer Inst 1999;91: 605–13.
17. Michaud DS, Pietinen P, Taylor PR, Virtanen M, Virtamo J, Albanes D. Intakes of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study. Br J Cancer 2002;87:960–5.
18. Mettlin C, Graham S. Dietary risk factors in human bladder cancer. Am J Epidemiol 1979;110:255–63.
19. Holick CN, De Vivo I, Feskanich D, Giovannucci E, Stampfer M, Michaud DS. Intake of fruits and vegetables, carotenoids, folate, and vitamins A, C, E and risk of bladder cancer among women (United States). Cancer Causes Control 2005;16:1135–45.
20. Zeegers MP, Goldbohm RA, van den Brandt PA. Consumption of vegetables and fruits and urothelial cancer incidence: a prospective study. Cancer Epidemiol Biomarkers Prev 2001;10:1121–8.
21. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001;56:5–51.
22. Fenwick GR, Heaney RK, Mullin WJ. Glucosinolates and their breakdown products in food and food plants. Crit Rev Food Sci Nutr 1983;18:123–201.
23. Rouzaud G, Young SA, Duncan AJ. Hydrolysis of glucosinolates to isothiocyanates after ingestion of raw or microwaved cabbage by human volunteers. Cancer Epidemiol Biomarkers Prev 2004;13: 125–31.
24. Getahun SM, Chung FL. Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress. Cancer Epidemiol Biomarkers Prev 1999;8:447–51.
25. Conaway CC, Getahun SM, Liebes LL, et al. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. Nutr Cancer 2000;38:168–78.
26. Shapiro TA, Fahey JW, Wade KL, Stephenson KK, Talalay P. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. Cancer Epidemiol Biomarkers Prev 1998;7:1091–100.
27. Byers T, Marshall J, Fiedler R, Zielezny M, Graham S. Assessing nutrient intake with an abbreviated dietary interview. Am J Epidemiol 1985;122:41–50.
28. Marcus PM, Hayes RB, Vineis P, et al. Cigarette smoking, *N*-acetyltransferase 2 acetylation status, and bladder cancer risk: a case-series meta-analysis of a gene-environment interaction. Cancer Epidemiol Biomarkers Prev 2000;9:461–7.
29. Hartge P, Silverman D, Hoover R, et al. Changing cigarette habits and bladder cancer risk: a case-control study. J Natl Cancer Inst 1987; 78:1119–25.
30. Augustine A, Hebert JR, Kabat GC, Wynder EL. Bladder cancer in relation to cigarette smoking. Cancer Res 1988;48:4405–8.
31. Castelao JE, Yuan JM, Skipper PL, et al. Gender- and smoking-related bladder cancer risk. J Natl Cancer Inst 2001;93:538–45.
32. Vineis P. Epidemiological models of carcinogenesis: the example of bladder cancer. Cancer Epidemiol Biomarkers Prev 1992;1: 149–53.
33. Block NL, Sigel MM, Lynne CM, Ng AB, Grosberg RA. The initiation, progress, and diagnosis of dog bladder cancer induced by 4-aminobiphenyl. Invest Urol 1978;16:50–4.
34. Poirier MC, Fullerton NF, Smith BA, Beland FA. DNA adduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. Carcinogenesis 1995;16:2917–21.
35. Tsuneoka Y, Dalton TP, Miller ML, et al. 4-aminobiphenyl-induced liver and urinary bladder DNA adduct formation in Cyp1a2(−/−) and Cyp1a2(+/+) mice. J Natl Cancer Inst 2003;95: 1227–37.
36. Bookland EA, Swaminathan S, Oyasu R, Gilchrist KW, Lindstrom M, Reznikoff CA. Tumorigenic transformation and neoplastic

progression of human uroepithelial cells after exposure *in vitro* to 4-aminobiphenyl or its metabolites. Cancer Res 1992;52:1606 – 14.

37. Airoldi L, Orsi F, Magagnotti C, et al. Determinants of 4-aminobiphenyl-DNA adducts in bladder cancer biopsies. Carcinogenesis 2002;23:861 – 6.
38. Curigliano G, Zhang YJ, Wang LY, et al. Immunohistochemical quantitation of 4-aminobiphenyl-DNA adducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. Carcinogenesis 1996;17:911 – 6.
39. Talaska G, al-Juburi AZ, Kadlubar FF. Smoking related carcinogen-DNA adducts in biopsy samples of human urinary bladder: identification of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. Proc Natl Acad Sci U S A 1991;88:5350 – 4.
40. Zhao H, Lin J, Grossman HB, Hernandez LM, Dinney CP, Wu X. Dietary isothiocyanates, GSTM1, GSTT1, NAT2 polymorphisms and bladder cancer risk. Int J Cancer 2007;120:2208 – 13.
41. Jiao D, Yu MC, Hanker JH, Low SH, Chung FL. Total isothiocyanate contents in cooked vegetables frequently consumed in Singapore. J Agric Food Chem 1998;46:1055 – 8.
42. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci U S A 1997;94:10367 – 72.