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ORIGINAL PAPER

Flavonoids and bladder cancer risk

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Abstract

Purpose Flavonoids have drawn attention because of their antioxidant capacity and anti-carcinogenic effect in various types of cancer. A limited number of studies has investigated their potential effect on the risk of bladder cancer, with inconsistent results.

Methods We analyzed data from an Italian case–control study including 690 incident bladder cancer cases and 665 controls admitted to the same network of hospitals for acute, non-neoplastic, non tobacco-related diseases. Subjects were interviewed using a reproducible and validated food-frequency questionnaire. We applied data on food and beverage composition to estimate the intake of isoflavones, anthocyanidins, flavan-3-ols, flavanones, flavones and flavonols. We estimated odds ratios (ORs) through multiple logistic regression models, including terms for potential confounding factors, including tobacco smoking and total energy intake.

Results We found an inverse association between isoflavones (OR for the highest compared to the lowest quintile of intake = 0.56, 95% CI 0.37–0.84) and flavones (OR = 0.64, 95% CI 0.44–0.95) and bladder cancer. Non-significant inverse association was found for flavan-3-ols (OR = 0.70), flavonols (OR = 0.85) and total flavonoids (OR = 0.76). The results were consistent for non-muscle-invasive and muscle-invasive bladder cancers.

Conclusions Our data indicate an inverse association between isoflavones and flavones with respect to bladder cancer risk.

Keywords Case–control study · Bladder cancer · Flavonoids · Risk factors

Background

Bladder cancer has been related to tobacco smoking, occupational exposure to aromatic amines [1], diabetes [2], infection with *Schistomosa haematobium* [3] and other

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urinary tract infections [4, 5]. However, these factors explain only about half of bladder cancers [6]. Genetic polymorphisms in genes involved in the detoxification of carcinogenic compounds have also been related [7]. Water containing arsenic increases the risk of bladder cancer [8], whereas there is some evidence that higher consumption of vegetables, fruit and tea reduce bladder cancer risk [9–12].

Flavonoids constitute a wide group of selective bioactive compounds and have been related to the risk of various cancers [13–16]. They may modulate many biological events in cancer such as apoptosis, vascularization, cell differentiation and proliferation [17]. The most common forms of these polyphenolic compounds are isoflavones, anthocyanidins, flavan-3-ols, flavanones, flavones and flavonols. They mainly come from dietary components of plant origin, such as vegetables, fruits, tea and red wine [13, 18]. A possible effect of flavonoids on bladder cancer is biologically plausible, considering that urine is an important route for the excretion of flavonoids and their metabolites, and these dietary bioactive components are consequently in direct contact with the mucosa of the bladder [13, 19]. However, only a few epidemiological studies estimated the association between flavonoids and bladder cancer providing inconsistent results [20–24].

The aim of the current study is to investigate the relation of various classes of flavonoids and total flavonoids with bladder cancer risk, analyzing data from a large multicentric case–control study conducted in Italy.

Materials and methods

Between 2003 and 2014, we conducted a case–control study on bladder cancer in four Italian centers: Milan and Aviano (in Northern Italy) and Catania and Naples (in Southern Italy) [25, 26]. Cases were 690 patients aged 25–80 (median 67 years) with incident bladder cancer (mostly transitional cell carcinoma), admitted to a network including major hospitals in the study areas. Overall, 460 cases (66.7%) were non muscle-invasive (NMIBC, TNM pTis/Ta/T1) and 159 (23.0%) were muscle-invasive (MIBC, other T); 307 (44.5%) were well-differentiated or low grade (G1, G2) and 312 (45.2%) were poorly differentiated or high grade (G3, G4). The controls included 665 patients frequency matched to cases by study center, sex, and 5-year-age group (median age 66 years). They were patients admitted to the same network of hospitals as cases for a wide spectrum of acute, non-neoplastic conditions unrelated to tobacco smoking or long-term diet modification. Overall, 28.9% of controls were admitted for traumas, 22.1% for non-traumatic orthopedic disorders, 39.3% for acute surgical conditions, and 9.8% for other illnesses. All subjects signed an informed consent, according to the recommendations of the Board of Ethics of the study hospitals.

Centrally trained interviewers, unblinded to case–control status, administered a structured questionnaire to cases and controls during their hospital stay. The questionnaire included personal and sociodemographic characteristics, anthropometric measures, lifestyle habits, including tobacco smoking and alcohol consumption, family history of cancer, personal medical history and, for women, menstrual and reproductive factors. A satisfactorily reproducible and validated food frequency questionnaire (FFQ) was used to assess the participants' usual diet [27, 28]. The FFQ included the average weekly consumption of 80 items on food and 15 on (alcoholic and non-alcoholic) beverages. Subjects were asked to indicate the average weekly frequency of consumption for each dietary item; intakes less than once a week, but at least once a month, were coded as 0.5 per week.

We obtained food and beverage content of six classes of flavonoids (isoflavones, anthocyanidins, flavan-3-ols, flavanones, flavones and flavonols) from databases developed by the USA Department of Agriculture [18, 29] integrated with other published data when needed [30–32]. Estimates for energy intake were based on an Italian food composition database [33].

Major flavonoids included in the six classes are epicatechin and catechin for flavan-3-ols; hesperetin and naringenin for flavanones; quercetin for flavonols; malvidin for anthocyanidins; cyanidin, apigenin and luteolin for flavones and genistein for isoflavones. In our population, the intake of the six classes of flavonoids derived mainly from the consumption of oranges and other citrus fruits, tea, wine, apples or pears, and pulses (Supplementary Table 1).

Statistical analysis

Odds ratios (ORs) for bladder cancer, and their corresponding 95% confidence intervals (95% CIs), were estimated using unconditional multiple logistic regression models including terms for sex, age (in quinquennia, categorically), calendar year of interview (continuously), study center, education level (<7; 7–11; ≥ 12 years, categorically), tobacco smoking (never; former; current: <15, 15–24, ≥ 25 cigarettes/day, categorically), family history of bladder cancer, and total energy intake (quintiles of the distribution of controls, categorically). We also performed adjustment for energy intake using the residuals regression method suggested by Willett and Stampfer [34], which includes ''calorie-adjusted'' flavonoid intakes, calculated as residuals of the regressions of flavonoids on energy. However, since the residual regression method and the logistic regression models including energy intake adjustment yielded similar results, only findings from the latter are presented. Flavonoids were entered in the models as quintiles of the distribution of controls. Tests for trend were based on the likelihood ratio test between models with and without a linear term for each class of flavonoids. Additional models were used to assess the potential modifying effect of age, sex, education, and smoking. Polytomous logistic regression was used to estimate separate ORs for type and grade of cancer.

Results

Table 1 gives selected characteristics of 690 cases of bladder cancer and 665 controls according to sex, age and other selected factors. Cases and controls had a similar distribution in terms of study center, sex and age. The groups were also comparable in terms of education level, whereas tobacco smoking was more frequent among patients with cancer than controls.

The mean daily intake was 44.4 µg for isoflavones, 20.3 mg for anthocyanidins, 57.7 mg for flavan-3-ols, 30.9 mg for flavanones, 1.0 mg for flavones and 19.5 mg for flavonols, and 129.3 for total flavonoids among cases, and 46.7 µg for isoflavones, 19.5 mg for anthocyanidins, 63.5 mg for flavan-3-ols, 29.6 mg for flavanones, 1.2 mg for flavones and 20.6 mg for flavonols, and 134.3 for total flavonoids among controls (Table 2).

Table 3 gives the ORs and the corresponding 95% CIs for bladder cancer according to quintiles of flavonoids. Inverse associations were found for isoflavones (OR for the highest vs. the lowest quintile of intake = 0.56, 95% CI 0.37–0.84; p for trend < 0.001) and flavones (OR = 0.64, 95% CI 0.44–0.95; p for trend < 0.001). Inverse associations, although non-significant, were also observed for flavan-3-ols (OR = 0.70, 95% CI 0.46–1.04), flavonols (OR = 0.85,

Table 1 Distribution of 690 cases of bladder cancer and 665 controls according to center, sex, age and other selected variables. Italy, 2003–2014

	Cases		Controls		*p* value[a]
	No	%	No	%	
Centre					0.34
Aviano	242	35.1	250	37.6	
Milan	241	34.9	238	35.8	
Naples	129	18.7	100	15.0	
Catania	78	11.3	77	11.6	
Sex					0.33
Male	595	86.2	561	84.4	
Female	95	13.8	104	15.6	
Age (years)					0.06
< 60	148	21.5	178	26.8	
60–64	107	15.5	119	17.9	
65–69	164	23.8	147	22.1	
70–74	155	22.5	124	18.7	
≥ 75	116	16.8	97	14.6	
Education (years)[b]					0.80
< 7	292	42.4	273	41.1	
7–11	224	32.5	215	32.3	
≥ 12	173	25.1	177	26.6	
Smoking[b]					< 0.0001
Never smokers	96	14.1	237	35.6	
Ex-smokers	310	45.5	284	42.7	
Current smokers					
< 15 Cigarettes/day	79	11.6	53	8.0	
15–24 Cigarettes/day	127	18.7	68	10.2	
≥ 25 Cigarettes/day	69	10.1	23	3.5	
Family history of bladder cancer					0.04
No	667	96.7	654	98.3	
Yes	23	3.3	11	1.7	

[a] *p* value for Chi square test

[b] The sum does not add up to the total because of some missing values

Table 2 Mean intake (and standard deviation) of each class of flavonoid and total flavonoids among 690 cases of bladder cancer and 665 controls

Flavonoid	Cases	Controls	*p* value[a]
Isoflavones (µg)	44.4 (25.9)	46.7 (23.2)	0.08
Anthocyanidins (mg)	20.3 (17.4)	19.5 (16.5)	0.38
Flavan-3-ols (mg)	57.7 (53.9)	63.5 (58)	0.06
Flavanones (mg)	30.9 (29.7)	29.6 (28.5)	0.41
Flavones (mg)	1.0 (1.7)	1.2 (1.6)	0.022
Flavonols (mg)	19.5 (9.4)	20.6 (10)	0.031
Total flavonoids (mg)	129.3 (79.1)	134.3 (81.0)	0.25

[a]*p* value for t-test

95% CI 0.53–1.36) and total flavonoids (OR = 0.76, 95% CI 0.50–1.14). The estimates did not substantially change when further adjusted for body mass index and alcohol consumption, adding one variable at a time as well all variables combined. We also considered the main occupational exposures to substances related to bladder cancer (i.e., aromatic amines). However, only few subjects (six cases and five controls) were exposed to these chemicals.

We also performed analysis by NMIBC and MIBC patients, and found no significant differences in all classes of flavonoids. In particular, the ORs for the highest versus the lowest quintile were 0.58 (95% CI 0.37–0.92) in NMIBC and 0.35 (95% CI 0.17–0.70) in MIBC for isoflavones, and 0.61 (95% CI 0.40–0.95) in NMIBC and 0.56 (95% CI 0.30–1.07) in MIBC for flavones (Table 4).

Table 3 Distribution of 690 cases of bladder cancer and 665 controls according to quintiles of intake and corresponding odds ratios (ORs) and 95% confidence intervals (95% CIs)

	Quintiles					
	1	2	3	4	5	*p* for trend
Isoflavones (µg)						
Upper cutpoint	28.3	38.2	48.4	62.1	–	
Cases	184	130	148	121	107	
OR[a] (95% CI)	1[b]	0.66 (0.48–0.99)	0.77 (0.54–1.10)	0.59 (0.41–0.87)	0.56 (0.37–0.84)	0.0044
Anthocyanidins (mg)						
Upper cutpoint	5.6	12.3	19.3	30.8	–	
Cases	148	123	142	118	159	
OR[a] (95% CI)	1[b]	0.80 (0.55–1.17)	0.93 (0.64–1.36)	0.75 (0.51–1.12)	1.07 (0.70–1.63)	0.9250
Flavan-3-ols (mg)						
Upper cutpoint	22.6	36.6	54.0	101.8	–	
Cases	162	154	110	162	102	
OR[a] (95% CI)	1[b]	0.95 (0.66–1.36)	0.68 (0.45–1.01)	1.08 (0.73–1.59)	0.70 (0.46–1.04)	0.2518
Flavanones (mg)						
Upper cutpoint	4.8	19.2	33.5	38.3	–	
Cases	142	145	139	93	171	
OR[a] (95% CI)	1[b]	0.98 (0.68–1.41)	0.86 (0.60–1.24)	0.73 (0.49–1.09)	1.18 (0.82–1.71)	0.7151
Flavones (mg)						
Upper cutpoint	0.1	0.3	0.7	2.0	–	
Cases	181	151	152	110	96	
OR[a] (95% CI)	1[b]	0.84 (0.59–1.19)	0.89 (0.62–1.27)	0.67 (0.46–0.98)	0.64 (0.44–0.95)	0.0144
Flavonols (mg)						
Upper cutpoint	12.3	16.6	20.9	27.8	–	
Cases	145	181	109	137	118	
OR[a] (95% CI)	1[b]	1.29 (0.88–1.87)	0.72 (0.47–1.09)	0.92 (0.60–1.41)	0.85 (0.53–1.36)	0.1720
Total flavonoids (mg)						
Upper cutpoint	71.9	104.2	140.3	187.0	–	
Cases	170	133	137	122	128	
OR[a] (95% CI)	1[b]	0.67 (0.46–0.97)	0.81 (0.55–1.18)	0.73 (0.50–1.09)	0.76 (0.50–1.14)	0.3201

[a]Estimates from unconditional logistic regression models adjusted for age, sex, study center, year of interview, education, smoking, family history of bladder cancer and total energy intake

[b]Reference category

When we separately performed analysis by grade, we found an OR of 0.69 (95% CI 0.41–1.13) in well-differentiated or low grade and 0.37 (95% CI 0.22–0.62) in poorly differentiated or high grade for isoflavones, and an OR of 0.68 (95% CI 0.42–1.10) in well-differentiated or low grade and 0.53 (95%CI 0.32–0.87) in poorly differentiated or high grade for flavones.

Table 5 gives the ORs for the highest versus the lowest quintile of isoflavones and flavones across strata of sex, age, education, and smoking status. All the ORs were below unity, and were not heterogeneous across strata of the covariates considered.

Discussion

This large multicenter study indicates, and better quantifies than previously available, that isoflavones and flavones are inversely associated to bladder cancer risk. For other classes of flavonoids and total flavonoids there was a non-significant relation with bladder cancer risk. The inverse associations were consistent in MIBC and NMIBC. Results were homogeneous across strata of sex, age, education and smoking.

A report from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, including 1575 incident bladder cancer cases, suggested an inverse association between flavonols and risk of bladder cancer (hazard ratio, HR = 0.75 for more than 38.5 mg/day vs. less than 12.9 mg/day of intake, 95% CI 0.61–0.91), in the absence, however, of association with total flavonoids [20].

Other epidemiological studies focused on selected individuals flavonoids. The Iowa Women's Health Study found no association for dietary catechins (a flavan-3-ol monomer) among a cohort of postmenopausal women, including 103 cases (relative risk, RR, for the highest vs the lowest quintile of consumption = 1.12, 95% CI 0.65–1.93) [23]. Another case–control study from Spain did not report any significant results for single flavones and flavonols, such as quercetin, kaempferol, myricetin and luteolin. The ORs for the highest versus the lowest quartile of intake were 1.21 (95% CI 0.8–1.9), 1.35 (95% CI 0.9–2.1), 0.82 (95% CI 0.6–1.2) and 0.95 (95% CI 0.6–1.4), respectively [21].

Some studies have tried to explore the link between soy intake, the main source of isoflavones in traditional Asian diet, and bladder cancer risk with controversial results [22, 24]. In two Chinese cohort studies, twofold increased risk was found in the highest quartile of soy consumption compared with the lowest one. In the same studies, similar results were obtained for intakes of soy isoflavones. A probable explanation for the positive association found in the Chinese studies is related to some carcinogenic products that are created by a prolonged contact between soybeans and chlorinated water in the tofu production [24]. Moreover,

Table 4 Distribution of 460 non-muscle-invasive (NMIBC) and 159 muscle-invasive (MIBC) bladder cancer cases and 665 controls according to quintiles of flavonoid intakes and corresponding odds ratios (ORs) and 95% confidence intervals (CIs)

	NMIBC		MIBC	
	No. cases: No. controls	OR (95% CI)[a]	No. cases: No. controls	OR (95% CI)[a]
Isoflavones				
I	124:133	1.00[b]	43:133	1.00[b]
II	90:133	0.75 (0.51–1.11)	29:133	0.67 (0.38–1.19)
III	94:133	0.71 (0.48–1.06)	39:133	0.87 (0.51–1.51)
IV	79:133	0.56 (0.37–0.85)	30:133	0.61 (0.33–1.10)
V	73:133	0.58 (0.37–0.92)	18:133	0.35 (0.17–0.70)
Anthocyanidins				
I	95:133	1.00[b]	33:133	1.00[b]
II	72:133	0.68 (0.44–1.04)	33:133	0.93 (0.51–1.70)
III	101:133	0.98 (0.64–1.49)	30:133	0.95 (0.51–1.76)
IV	81:133	0.73 (0.47–1.13)	25:133	0.70 (0.36–1.35)
V	111:133	0.98 (0.62–1.56)	38:133	1.20 (0.62–2.34)
Flavan-3-ols				
I	105:133	1.00[b]	33:133	1.00[b]
II	99:133	0.92 (0.61–1.38)	41:133	1.19 (0.67–2.12)
III	77:133	0.65 (0.42–1.01)	23:133	0.64 (0.32–1.24)
IV	108:133	0.99 (0.64–1.53)	40:133	1.27 (0.68–2.36)
V	71:133	0.72 (0.46–1.14)	22:133	0.65 (0.33–1.29)
Flavanones				
I	92:134	1.00[b]	35:134	1.00[b]
II	97:132	0.95 (0.63–1.43)	31:132	0.78 (0.43–1.41)
III	100:144	0.91 (0.61–1.36)	31:144	0.79 (0.44–1.42)
IV	62:120	0.75 (0.48–1.19)	24:120	0.64 (0.33–1.21)
V	109:135	1.17 (0.78–1.78)	38:135	1.10 (0.61–1.96)
Flavones				
I	122:133	1.00[b]	41:133	1.00[b]
II	97:133	0.75 (0.51–1.12)	39:133	1.00 (0.57–1.73)
III	101:133	0.85 (0.57–1.26)	34:133	0.83 (0.47–1.46)
IV	76:133	0.66 (0.44–1.01)	24:133	0.57 (0.31–1.07)
V	64:133	0.61 (0.40–0.95)	21:133	0.56 (0.30–1.07)
Flavonols				
I	96:133	1.00[b]	28:133	1.00[b]
II	107:133	1.11 (0.73–1.69)	60:133	2.10 (1.15–3.84)
III	67:133	0.66 (0.42–1.05)	26:133	0.72 (0.36–1.46)
IV	97:133	0.87 (0.54–1.38)	27:133	0.75 (0.36–1.58)
V	93:133	0.86 (0.51–1.44)	18:133	0.52 (0.22–1.23)
Total flavonoids				
I	106:133	1.00[b]	38:133	1.00[b]
II	82:133	0.62 (0.40–0.94)	39:133	0.92 (0.52–1.63)
III	101:133	0.88 (0.57–1.34)	26:133	0.65 (0.34–1.22)
IV	83:133	0.76 (0.49–1.18)	29:133	0.71 (0.38–1.33)
V	88:133	0.77 (0.48–1.22)	27:133	0.65 (0.34–1.27)

[a]Estimates from unconditional logistic regression models adjusted for age, sex, study center, year of interview, education, smoking, family history of bladder cancer and total energy intake

Table 4 (continued)

[b]Reference category

Table 5 Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of bladder cancer according to intake of the highest versus the lowest quintile of selected flavonoids in strata of covariates among 690 cases and 665 controls

	No. of cases	OR (95% CI)[a]	
		Isoflavones	Flavones
Sex			
Male	595	0.60 (0.39–0.94)	0.67 (0.44–1.03)
Female	95	0.31 (0.10–0.96)	0.38 (0.13–1.16)
Age			
≤65	283	0.82 (0.44–1.53)	0.68 (0.36–1.25)
>65	407	0.47 (0.27–0.82)	0.57 (0.34–0.94)
Education level (years)[b]			
Low (<7)	292	0.83 (0.43–1.58)	0.60 (0.32–1.12)
Intermediate/high (≥7)	397	0.46 (0.27–0.80)	0.65 (0.39–1.08)
Smoking habits[b]			
Never	96	0.69 (0.28–1.67)	0.57 (0.24–1.31)
Former	310	0.49 (0.28–0.89)	0.65 (0.37–1.14)
Current	275	0.57 (0.24–1.34)	0.47 (0.21–1.04)

[a]V quintile as compared to I. Estimates from unconditional logistic regression models adjusted for age, sex, study center, year of interview, education, smoking, family history of bladder cancer and total energy intake, when appropriate

[b]The sum does not add up to the total because of some missing values

results of the two Chinese studies are based on a very low number of cases (61 each) [22, 24].

Moreover, supporting our results, a strong inverse association between beans, a major source of isoflavones in Latin American diet, and bladder cancer risk was found in two case–control studies [35, 36].

Flavonoids and their metabolites are in direct contact with bladder tissue, possibly causing a protective effect against tumor process [22]. Among all classes of flavonoids, isoflavones are those with the highest percentage in the urinary excretion [13]. Genistein plays a favourable chemopreventive role in many stages of carcinogenesis [37]. This isoflavone was found to induce G2/M cell cycle arrest [38] and dose-dependently apoptosis, to downregulate NF-KB pathway in bladder cancer cells [39], and to inhibit EGFR activity and phosphorylation and EGF mediated responses [40]. Genistein selectively inhibits the HER-2/neu-related signalling pathway [41] and the growth of poorly differentiated and highly metastatic bladder cancer cell line 253J B-V in vitro [19]. In addition, it showed a suppressor effect on multiple angiogenesis of human bladder cancer cells in vitro [42, 43].

Luteolin and apigenin, belonging to the flavone class, showed inhibition of the growth and induction of apoptosis and were able to induce cytotoxicity in bladder carcinoma cells in a dose- and time-dependent manner [44]. In particular, luteolin revealed an anti-proliferative activity with a dose-dependent inhibition of NAT activity and gene expression (NAT1 mRNA) in bladder cancer cells [45, 46]. The bladder cancer preventive effects of apigenin have been shown by various molecular mechanisms, including inhibition of proliferation of T-24 human bladder cells via blocking cell cycle progression in the subG1 phase and induction of apoptosis, as it triggers the mitochondrial apoptotic pathway by regulating the expression of Bcl-2 family proteins, causing the release of cytochrome c and activating caspase-9, caspase-7, caspase-3 and PARP [47]. It inhibits cell migration through MAPK pathways in human bladder smooth muscle cells [48], and promotes apoptosis via PI3K/Akt pathway and G2/M phase arrest in T-24 bladder cancer cells [49].

Flavones are abundant in citrus fruits and leafy vegetables, celery, parsley, tea and grains [18]. In our study, flavones comes mainly from spinach and chard, vegetables, beans and tea. Fruit and vegetable consumption is related to a reduced risk of bladder cancer [10–12, 50–52]. Mediterranean diet, characterized by the high intake of fruits and vegetables, including those rich in flavonols, has been found to have a protective role in invasive urothelial cell carcinoma, the most common form of bladder cancer [53]. In addition, dietary patterns with a low consumption of fruits and vegetables, like western pattern diet or with a high Dietary Inflammatory Index (DII), showed a detrimental role on bladder cancer risk [26, 54].

Limitations of the study are concerns about case–control studies. The exclusion from the control group of subjects with non-neoplastic conditions or with any potential long-term changes in diet, the comparable catchment areas and the almost complete participation rate of cases and controls can limit possible selection bias. The fact that cases and controls were unaware of any particular dietary hypothesis in bladder cancer etiology reduces potential recall bias. In addition, the questionnaire was administered to cases and controls by the same interviewers under similar conditions in a hospital setting, thus minimizing information bias. Among other strengths of the study are the large sample size, the nearly complete participation of identified cases and controls, and the use of a reproducible and validated FFQ although designed to investigate other vegetable components such as vitamins and minerals but not flavonoids [27, 28]. A possible limitation due to the adaptability of the U.S. food composition data to the Italian foods was resolved using a standardised methodology [55]. With reference to measurement error, no adequate studies using biomarkers of flavonoid intake are available. Therefore, it is difficult to evaluate the precision of exposure measurement due to the variation of the food amounts in the recipes and the variability in flavonoid content attributable to several factors, i.e., heat and sunlight. With reference to information bias, it is not unlikely that patients modified their diet because of symptoms, and this is an inherent limitation to the study design. However, symptoms of acute control conditions and of bladder cancer (e.g., frequent urination, hematuria, low back pain) do not necessarily lead to substantial changes in diet. It is also unlikely that any symptoms of bladder cancer lead to a reduced consumption of selected flavonoids (i.e., reverse causation).

We were also able to adjust the risk estimates for total energy intake and major recognized risk factors for bladder cancer, such as tobacco smoking and family history of bladder cancer. We used unconditional regression. As a sensitivity analysis, we fitted conditional models, too. No material difference was observed. The ORs for the highest quintile were 0.57 (95% CI 0.38–0.85) for isoflavones, 1.11 (95% CI 0.73–1.69) for anthocyanidins, 0.68 (95% CI 0.45–1.01) for flavan-3-ols, 1.20 (95% CI 0.82–1.74) for flavanones, 0.63 (95% CI 0.42–0.92) for flavones, 0.82 (95% CI 0.51–1.32) for flavonols, and 0.75 (95% CI 0.50–1.14) for total flavonoids. Likewise, including energy intake as a continuous variable, the results did not materially change. The ORs for the highest quintile were 0.56 (95% CI 0.37–0.84) for isoflavones, 1.06 (95% CI 0.70–1.61) for anthocyanidins, 0.71 (95% CI 0.47–1.05) for flavan-3-ols, 1.18 (95% CI 0.82–1.71) for flavanones, 0.65 (95% CI 0.44–0.96) for flavones, 0.82 (95% CI 0.51–1.32) for flavonols, and 0.75 (95% CI 0.50–1.12) for total flavonoids. Moreover, we performed stratified analyses according to various risk factors, including smoking. Risk estimates were not significantly heterogeneous across strata of different smoking habits. As an additional sensitivity analysis, when smoking was excluded from the main models, the ORs for the highest versus the lowest quintile were 0.45 (95% CI 0.30–0.66) for isoflavones and 0.52 (95% CI 0.36–0.75) for flavones.

In conclusion, this is the first epidemiological study indicating an inverse association of dietary intake of isoflavones and flavones in relation to bladder cancer risk. Other classes and total flavonoids did not yield any significant results.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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