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Food, nutrient and heterocyclic amine intake and the risk of bladder cancer

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ABSTRACT

Fruit and vegetable intake has been linked to bladder cancer risk; however, evidence for other foods or specific dietary factors is inconclusive. The association between diet and bladder cancer risk was evaluated among 912 incident bladder cancer cases and 873 controls in Spain. Data were consistent with a reduced bladder cancer risk associated with high fruit intake; however, the association was significant only among current smokers (OR (95% CI) for 5th versus 1st quintile: 0.5 (0.3–0.9), p trend = 0.009). Evaluation of food subgroups showed significant inverse associations with high intakes of berries, *Liliaceae* vegetables and yellow-orange vegetables. The latter association was stronger among individuals with the *GSTM1* present than the null genotype (0.4 (0.2, 0.7) and 0.9 (0.6, 1.3), respectively; p for interaction = 0.04). Meat or fish intake, their cooking methods or level of doneness, or heterocyclic amine intakes were not significantly associated with risk. Intake of folate, other B-vitamins (B12, B6, B2) and retinol was also associated with a reduced risk, the strongest associations being for vitamin B6 (0.6 (0.4, 0.8) p trend = 0.0006) and retinol (0.6 (0.4–0.9) p trend = 0.004). Our findings indicate that fruit and vegetable intake, as well as B-vitamin and retinol intake might be associated with a reduced bladder cancer risk.

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1. Introduction

While cigarette smoking and certain occupational hazards are well-established risk factors for bladder cancer,[1] laboratory and observational epidemiological studies suggest that dietary factors may also influence bladder carcinogenesis.[2–4] The evidence for an inverse association is strongest for fruit and vegetable intake, and weaker for other food groups, and

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for all nutrients.[2,5–12] Carcinogens formed during cooking, such as heterocyclic amines (HCAs), or resulting from meat preservation methods have been suggested to increase bladder cancer risk; however, evidence is inconclusive.[13] In the most recently published systematic review on diet and bladder cancer, it was concluded that total fruit consumption rather than total vegetable intake is responsible for the decrease in bladder cancer risk.[14] Evaluation of nutrient intake in this review indicated a possible moderate inverse association with vitamin E, with weaker or no evidence for vitamin A and vitamin C intakes.

Polymorphisms in *NAT2* and *GSTM1* genes are associated with increased bladder cancer risk.[15] *NAT2* encodes the *N*-acetyltransferase 2 enzyme responsible for detoxification of aromatic amines by *N*-acetylation or activation by *O*-acetylation,[16] and about 50% of Caucasian individuals have a polymorphism resulting in decreased enzyme activity (slow acetylators). Therefore, this polymorphism could modify the potential association between food carcinogen intake, such HCA, and bladder cancer risk.[17] *GSTM1* encodes the glutathione *S*-transferase M1 enzyme responsible for detoxification of carcinogens such as polycyclic aromatic hydrocarbons and reactive oxygen species,[18] and about 50% of Caucasian individuals have a deletion in both copies of the gene resulting in lack of enzyme activity (null genotype). Thus, this polymorphism could modify the association between antioxidant intake, food carcinogens and bladder cancer risk.

This report examines the association of bladder cancer risk with food and nutrient intake (including fish and meat cooking methods, doneness level of meats and HCA intake), in a large hospital-based case-control study conducted in Spain. In addition, it evaluates whether the dietary associations are modified by cigarette smoking, and genotypes in the *NAT2* and *GSTM1* genes.

2. Materials and methods

2.1. Study population

The design of the Spanish Bladder Cancer Study has been previously described.[15] Briefly, this is a hospital-based case-control study conducted in 1998–2001 in five different areas in Spain. Cases were patients newly diagnosed with histologically confirmed bladder cancer in 18 participating hospitals. Controls without a previous history of cancer were selected among patients from the same hospitals with diagnoses believed to be unrelated to the exposures of interest, matched to the cases on age (within a 5-year window), gender, race and study hospital. The most common reasons for hospitalisation for control patients were hernias (36%), fractures (23%), hydrocele (12%) and other abdominal surgery (11%).

A total of 1219 cases (84% of eligible subjects) and 1271 controls (88% of eligible subjects) agreed to participate in the study and were interviewed for known or suspected risk factors (excluding diet) at the hospital. Of these subjects, 917 cases and 875 controls completed a separate food frequency questionnaire (FFQ). Among subjects completing the FFQ, 3 cases with unsatisfactory interview, and 2 cases and 2 controls with missing information on smoking status were excluded from the analyses. After exclusions, the present analyses included a total of 912 cases and 873 controls. Comparison of demographic and risk factor characteristics for subjects with and without dietary information showed a higher proportion of males, never smokers, higher education level and residents of Tenerife and Elche area (data not shown) among individuals answering the FFQ. Differences were similar in direction and magnitude for cases and controls.

Both cases and controls provided informed consent to the study in accordance with the National Cancer Institute and local Institutional Review Boards.

2.2. Dietary assessment

Food intake over the 5 years before diagnosis for cases and before interview for controls was estimated using a modified semi-quantitative FFQ of 127 items, previously validated in Spain.[19] Modifications included the addition of regional food items frequently consumed in the study areas and the inclusion of a cooking practice module for meats and fish (see below). Portion sizes were specified for each food item, and subjects reported how often they consumed the specified quantity. Forty-nine percent of the FFQs were administered with the help of the spouse or other relative, 34% were self-administered and 17% were administered by the interviewer. Thirty-nine percent of FFQ were completed while in the hospital and 61% were completed at home few days after discharge.

Fruits were categorised as citric, non-citric and berries; and vegetables as *Brassica* or cruciferous, *Liliaceae* , dark-green and yellow-orange (Supplementary Table 1). Information on consumption of vitamin and mineral supplements and on important changes on dietary habits through lifetime was also collected within the FFQ. Data on nutrient composition of foods were obtained from a Spanish food composition table.[20]

Meats were categorised into red (beef, veal, lamb, pork) and white (chicken) meat, and fish into white (e.g. cod, flounder) and dark (e.g. tuna, salmon) meat fish. The meat and fish module collected information on whether the meat or fish was gridled/pan-fried, deep-fried, boiled/stewed/in sauce/microwaved, baked/roasted/oven-broiled or grilled/barbecued. Data on doneness level of meats were collected with the help of a set of three photographs that indicated both the internal colouring and external browning for chicken, beef/veal steaks and pork chops. Data on HCA intake (2-amino-3,8-dimethylimadazol [4,5-f] quinoxaline – MeIQx-, 2-amino-1-methyl-6-phenylimadazol [4,5-b] pyridine – PhIP-, 2-amino-3,4,8-trimethylimidazol [4,5-f] quinoxaline – DiMeIQx-) and mutagenic activity were obtained from the CHARRED (Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease; http://charred.cancer.gov/software/) software package and missing values were completed by means of a database compiled by Jakszyn et al.[21] HCA and mutagenic activity were estimated using responses from the FFQ and CHARRED. We used frequency and portion size information from the FFQ to estimate gram consumption of each meat item by cooking technique and doneness level. Grams of meat were multiplied by the HCA concentration or mutagenic activity measured for each meat cooking technique/doneness level.

2.3. Genotyping

We obtained genomic DNA for genotype analyses from 889 cases and 819 controls included in this report. Six of these participants were excluded from genotype analyses because of DNA quality control difficulties. Genotype assays for *NAT2* and *GSTM1* were performed at the core genotyping facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute (http://snp500cancer.nci.nih.gov), as previously described.[15]

2.4. Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated using logistic regression models for quintiles or food or nutrient intake, adjusting for age in 5 year categories, gender, region and smoking habits (smoking status and duration of smoking). Analyses were further adjusted for total energy intake using the nutrient density model (19), and for other dietary variables. Further adjustment for educational level, place of completion of the FFQ and type of assistance received to complete the FFQ did not appreciably change estimates of risk and, therefore, are not presented in this report. Quintiles of intake were determined on the basis of the distribution among controls, and the lowest quintile was used as the reference category. Log-linear monotonic trend for increasing quartiles was tested by weighting each category by its median value of intake in the control population.

3. Results

Study subjects had a mean (±SD) age of 65.3 (±10.2) years for cases and 64.0 (±9.9) years for controls, and all subjects but one case and one control were Caucasian. Eighty-eight percent of cases and 89% of controls were male, and a higher proportion of cases than controls were cigarette smokers (85.5% versus 72.4%, respectively). Associations between smoking and bladder cancer in the study population were similar to those previously reported in the full study.[22] The mean (SD) intake in the control population for different food groups was 461 (365) g of total fruits, 290 (254) g of total vegetables, 210 (117) g of total meat and 80 (63) g of total fish (please see Supplementary Table 2 for more detailed information on the intake distribution of food groups, nutrients and HCAs). These figures were similar to those of Spanish population surveys in representative populations.[23] The range of dietary intake of HCAs was comparable to the range estimated in US populations.[24,25]

Data were consistent with a reduced bladder cancer risk associated with total fruit and vegetable intake; however, the test for trend was not significant (Table 1). Assessment of fruit and vegetable subgroups revealed a significant association with intake of berries, *Liliaceae* vegetables and yellow-orange vegetables. The inverse association with *Liliaceae* vegetables was also seen for consumption of the genus *Allium* (leek and onion), which belongs to the family *Liliaceae*, and for individual foods of this family (data not shown). No significant associations were found for the genus *Brassica* (cruciferous) or for dark-green vegetables (Table 1). Other selected individual vegetables (tomato, carrot) were also analysed and were not found to be associated with risk (data not shown). Data did not support a decreased risk with increasing intake of juices (data not shown).

Stratification by smoking status showed a significant reduction in bladder cancer risk for high versus low fruit intake among current but not among never or former smokers; however, the test for interaction was not statistically significant (Table 2). The only significant interaction was for berry intake, which showed a stronger protection for never and former smokers than current smokers (Table 2). The associations between the *NAT2* slow versus rapid/intermediate and *GSTM1* null versus present genotypes and bladder cancer risk in this study population were similar to those found in the whole study population.[15] Here we examined the potential modification of dietary associations by these two genotypes. The association with yellow-orange vegetable intake was significantly stronger among individuals with the *GSTM1* present than the null genotype (ORs (95% CI) for 5th versus 1st: 0.4 (0.2, 0.7) and 0.9 (0.6, 1.3), respectively; p for interaction = 0.04; Table 2). The joint association for the highest level of yellow-orange vegetable intake and *GSTM1* present genotype compared to the lowest level of intake and *GSTM1* null genotype was 0.40 (0.24, 0.68). The inverse association with total fruit intake was also stronger for subjects with the *GSTM1* present genotype, although the interaction was not statistically significant. None of the other groups of fruits and vegetables were modified by the *GSTM1* genotype (including *Brassilica* or cruciferous vegetables, p for interaction = 0.95; Table 2), nor by *NAT2* genotype or smoking habits (data not shown).

Meat and fish intake was unrelated to the risk of bladder cancer (Table 3). With respect to cooking methods and doneness level, red meat was consumed preferably pan-fried (average of 42%) or deep-fried (32%), with grilled or barbequed red meat consumption being very low. Red meat was cooked mostly medium (45%) or well done (36%). White meat was consumed preferably deep-fried (43%) or pan-fried (33%), and cooked well-done (86%). White fish was consumed preferably boiled/stewed/baked (78%), while dark fish was mainly consumed baked or stewed (51%). There was no evidence of an increased risk of bladder cancer with any of the cooking methods for red meat, white meat, white fish and dark fish, including pan-fried, broiled and barbequed, nor for level of meat doneness (data not shown).

Although there was a suggestion for a modest increase in risk for subjects with high intakes of HCAs (MeIQx, PhiP, DiMeIQx) compared to the lowest levels of intake, or high versus lower mutagenic activity, differences were not statistically significant, and there was no evidence of a trend with increasing levels of intake (Table 3). Assessment of interactions with *NAT2* genotype suggested a stronger association for slow *NAT2* than rapid/intermediate acetylators e.g. ORs (95% CI) for 5th versus 1st quintile of DiMeIQx: 1.4 (0.9, 2.1) and 1.0 (0.6, 1.6), respectively; however, the interaction was not statistically significant (p = 0.33).

With respect to nutrients, intake of vitamins B12, B6, B2, and retinol was associated with significant decreases in risk (Table 4). Although folate intake was not significantly associated with risk (p-trend = 0.06), data were consistent with a decrease in risk for higher intakes (OR 95% CI for high versus

Table 1 – Association between fruit and vegetable intake and bladder cancer risk (912 cases and 873 controls)

Quintiles of intake (Q)	Median (g/day/kcal)	Cases *N*	Controls *N*	OR[a]	95% CI[a]	
Total fruits and vegetables						
Q1 (<181)	126	225	172	1.0		
Q2 (181–253)	216	161	173	0.7	0.5	1.0
Q3 (254–344)	299	181	171	0.9	0.7	1.2
Q4 (345–457)	397	172	173	0.8	0.6	1.1
Q5 (>457)	559	168	172	0.8	0.6	1.1
p for trend				0.33		
Total fruits						
Q1 (<91)	56	229	172	1.0		
Q2 (91–142)	118	161	173	0.8	0.6	1.1
Q3 (143–203)	176	175	172	0.8	0.6	1.1
Q4 (204–299)	240	180	173	0.8	0.6	1.2
Q5 (>299)	381	163	172	0.8	0.6	1.1
p for trend				0.23		
Citric fruits						
Q1 (<12)	3	213	174	1.0		
Q2 (12–31)	22	162	176	0.7	0.5	1.0
Q3 (32–61)	47	182	174	1.0	0.7	1.3
Q4 (62–107)	77	155	174	0.7	0.5	1.0
Q5 (>107)	154	200	175	1.0	0.7	1.3
p for trend				0.73		
Non-citric fruits						
Q1 (<56)	33	219	172	1.0		
Q2 (56–92)	77	195	173	1.0	0.8	1.4
Q3 (93–135)	112	172	172	0.8	0.6	1.1
Q4 (136–198)	162	155	173	0.8	0.6	1.1
Q5 (>198)	264	167	172	0.9	0.6	1.2
p for trend				0.22		
Berries						
Q1 (0)	0	337	283	1.0		
Q2 (<1)	1	75	66	1.0	0.7	1.5
Q3 (2–4)	2	203	175	1.0	0.8	1.4
Q4 (5–8)	6	147	175	0.7	0.6	1.0
Q5 (>8)	12	150	174	0.8	0.6	1.0
p for trend				0.03		
Total vegetables						
Q1 (<54)	31	184	173	1.0		
Q2 (54–92)	73	213	172	1.2	0.9	1.7
Q3 (93–129)	108	186	172	1.1	0.8	1.6
Q4 (130–190)	158	171	173	1.0	0.7	1.4
Q5 (>190)	245	153	172	0.9	0.6	1.2
p for trend				0.17		
Brassica vegetables						
Q1 (0)	0	292	242	1.0		
Q2 (<6)	5	96	107	0.7	0.5	1.0
Q3 (7–12)	7	190	175	1.0	0.7	1.3
Q4 (13–21)	17	154	175	0.7	0.5	1.0
Q5 (>21)	31	180	174	0.9	0.7	1.2
p for trend				0.32		
Liliaceae vegetables						
Q1 (<2)	0	205	175	1.0		
Q2 (2–6)	3	186	175	0.9	0.7	1.2
Q3 (7–11)	8	202	174	1.0	0.7	1.3
Q4 (12–20)	15	165	174	0.8	0.6	1.1
Q5 (>20)	28	154	175	0.7	0.5	1.0
p for trend				0.04		
Dark-green vegetables						
Q1 (<15)	7	185	175	1.0		
Q2 (16–30)	22	202	175	1.2	0.8	1.6

Table 1 (*continued*)

Quintiles of intake (Q)	Median (g/day/kcal)	Cases *N*	Controls *N*	OR[a]	95% CI[a]	
Q3 (31–49)	39	172	174	0.9	0.7	1.2
Q4 (50–77)	60	183	174	1.0	0.7	1.4
Q5 (>77)	107	170	175	1.0	0.7	1.4
p for trend				0.72		
Yellow-orange vegetables						
Q1 (0)	0	233	185	1.0		
Q2 (<4)	2	181	165	0.9	0.6	1.2
Q3 (5–9)	6	176	174	0.8	0.6	1.1
Q4 (10–17)	12	157	174	0.7	0.5	1.0
Q5 (>17)	28	165	175	0.7	0.5	1.0
p for trend				0.04		

a Adjusted for age (<55, 55–64, 65–69, 70–74, >74 years old), gender, region (Barcelona, Bages/Vallès, Elche, Tenerife, Asturias), smoking status (never, occasional, former, current) and duration of smoking (<20, 20– <30, 30–<40, 40–<50, ⩾50 years).

Table 2 – Association between selected food items and bladder cancer risk, stratified by smoking status (912 cases and 873 controls) and GSTM1 genotype (886 cases and 816 controls with DNA samples)

Foot item	Non-smokers				Former smokers					Current smokers				
	OR	95% CI		*p* Trend	OR	95% CI		*p* Trend	*p* Inter	OR	95% CI		*p* Trend	*p* Inter
Total fruit and vegetable intake	0.6	0.3	1.2	0.17	0.9	0.6	1.5	0.86	0.26	0.8	0.4	1.5	0.19	0.52
Total fruit intake	0.7	0.3	1.5	0.39	1.0	0.6	1.5	0.74	0.49	0.5	0.3	0.9	0.009	0.43
Berries	0.5	0.3	1.0	0.03	0.7	0.5	1.1	0.14	0.14	1.0	0.6	1.8	0.65	<0.001
Total vegetable intake	0.8	0.4	1.7	0.18	0.8	0.5	1.4	0.22	0.87	1.2	0.6	2.2	0.83	0.40
Brasssica vegetables	1.2	0.6	2.2	0.72	0.9	0.6	1.3	0.58	0.45	0.7	0.4	1.3	0.22	0.29
Dark green vegetables	1.0	0.5	2.1	0.91	1.0	0.6	1.6	0.75	0.96	0.8	0.5	1.6	0.85	0.70
Yellow orange vegetables	0.7	0.4	1.5	0.42	0.6	0.4	1.0	0.14	0.76	0.7	0.4	1.3	0.25	0.96
	GSTM1 present				*GSTM1* null									
Total fruit and vegetable intake	0.7	0.4	1.1	0.15	0.9	0.6	1.5	0.92	0.24					
Total fruit intake	0.5	0.3	0.9	0.06	1.0	0.6	1.5	0.96	0.15					
Berries	0.9	0.6	1.4	0.46	0.6	0.4	0.9	0.01	0.28					
Total vegetable intake	0.8	0.5	1.4	0.19	1.1	0.7	1.7	0.81	0.39					
Brasssica vegetables	0.9	0.6	1.5	0.61	0.9	0.6	1.3	0.46	0.95					
Dark green vegetables	0.7	0.4	1.2	0.35	1.1	0.7	1.8	0.88	0.39					
Yellow orange vegetables	0.4	0.2	0.7	0.003	0.9	0.6	1.3	0.46	0.04					

Adjusted for age (<55, 55–64, 65–69, 70–74, >74 years old), gender, region (Barcelona, Vallès, Elche, Tenerife, Asturias), smoking status (never, occasional, former, current) and duration of smoking (<20, 20–<30, 30–<40, 40–<50, ⩾50 years).
P values for interaction of a log-linear trend with increasing quintiles of intake; for smoking status, never-smokers is the reference category. OR and 95% CI are shown for the highest versus the lowest quintile of intake.

lowest quintile 0.7 (0.5–1.0); Table 4). Folate intake was highly correlated with vitamins B6 (Spearman $r^2 = 0.86$) and B2 ($r^2 = 0.70$), showing weaker correlations with vitamin B12 ($r^2 = 0.49$) and retinol ($r^2 = 0.41$). Intakes of vitamins B6 and B2 were highly correlated ($r^2 = 0.78$), and moderately correlated with vitamin B12 intake ($r^2 = 0.60$ for B6–B12 and $r^2 = 0.62$ for B2–B12). When all B-vitamins were included in the same model, only vitamin B6 intake remained significant (OR's (95% CI) for 5th versus 1st quintile: 0.6 (0.4, 1.0)). Retinol intake was highly correlated with vitamin B12 ($r^2 = 0.78$), with weaker correlations with vitamin B2 ($r^2 = 0.60$) and B6 ($r^2 = 0.43$) intakes. Addition of retinol intake to the model with vitamins B12, B6 and B2 showed significant inverse associations with both vitamin B6 and retinol intake (data not shown).

High intakes of vitamin C, vitamin E and total carotenoids were also associated with decreases in risk; however, associations were not statistically significant (Table 4). Vitamin intake from supplements was ascertained within the FFQ; however, less than 5% of subjects reported taking supplements regularly and, thus, it was not possible to assess their effect. Our data did not support an association with

Table 3 – Association between meat, fish and heterocyclic amine intake and bladder cancer risk (912 cases and 873 controls)

Quintiles of intake (Q)	Median (g/day/kcal)	Cases *N*	Controls *N*	OR[a]	95% CI[a]	
Total meat						
Q1 (<57)	46	188	175	1.0		
Q2 (57–79)	68	224	175	1.2	0.9	1.6
Q3 (80–100)	89	197	173	1.1	0.8	1.5
Q4 (101–126)	111	163	176	1.0	0.7	1.3
Q5 (>126)	150	140	174	0.8	0.6	1.1
p for trend				0.10		
Total red meat						
Q1 (<20)	14	184	175	1.0		
Q2 (20–32)	26	211	175	1.1	0.8	1.5
Q3 (33–43)	37	188	173	1.1	0.8	1.5
Q4 (44–58)	50	180	175	1.0	0.7	1.3
Q5 (>58)	70	149	175	0.8	0.6	1.1
p for trend				0.09		
Total white meat						
Q1 (<26)	18	193	175	1.0		
Q2 (26–38)	32	209	174	1.1	0.8	1.5
Q3 (39–51)	45	183	175	1.0	0.7	1.4
Q4 (52–72)	59	185	174	1.1	0.8	1.5
Q5 (>72)	91	142	175	0.9	0.6	1.2
p for trend				0.29		
Processed red meat						
Q1 (<4)	2	158	174	1.0		
Q2 (4–9)	7	212	176	1.4	1.0	1.9
Q3 (10–12)	11	172	173	1.2	0.9	1.7
Q4 (13–18)	15	177	175	1.2	0.8	1.6
Q5 (>18)	24	193	175	1.2	0.9	1.7
p for trend				0.66		
Total fish						
Q1 (<15)	9	182	174	1.0		
Q2 (15–26)	20	225	175	1.2	0.9	1.6
Q3 (27–36)	31	163	175	0.9	0.6	1.2
Q4 (37–53)	43	194	175	1.2	0.8	1.6
Q5 (>53)	70	148	174	0.9	0.6	1.2
p for trend				0.36		
Heterocyclic amine intake from all meat types						
MeIQx5						
Q1 (<6)	2	187	174	1.0		
Q2 (6–12)	8	170	175	1.1	0.8	1.5
Q3 (13–21)	16	200	175	1.2	0.9	1.6
Q4 (22–37)	27	171	174	1.0	0.7	1.4
Q5 (>37)	55	184	175	1.2	0.8	1.6
p for trend				0.57		
DiMeIQx6						
Q1 (<0.01)	0.0	179	175	1.0		
Q2 (0.01–0.6)	0.3	205	174	1.2	0.9	1.6
Q3 (0.7–1.5)	1.1	164	175	1.0	0.7	1.4
Q4 (1.6–3.1)	2.1	172	175	1.0	0.8	1.5
Q5 (>31)	5.3	192	174	1.3	0.9	1.8
p for trend				0.22		
PhiP7						
Q1 (<9)	1	179	174	1.0		
Q2 (9–29)	18	218	175	1.3	1.0	1.8
Q3 (30–54)	40	157	175	1.0	0.7	1.4
Q4 (55–106)	76	178	174	1.2	0.8	1.7
Q5 (>106)	171	180	175	1.2	0.8	1.7
p for trend				0.75		

a Adjusted for age (<55, 55–64, 65–69, 70–74, >74 years old), gender, region (Barcelona, Vallès, Elche, Tenerife, Asturias), smoking status (never, occasional, former, current), duration of smoking (<20, 20–<30, 30–<40, 40–<50, ⩾50 years) and quintiles of fruit and vegetable intake.

Table 4 – Association between nutrient intake and bladder cancer risk (912 cases and 873 controls)

Quintiles of intake (Q)	Median	Cases *N*	Controls *N*	OR[a]	95% CI[a]	
Vitamin C (g/kcal/day)						
Q1 (<49)	38	219	175			
Q2 (49–68)	57	177	175	0.9	0.6	1.2
Q3 (69–91)	79	184	174	0.9	0.7	1.3
Q4 (92–124)	106	149	174	0.8	0.5	1.0
Q5 (>124)	154	183	175	0.9	0.7	1.3
p for trend				0.53		
Folate (μg/kcal/day)						
Q1 (<130)	114	231	174	1.0		
Q2 (130–156)	144	183	176	0.8	0.6	1.1
Q3 (157–182)	167	167	174	0.8	0.6	1.1
Q4 (183–222)	198	174	175	0.8	0.6	1.1
Q5 (>222)	259	157	174	0.7	0.5	1.0
p for trend				0.06		
Vitamin B12 (μg/kcal/day)						
Q1 (<2.6)	2.0	209	174			
Q2 (2.6–3.4)	3.0	197	175	1.0	0.7	1.3
Q3 (3.5–4.4)	3.9	179	175	0.9	0.7	1.2
Q4 (4.5–6.2)	5.1	179	174	0.9	0.6	1.2
Q5 (>6.2)	8.2	148	175	0.7	0.5	0.9
p for trend				0.009		
Vitamin B6 (μg/kcal/day)						
Q1 (<0.8)	0.7	244	175			
Q2 (0.8–0.9)	0.9	192	174	0.9	0.6	1.2
Q3 (1.0–1.1)	1.0	201	174	0.9	0.6	1.2
Q4 (1.1–1.2)	1.2	133	176	0.6	0.4	0.8
Q5 (>1.2)	1.4	142	174	0.6	0.4	0.8
p for trend				0.0006		
Vitamin B2 (μg/kcal/day)						
Q1 (<0.7)	0.7	214	174			
Q2 (0.7–0.9)	0.8	195	175	1.1	0.8	1.5
Q3 (0.9–1.0)	0.9	188	175	1.0	0.7	1.3
Q4 (1.0–1.2)	1.1	157	175	0.8	0.6	1.1
Q5 (>1.2)	1.3	158	174	0.8	0.5	1.0
p for trend				0.02		
Vitamin E (g/kcal/day)						
Q1 (<3.9)	3.3	215	174			
Q2 (3.9–4.8)	4.3	194	176	0.9	0.7	1.3
Q3 (4.9–5.7)	5.2	174	173	0.8	0.6	1.1
Q4 (5.8–6.8)	6.1	142	176	0.7	0.5	0.9
Q5 (>6.8)	8.2	187	174	0.9	0.7	1.3
p for trend				0.48		
Retinol (μg/kcal/day)						
Q1 (<115)	86	205	175			
Q2 (115–165)	141	155	174	0.8	0.6	1.1
Q3 (166–274)	203	210	174	1.0	0.7	1.4
Q4 (275–548)	389	191	176	0.9	0.6	1.2
Q5 (>548)	838	151	174	0.6	0.4	0.9
p for trend				0.004		
Total carotenoids (μg/kcal/day)						
Q1 (<612)	424	218	175			
Q2 (612–996)	788	183	175	0.9	0.6	1.2
Q3 (997–1421)	1205	154	174	0.7	0.5	1.0
Q4 (1422–2175)	1762	191	175	0.9	0.7	1.3
Q5 (>2175)	2857	166	174	0.8	0.5	1.1
p for trend				0.31		
Total fat (g/kcal/day)						
Q1 (<32)	28	181	174			
Q2 (32–37)	35	181	175	1.0	0.7	1.4

(*continued on next page*)

Table 4 – *continued*

Quintiles of intake (Q)	Median	Cases *N*	Controls *N*	OR[a]	95% CI[a]	
Q3 (38–41)	39	191	175	1.1	0.8	1.5
Q4 (42–46)	44	178	175	1.0	0.7	1.3
Q5 (>46)	49	181	174	1.0	0.7	1.4
p for trend				0.92		

a Adjusted for age (<55, 55–64, 65–69, 70–74, >74 years old), gender, region (Barcelona, Vallès, Elche, Tenerife, Asturias), smoking status (never, occasional, former, current) and duration of smoking (<20, 20–<30, 30–<40, 40–<50, ⩾50 years).

total fat (Table 4), nor with specific fatty acids (monounsaturated, polyunsaturated and saturated) and cholesterol intake (data not shown). These associations were not modified by cigarette smoking, *NAT2* or *GSTM1* genotypes (data not shown).

4. Discussion

Data from this large case-control study indicated that intake of selected fruits and vegetables could be inversely related to bladder cancer, and suggested that the reduction in risk from yellow-orange vegetable intake might be particularly strong among individuals with the *GSTM1* present genotype. In addition, intakes of folate, other B-vitamins and retinol were the main dietary nutrients inversely associated with bladder cancer.

Fruits and vegetables contain multiple antioxidant and chemopreventive compounds[26,27] and have been related to reduced levels of DNA adducts in the bladder.[28] Total fruit intake has been found to reduce bladder cancer risk in most studies,[5,10,29–32] but not all.[6] Our data were consistent with an overall reduction in risk from total fruit intake; however, the association was significant only among current smokers, as previously reported.[8,29] Berry intake was the only fruit subgroup with a significant overall association; however, this is not supported by a previous study.[6] The reduced risk associated with high intake of yellow-orange vegetables observed in our study was consistent with three previous case-control studies,[8,33,34] but not with a cohort study.[35] We also found *Liliaceae* vegetable (asparagus, leeks and onions) intake to be inversely associated with bladder cancer risk, which is interesting given the cancer-preventive properties of this group of vegetables.[36] A reduced bladder cancer risk associated with fruit and vegetable intake has been more consistently found in case-control studies than prospective studies,[5,6] suggesting that recall bias in case-control studies could at least partly explain the observed associations.

In addition to antioxidant compounds, fruits and vegetables contain phytochemicals such as isothiocyanate and allium which are substrates and can induce glutathione transferases.[27] Therefore, it has been suggested that subjects with the *GSTM1* null genotype (i.e. lacking enzyme activity) could receive a greater benefit from fruit and vegetable consumption. However, our data indicated a stronger reduction in risk from yellow-orange vegetable intake among subjects with the *GSTM1* present than null genotype. Thus, this interaction could be a false positive finding or could act through different mechanisms than previously suggested.

Our data did not support an increased bladder cancer risk with increasing consumption of meat, in agreement with previous studies,[10,37,38] and did not support an association with cooking methods or level of doneness, as it had been previously suggested.[39,40] Estimation of levels of HCA intake based on cooking methods and level of doneness suggested a small increase in risk for high levels of consumption, although our findings were not statistically significant. The only previous study that examined the association of HCA intake with bladder cancer also found a non-significant increase in risk.[41] Assessment of HCA exposure is difficult,[42] and it is possible that high misclassification in HCA intake estimation would underestimate real associations with risk.

High intakes of folate and other B-vitamins (B12, B6 and B2) were associated with significant decreases in bladder cancer risk. However, folate intake from diet alone was not found to protect against bladder cancer risk in a previous case-control study,[40] nor in two cohort studies.[6,11] Assessment of vitamin intakes from both food and supplement use in a case-control study showed a protective effect of folate,[40] while a cohort study showed no association for folate nor for vitamin B12.[35] Therefore, further evidence is needed to clarify this potential association.

In our study, subjects in the high quintile of consumption of retinol intake (median intake of 838 μg per day per Kcal or 2057 total μg per day) had a significant decrease in bladder cancer risk. This association is plausible because retinol is important in cell differentiation and has an inhibitory effect on bladder carcinogenesis[43–45]; however, it is not supported by the majority of previous studies.[4,6,11,12].

Strengths of this study are its relatively large sample size, inclusion of only incident cases, careful selection of control diagnoses to minimise selection bias, use of a comprehensive FFQ, and wide ranges of intakes. We did not observe significant differences in the distribution of dietary variables among controls with different diagnosis (data not shown), indicating that the choice of controls is unlikely to substantially bias our results. Cases and controls were matched in hospital and selected from departments with similar referral patterns to minimise selection bias. Although the study had some of the highest participation rates observed in hospital-based studies (84% for controls and 88% for cases), selection bias due to differences between participants and non-participants cannot be ruled out. Some demographic and risk factor characteristics were different for study participants completing and not completing the FFQ; however, differences were in the same direction for cases and controls, and the strength and magnitude of association of established risk factors were similar for participants and no participants in the FFQ (data

not shown), indicating that selection bias is unlikely to be substantial. A potential problem of our study is differential recall of previous diet between cases and controls, or pre-clinical disease affecting recent diet. However, all cases were incident and most of them were diagnosed as superficial tumours, for which general symptoms and subsequent change in habits are unlikely. Another concern is possible non-differential misclassification of dietary intake, and additional measurement error on nutrient and HCA intake estimation, which could have underestimated the observed associations. Estimates of HCA intake were obtained based on information on frequency and portion size of meat consumption, cooking technique and doneness level, and a database for HCAs. Therefore, error in measuring any of these variables would contribute error towards our estimates of HCA intake. We did not have information on occupation exposure to aromatics amines, a well-established risk factor for bladder cancer, at the time of the analyses. Therefore, if related to dietary intake, could have been confounded the observed associations.

In conclusion, the findings of this large study indicate that fruit and vegetable intake might be inversely associated with bladder cancer, and suggest that the inverse association with yellow-orange vegetable intake might be particularly strong among individuals with the *GSTM1* present genotype. Intakes of B-vitamins, in addition to retinol, were the main dietary nutrients associated with reductions in risk in our study; however, additional evidence from independent study populations is necessary to clarify these potential associations.

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Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2007.05.007.

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