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Minerals and vitamins and the risk of bladder cancer: results from the New Hampshire Study

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Abstract

Objective Although the effect of fruit and vegetables on the risk of bladder cancer has been widely studied, little is known about their micronutrient components. Our aim was to investigate associations between minerals and vitamins and bladder cancer.

Methods A case–control study was conducted in New Hampshire, USA. Dietary data were collected from 322 cases and 239 controls using a 121-item food frequency questionnaire. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression adjusting for sex, age, smoking characteristics, and energy intake.

Results The ORs (95% CI) for highest quartile versus lowest quartile for total intake of vitamin E was 0.66 (0.36–1.20; p trend = 0.09) and 0.49 (0.21–1.17; p trend = 0.13) for dietary phosphorus. The odds of bladder cancer for heavy smokers with the highest total intake of vitamin E, carotenoids, and niacin were 0.58 (0.34–0.99), 0.62 (0.36–1.09), and 0.66 (0.39–1.14), respectively. Higher total intakes of carotenoids, vitamin D, thiamin, niacin, and vitamin E were inversely related to bladder cancer risk among older individuals.

Conclusion Our findings suggest further investigation of the effect of vitamin E, carotenoids, vitamin D, thiamin, and niacin on bladder cancer risk may be warranted. Future studies should focus on high risk groups such as heavy smokers and older individuals.

Keywords Bladder cancer · Minerals · Vitamins · Carotenoids · Folate

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Introduction

In 2002, a global study reported that the US had among the highest reported age standardized incidence rate of bladder cancer (24.1/100,000) [1]. The National Cancer Institute (NCI) estimated that in 2009, there would be approximately 70,980 new cases and 14,330 deaths from bladder cancer in the USA [2].

Despite reduced exposure to established risk factors such as smoking, aromatic arylamines (occupation), and

schistosomal infection, the incidence of bladder cancer in the USA remains high [3]. This is consistent with other studies suggesting that these risk factors only partly explain bladder cancer etiology [4, 5]. One etiologic study [6] investigating regional variation in bladder cancer rates across the USA also considered diet as a possible risk factor.

Several studies [7–14] have reported that dietary factors such as fruit and vegetables may be protective against bladder cancer. However, there is a lack of consistent evidence between the intake of related micronutrient components of fruits and vegetables and reduced risk of the disease [5]. The Second Expert Report on nutrition and cancer by the World Cancer Research Fund/American Institute of Cancer Research states that the evidence was too limited to conclude that any food or nutrient directly influences the risk of bladder cancer [15]. Conversely, a recent systematic review [16] as part of a WHO consultancy report found that nutrient components of specific fruits and vegetables have a possible inverse association with bladder cancer. It is these dietary micronutrients or their metabolites that actually come into direct contact with the bladder epithelium and thus in theory could be protective [10].

Vitamin A is obtained from the diet as preformed vitamin A from animal products and as carotenoids including both provitamin A and non-provitamin A carotenoids from plant foods [15]. An inverse association between vitamin A intake and bladder cancer has been reported in some studies [17, 18], but not others [3, 19–21]. Inconsistencies may be due to many earlier studies not distinguishing between these dietary sources [10]. Some recent studies suggest that it is the carotenoids [8, 22–24] (α-carotene [8], β-carotene [8, 25], β-cryptoxanthin [21], lycopene [8], lutein/zeaxanthin [8], and not retinol [10, 23, 26] that influences the risk of bladder cancer. Although, other studies have reported the opposite effects, with some finding that retinol may actually be protective [18, 27–29] and others that the carotenoids have no effect [3, 10, 19, 30, 31].

The relationship between bladder cancer and vitamin C and E also appears to be unclear with some studies reporting potential inverse associations (vitamin C: [29, 32, 33]; vitamin E: [20, 27]) and others no associations at all (vitamin C: [3, 19, 21, 23, 25]; vitamin E: [3, 19, 21, 23, 25, 31]).

Deficiencies in folic acid, vitamins B3 (niacin), B6, B12, have been reported to mimic radiation damage to DNA and possibly lead to the initiation of cancer [34]. However, there is only limited literature available on the association between water-soluble B-group vitamins and risk of bladder cancer, and the findings have been inconsistent [20, 21, 23, 25, 28, 35].

Cohen [36] reviewed the effect of diet on bladder cancer risk. He concluded that the potential for certain dietary minerals to influence urine composition and hence bladder carcinogenesis observed in animal studies needed further evaluation before extrapolation could be made to human populations. To date, few epidemiological studies have investigated the effect of dietary minerals such as sodium [20, 23], potassium [20], calcium [20, 23, 26], magnesium [20], and iron [20, 26] on the risk of developing bladder cancer.

Micronutrient deficiencies could provide a possible explanation for why an estimated 25% of the US population who consume the least fruit and vegetables have double the cancer rate [34]. The aim of our study was to investigate the association between major dietary minerals and vitamins and the risk of bladder cancer in a US population from a region with a high incidence rate [37].

Methods

Study population

A population-based case–control study was conducted in New Hampshire, USA. Details of the study have been reported previously [38, 39]. Briefly, bladder cancer cases were identified from the New Hampshire State Department of Health and Human Services Cancer Registry as histologically confirmed, primary bladder cancer diagnosed between 1 July 1998 and 31 December 2001. To be eligible for inclusion in the study, all cases had to be New Hampshire residents aged between 25 and 74, have a listed phone number and speak English. Physician consent was obtained before contacting potential participants. Of the 472 potentially eligible cases we contacted, a total of 398 (84%) were interviewed for the study. For efficiency, controls were shared with another study on non-melanoma cancer covering a reference period from 1 July 1997 to 30 March 2000 [40]. Controls less than 65 years of age were selected from lists obtained from the New Hampshire Department of Transportation. Controls 65 years of age and older were chosen from data files provided by the Centers for Medicare & Medicaid Services (CMS) of New Hampshire. Controls were randomly assigned a comparable reference date to case group dates of diagnoses [41]. A total of 526 controls (76%) were interviewed from a potential 694 confirmed eligible participants.

Data collection

Extensive in-person interviews were conducted with consenting participants, usually in the homes of the participants. Data were collected on participants' socio-demographic information such as education level, residence, occupation (history), medical history, lifestyle factors (including tobacco smoking), household water supply, and family history of

cancer. Interviewers were blind to case/control status and interviews were tape recorded with subjects' consent (less than 5% refused to have the interview taped) to ensure consistent quality of the interview and clarification of details. Cases were also asked if they held a driver's license or a Medicare enrollment card for comparability with controls [42].

Dietary data

Interviews which included dietary assessment took place between 2000 and 2003 for both cases and controls (for 95% of subjects). As data were unavailable for 150 cases and 455 controls; a total of 322 cases and 239 controls were included in the dietary analyses of this study. Subjects from whom dietary data were collected were comparable to those who did not provide dietary data with respect to age, sex, and smoking history (data not shown).

Dietary information was collected from a 121-item semi-quantitative food frequency questionnaire (FFQ) that was developed by Willett et al. for the Nurses' Health Study [43]. Participants were asked about their usual dietary intake for the previous 12-month period. Foods items were assigned to seven broad categories. These included dairy, fruit, vegetables, eggs and meat, breads, beverages, and baked goods. There was a list of nine common mineral and vitamin supplements to choose from which included the following: multiple vitamins (participants were asked to report the individual vitamins within this category), vitamins A, C, B6 and E, selenium, iron, zinc, and calcium. Additional questions covered folic acid, vitamin D, vitamin B complex, cod liver oil, omega 3 fatty acids, iodine, copper, Brewer's yeast, β-carotene, magnesium, and an open ended option, "other." Participants were provided with options for duration of supplement intake ranging from "0" to 10+ years.

Frequency options ranged from never to six or more times a day. Nutrient intake was calculated by multiplying frequency of each food item on the FFQ by the nutrient content for the specific portion size [44]. Food composition data were based primarily on data from the United States Department of Agriculture (USDA) [45].

We investigated vitamins that had a biologically plausible association with bladder cancer and "macro-minerals", which are required in quantities greater than 100 mg per day e.g., sodium, potassium, calcium, phosphorus, and magnesium [20]. An exception was iron, which was included due to its physiological function and potential to influence the risk of bladder cancer [34].

Statistical analysis

We investigated the association between total mineral and vitamin intake, which included micronutrients from both dietary and supplemental sources and bladder cancer risk. The intake of carotenoids, folate, and vitamin B12 were measured in micrograms (µg) per day. All other vitamins including, B1 (thiamin), B2 (riboflavin), B3 (niacin), vitamin C and vitamin E, and minerals (sodium, potassium, calcium, phosphorus, magnesium, and iron) were calculated in milligrams (mg) per day except for vitamin D intake, which was calculated in international units (IU). The data were analyzed in quartiles based on the control group distributions. ORs and corresponding 95% CIs were calculated using logistic regression analysis. Adjustment was made for a number of possible confounding variables including sex, age, cigarette smoking status (current/non-current smoker), pack years of cigarette smoking (categories: $0 < 10$, $10 < 20$, $20 < 30$, $30 < 40$, $40 < 50$, ≥ 50; calculated by dividing the number of cigarettes smoked per day by 20 and multiplying by the number of years smoked) and total energy intake (quartiles for kcal/day). Additional adjustment for other potential confounders, total fat intake (fat-soluble vitamins), alcohol and coffee consumption (water-soluble vitamins) did not alter the associations between micronutrients and bladder cancer. Minerals and vitamins were dichotomized according to median intakes by controls. Odds ratios and 95% CIs were calculated to investigate potential differences between invasive and non-invasive bladder cancer. We also performed additional analyses to assess the effects of minerals and vitamins from both dietary and supplemental sources separately. Intake of nutrients from dietary sources were analyzed in quartiles using the same statistical model for total intake of minerals and vitamins. We estimated the intake of minerals and vitamins from supplements by subtracting dietary intake from total nutrient intake. Due to the relatively small contribution to mineral and vitamin intake by supplements in this study population, these nutrients were dichotomized according to average intake of controls. Likelihood ratio tests were conducted to test for trend by assigning an integer to each quartile of the nutrient e.g., 1–4 then fit the term as a continuous variable in the model. The joint effects of specific nutrients and smoking status/history and age were also investigated. Nutrients and age were dichotomized into high and low categories based on median values of the controls. Smoking status was dichotomized into never/ever smoker, cigarettes per day into high and low categories ($<20/\geq 20$ cigarettes per day) and duration of smoking (<27 years/≥ 27 years). We used the likelihood ratio test to evaluate interaction, i.e., comparing the log likelihood of regression models with and without the interaction term included. The false-positive report probability (FPRP) was employed to assess the probability of no true association between nutrients and the risk of bladder cancer given a statistically significant finding [46]. This test takes into account three factors that determine the

probability that a statistically significant result may actually be a false-positive finding. These are (1) prior probability of an association; (2) the alpha-level, and (3) statistical power to detect an odds ratio for the alternative hypothesis at a given alpha-level or p value. We used previously reported arbitrary values for these factors: 0.05 (alpha-level), 0.25 (prior probability), and 0.5 (false-positive report probability) [46, 47].

All reported p values were two-sided and values less than or equal to 0.05 were considered to be statistically significant. The statistical software package Stata/SE 10.0 (Stata Corporation, College Station, TX, USA) was used for all statistical analyses.

Results

Study characteristics

Table 1 presents the frequency distributions of selected study characteristics for the 322 cases and 239 controls for

Table 1 Distribution of bladder cancer cases and controls by demographic characteristics and bladder cancer risk factors

	Cases number (%)	Controls number (%)
Total	322	239
Sex*		
Men	237 (74)	138 (58)
Women	85 (26)	101 (42)
Age (years) [mean (SD)]	62 (9.2)	60.7 (10.6)
Smoking status*		
Never smokers	56 (17.4)	95 (39.7)
Ex-smokers	162 (50.3)	103 (43.1)
Current smokers	104 (32.3)	41 (17.2)
Years smoking [mean (SD)]*	32.2 (14)	27.4 (15)
Cigarettes/day [mean (SD)]*	26.7 (14.8)	22.3 (15.6)
Total calories kcal/day [mean (SD)]	1962.56 (790.72)	1887.74 (706.64)
Total fat g/day [mean (SD)]	71.74 (32.96)	69.77 (31.76)
Total alcohol g/day [mean (SD)]	7.91 (15.84)	8.29 (14.45)
Total coffee g/day [mean (SD)]	227.41 (210.13)	216.57 (192.88)
Tumor stage at diagnosis		
Carcinoma in situ (%)	16 (5)	
Non-invasive/low grade (%)	190 (59)	
Non-invasive/high grade (%)	23 (7)	
Invasive (%)	93 (29)	

SD standard deviation

* Statistically significant differences between cases and controls; $p < 0.05$

whom dietary data were available for analyses in this study. Bladder cancer cases consisted of approximately three times more men (74%) than women. A majority of both cases and controls were in the older age group (60 years and over). There was a higher prevalence of current smokers and greater number of both cigarettes smoked per day and years smoked among cases compared with the controls. We detected no statistically significant differences between cases and controls for calorie, total fat, alcohol, or coffee intake.

Total intake of minerals and vitamins and the risk of bladder cancer

Table 2 shows the ORs and 95% CIs for bladder cancer and total intake (including dietary and supplemental sources) of fat-soluble and water-soluble vitamins. We observed a borderline statistically significant inverse association between total intake of vitamin E and bladder cancer (highest vs. lowest quartile, OR: 0.66; 95% CI: 0.36–1.20; p trend = 0.09). No statistically significant ORs were observed when comparing the highest quartile of intake with the lowest quartile for carotenoids as a group or for any of the individual carotenoids, α-carotene, β-carotene, β-cryptoxanthin, lycopene, or lutein/zeaxanthin. Although the point estimate for vitamin D was well below one, this also did not reach statistical significance (OR: 0.58; 95% CI: 0.31–1.06; p trend = 0.22).

Among the water-soluble vitamins, the OR for niacin and bladder cancer was of borderline statistical significance (highest vs. lowest quartile OR: 0.56; 95% CI: 0.31–1.02). No other associations were observed between bladder cancer and any other B-group or water-soluble vitamins, which included thiamin, riboflavin, B6, B12, and folate or vitamin C. Similarly, no associations were observed between bladder cancer and total intake for any of the minerals reported in Table 3, including sodium, potassium, magnesium, calcium, phosphorus, and iron. Nor were there any detectable differences in the effects of any of the minerals and vitamins between invasive and non-invasive bladder cancer.

Analyses were repeated for all minerals and vitamins using measures taken from both dietary sources (excluding supplements) and supplements alone (data not shown). The only nutrient of interest to emerge from dietary sources alone when comparing highest quartiles of intake with lowest quartiles of intake, although not statistically significant was phosphorus (OR: 0.49; 95% CI: 0.21–1.17; p trend = 0.13). No other associations or trends could be observed for any of the other dietary or supplemental sources of minerals and vitamins.

We continued analyses for minerals and vitamins where an association had been indicated either from borderline

Table 2 Calculated odds ratios (95%) for total intake of vitamins and bladder cancer

Daily intake	Q1	Q2	Q3	Q4	p Trend
Alpha-carotene (µg)	3.4–404	404.1–647.13	647.14–1104.70	≥1104.71	0.26
Cases/controls	76/59	89/60	77/61	77/59	
ORs (95% CI)	1.00	1.33 (0.77–2.30)	1.19 (0.68–2.11)	1.48 (0.83–2.64)	
Beta-carotene (µg)	292.29–2294.21	2294.22–3773.80	3773.81–5517.20	≥5517.21	0.90
Cases/controls	94/59	78/61	65/60	79/59	
ORs (95% CI)	1.00	0.87 (0.50–1.50)	0.80 (0.46–1.39)	0.99 (0.56–1.72)	
Beta-cryptoxanthin (µg)	0–52.60	52.61–125.77	125.78–215.71	≥215.72	0.65
Cases/controls	109/59	76/61	59/58	78/61	
ORs (95% CI)	1.00	0.69 (0.40–1.20)	0.66 (0.37–1.16)	0.87 (0.50–1.53)	
Lycopene (µg)	0–3092.68	3092.69–4827	4828–7746.43	≥7746.44	0.74
Cases/controls	89/59	73/60	72/60	88/60	
ORs (95% CI)	1.00	0.96 (0.55–1.66)	0.70 (0.39–1.24)	0.98 (0.55–1.73)	
Lutein (µg)	0.93–1261.48	1261.49–1942.44	1942.45–3134.85	≥3134.86	0.66
Cases/controls	116/58	61/61	74/60	70/59	
ORs (95% CI)	1.00	0.52 (0.30–0.90)	0.76 (0.45–1.31)	0.80 (0.46–1.40)	
Total carotenoids (µg)	398.26–8879.22	8879.23–12900.60	12900.61–17932.72	≥17932.73	0.47
Cases/controls	107/59	73/60	52/60	87/60	
ORs (95% CI)	1.00	0.70 (0.41–1.21)	0.49 (0.27–0.86)	0.90 (0.51–1.59)	
Vitamin D (IU)	15.53–171.74	171.75–388.90	388.91–641.12	≥641.13	0.22
Cases/controls	89/59	72/60	100/60	61/60	
ORs (95% CI)	1.00	0.78 (0.44–1.38)	1.03 (0.60–1.76)	0.58 (0.31–1.06)	
Vitamin E (mg)	1.13–7.26	7.27–20.94	20.95–193.35	≥193.36	0.09
Cases/controls	77/57	95/58	81/57	54/57	
ORs (95% CI)	1.00	1.19 (0.68–2.08)	0.88 (0.49–1.56)	0.66 (0.36–1.20)	
Thiamin (mg)	0.21–1.32	1.33–2.11	2.12–3.34	≥3.35	0.50
Cases/controls	91/58	63/59	99/63	67/58	
ORs (95% CI)	1.00	0.53 (0.29–0.96)	0.97 (0.56–1.68)	0.63 (0.35–1.14)	
Riboflavin (mg)	0.25–1.63	1.64–2.75	2.76–4.04	≥4.05	0.61
Cases/controls	85/60	74/59	91/61	71/59	
ORs (95% CI)	1.00	0.73 (0.41–1.29)	1.02 (0.58–1.77)	0.75 (0.42–1.34)	
Niacin (B3) (mg)	5.77–21.91	21.92–33.07	33.08–46.50	≥46.51	0.23
Cases/controls	90/58	68/60	93/60	67/60	
ORs (95% CI)	1.00	0.59 (0.33–1.06)	0.94 (0.54–1.64)	0.56 (0.31–1.02)	
Vitamin B6 (mg)	0.32–1.94	1.95–3.28	3.29–4.88	≥4.89	0.41
Cases/controls	93/59	75/59	77/61	75/59	
ORs (95% CI)	1.00	0.67 (0.38–1.18)	0.82 (0.47–1.44)	0.72 (0.41–1.27)	
Vitamin B12 (µg)	0.75–5.51	5.52–10.82	10.83–18.0	≥18.1	0.75
Cases/controls	79/60	89/60	65/60	87/59	
ORs (95% CI)	1.00	1.30 (0.75–2.26)	0.84 (0.47–1.51)	1.05 (0.60–1.84)	
Folate (µg)	48.41–339.03	339.04–579.10	579.11–819.50	≥819.51	0.43
Cases/controls	82/59	82/61	85/59	71/60	
ORs (95% CI)	1.00	0.83 (0.47–1.46)	0.91 (0.53–1.56)	0.74 (0.40–1.35)	
Vitamin C (mg)	14.61–113.03	113.04–190.25	190.26–366.10	≥366.11	0.42
Cases/controls	98/58	78/60	80/61	62/59	
ORs (95% CI)	1.00	0.83 (0.48–1.43)	0.84 (0.48–1.45)	0.78 (0.45–1.38)	

OR odds ratios, *CI* confidence intervals, adjusted for age, sex, smoking status: current versus non-current smoker, pack years smoked (categories: 0–10, 10–20, 20–30, 30–40, 40–50, 50+), and total energy intake (quartiles kcal/day)

Table 3 Calculated odds ratios (95%) for total intake of minerals and bladder cancer

Daily intake	Q1	Q2	Q3	Q4	p Trend
Sodium (mg)	344.61–1484.83	1484.84–1898.64	1898.65–2372.65	≥2372.66	0.32
Cases/controls	78/58	64/60	59/60	119/60	
ORs (95% CI)	1.00	1.17 (0.61–2.23)	1.00 (0.48–2.12)	1.66 (0.70–3.92)	
Potassium (mg)	535.63–2382.5	2382.6–2874.24	2874.25–3781.33	≥3781.34	0.58
Cases/controls	101/59	45/60	95/61	80/59	
ORs (95% CI)	1.00	0.50 (0.27–0.91)	1.03 (0.53–2.0)	0.65 (0.29–1.45)	
Calcium (mg)	86.63–600.36	600.37–918.23	918.24–1346.4	≥1346.5	0.27
Cases/controls	99/59	73/60	90/59	60/60	
ORs (95% CI)	1.00	0.60 (0.35–1.05)	0.88 (0.50–1.53)	0.60 (0.32–1.13)	
Magnesium (mg)	58.76–259.03	259.04–328.08	328.09–418.58	≥418.59	0.20
Cases/controls	95/59	62/61	83/60	80/59	
ORs (95% CI)	1.00	0.65 (0.36–1.15)	0.79 (0.43–1.44)	0.56 (0.27–1.16)	
Phosphorus (mg)	177.85–965.2	965.3–1195.67	1195.68–1556.5	≥1556.6	0.46
Cases/controls	93/60	65/59	81/61	83/59	
ORs (95% CI)	1.00	1.02 (0.55–1.92)	0.93 (0.44–1.98)	0.70 (0.28–1.75)	
Iron (mg)	2.12–11.25	11.26–15.56	15.57–23.20	≥23.21	0.93
Cases/controls	82/59	75/60	77/60	86/60	
ORs (95% CI)	1.00	1.04 (0.57–1.90)	0.92 (0.49–1.73)	1.06 (0.57–1.96)	

OR odds ratios, *CI* confidence intervals, adjusted for age, sex, smoking status: current versus non-current smoker, pack years smoked (categories: 0–10, 10–20, 20–30, 30–40, 40–50, 50+), and total energy intake (quartiles kcal/day)

associations and biological plausibility or from previous studies. Minerals and vitamins that met the criteria were investigated for interactions with smoking characteristics and age, which were both adjusted for each other. The joint effects of nutrient intake and established risk factors e.g., smoking status (ever/never), cigarettes per day (<20/≥20/day), duration of smoking (<27 years/≥27 years), and age (<63 years/≥63 years) are presented in Table 4. The OR for heavy cigarette smokers (≥20 cigarettes per day) in the highest group for total intake of vitamin E, was 0.58 (95%: 0.34–0.99; *p* interaction 0.03). Inverse associations of borderline statistical significance were also observed among heavy smokers for the highest intake of total carotenoids (OR: 0.62; 95% CI: 0.36–1.09; *p* interaction 0.08) and niacin (OR: 0.66; 95% CI: 0.39–1.14; *p* interaction 0.08). Interaction between thiamin intake and the number of cigarettes smoked per day was also of borderline statistical significance. Possible reductions in risk of bladder cancer for older participants were also associated with higher intakes of total carotenoids (OR: 0.59; 95% CI: 0.35–0.99), vitamin D, thiamin, and niacin (*p* interaction: 0.04, 0.02, and 0.03, respectively). No other statistically significant associations were observed, although there were reduced odds of bladder cancer among the older age group for the highest intake of vitamin E (OR: 0.61; 95% CI: 0.37–1.02) and interactions between vitamin C and

ever-smoking (*p* = 0.08) and vitamin C and age (*p* = 0.08) were borderline (data not shown).

The statistically significant inverse associations observed between total intake of carotenoids, vitamin D, thiamin, and niacin and the risk of bladder cancer for older individuals remained robust following the FPRP test. Each inverse association had less than a 50% likelihood of being false positive for an odds ratio of 1.5 and based on a prior probability of 25%,

Discussion

Main findings

We investigated an extensive range of micronutrients in relation to the risk of bladder cancer. Possible inverse associations of borderline statistical significance were observed between bladder cancer and total intake of vitamin E and dietary phosphorus. We found some evidence that total intake of vitamin E, total carotenoids, niacin and possibly thiamin might be modified by cigarette smoking in that inverse associations were observed largely among heaviest smokers. Higher intakes of carotenoids, vitamin D, thiamin, niacin, and vitamin E were also associated with reduced odds of bladder cancer in the older age group. No

Table 4 Odds ratios (95% confidence intervals) for the joint effects of total intake of selected nutrients (highest vs. lowest) and smoking characteristics and age

	Carotenoids			Vitamin D			Vitamin E			Thiamin			Niacin		
	<12900.6 µg Cases/controls	≥12900.6 µg Cases/controls	ORs (95% CI)	<388.91 IU Cases/controls	≥388.91 IU Cases/controls	ORs (95% CI)	<20.7 (mg) Cases/controls	≥20.7 (mg) Cases/controls	ORs (95% CI)	<2.12 (mg) Cases/controls	≥2.12 (mg) Cases/controls	ORs (95% CI)	<33.08 (mg) Cases/controls	≥33.08 (mg) Cases/controls	ORs (95% CI)
Smoking status															
Never*	28/43	28/52	0.66 (0.32–1.35)	29/54	27/41	1.04 (0.50–2.15)	33/51	20/42	0.64 (0.31–1.33)	23/50	33/44	1.35 (0.66–2.77)	27/53	29/41	1.10 (0.54–2.26)
Ever*	155/76	111/68	0.75 (0.48–1.17)	132/65	134/79	0.82 (0.54–1.24)	140/64	117/73	0.73 (0.48–1.13)	134/70	132/74	0.91 (0.59–1.39)	135/66	131/77	0.80 (0.52–1.23)
p Interaction			0.98			0.41			0.87			0.27			0.26
Cigarettes/day															
<20	25/30	26/23	1.65 (0.68–3.99)	19/26	32/27	1.58 (0.66–3.79)	20/27	31/23	1.57 (0.63–3.88)	19/28	32/25	1.85 (0.78–4.41)	20/27	31/25	1.64 (0.68–3.95)
≥20	127/44	84/44	0.62 (0.36–1.09)	111/38	100/50	0.73 (0.43–1.23)	118/36	85/48	0.58 (0.34–0.99)	114/41	97/47	0.78 (0.46–1.34)	114/38	97/50	0.66 (0.39–1.14)
p Interaction			0.08			0.14			0.03			0.10			0.08
Years smoking															
<27	51/38	45/34	1.19 (0.58–2.47)	49/32	47/40	0.78 (0.40–1.52)	49/36	45/32	1.08 (0.54–2.16)	46/30	50/42	0.71 (0.35–1.43)	47/30	49/41	0.70 (0.35–1.41)
≥27	101/37	64/33	0.62 (0.33–1.16)	81/32	84/38	1.00 (0.55–1.81)	89/27	70/40	0.61 (0.33–1.12)	87/39	78/31	1.41 (0.76–2.59)	87/35	78/35	1.02 (0.56–1.85)
p Interaction			0.20			0.58			0.24			0.19			0.51
Age															
<63	73/59	69/54	0.91 (0.49–1.68)	76/69	66/44	1.55 (0.88–2.74)	79/61	56/47	1.12 (0.62–2.01)	69/65	73/47	2.02 (1.11–3.68)	69/63	73/48	1.59 (0.88–2.87)
≥63	110/60	70/66	0.59 (0.35–0.99)	85/50	95/76	0.65 (0.40–1.08)	94/54	81/68	0.61 (0.37–1.02)	88/55	92/71	0.75 (0.45–1.24)	93/56	87/70	0.64 (0.38–1.07)
p Interaction			0.18			0.04			0.18			0.02			0.03

IU international units, mg milligrams, low intakes were categorized below median and high intakes above medians

Adjusted for age, sex, smoking status (current/non-current), number of cigarettes per day, and years of smoking

* Adjusted for age, sex, and kcal only

other associations were apparent between bladder cancer and any other mineral or vitamin.

Previous findings

The potentially protective effect of vitamin E that we observed against developing bladder cancer was consistent with findings from two other US studies [20, 27]. However, we detected no association between risk of bladder cancer overall and the intake of carotenoids either as individual carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin) or collectively as total carotenoid intake. Our findings were in agreement with an intervention study [31], a meta-analysis [10], two prospective studies [3, 20], and one case–control study [30]. Conversely, four case–control studies [8, 22, 23, 48] observed inverse associations with carotenoid intake and two other prospective studies [21, 25] found specific carotenoids, dietary beta-carotene [25] and beta-cryptoxanthin [21] to be possibly protective.

Like previous studies, we investigated the most common carotenoids from an estimated 40 carotenoids present in the human diet [22]. Each carotenoid is considered to have a specific biological function [22]. While some carotenoids have potential to form vitamin A (provitamin A carotenoids include, α-carotene, β-carotene, and β-cryptoxanthin) others do not have this capability (non-provitamin A carotenoids are lycopene, lutein, and zeaxanthin) [15]. Contrary to an earlier study [22], we did not find either of these two categories of carotenoids to be associated with the risk of bladder cancer (data not shown). Any chemopreventive effect may be difficult to detect for individual carotenoids either due to the relatively small sample size, small size of the effect or because carotenoids possibly only work in synergy with each other or other dietary factors [49].

Where a relatively large prospective study of US men [20] and a case–control study [23] both reported no association between vitamin D and bladder cancer, we observed reduced odds of bladder cancer with higher intake of vitamin D; although this was not statistically significant. The anti-proliferative effect of vitamin D has been shown in various in vitro and in vivo investigations [50]. However, as vitamin D is available from both dietary sources and UV-B radiation [50], it is difficult to estimate the full effect of vitamin D without taking into account sunlight exposure.

Although B-group vitamins are thought to possibly offer protection via their role in genomic stability, DNA repair, and regulation of cell division [15], we did not detect any associations between any B-group vitamin and bladder cancer overall. This was consistent with three previous prospective studies [20, 21, 25] and one retrospective study

[23] but disagreed with two other recent case–control studies [28, 35]. We also failed to detect any association between bladder cancer and another water-soluble vitamin with antioxidant potential, vitamin C. Once again, there were conflicting reports relating to the effect of vitamin C on bladder cancer risk among the various studies [3, 8, 19–21, 23, 29, 32, 33]. When reviewing the existing literature on vitamin C and many of the other vitamins, any evidence for an association generally appeared to be weaker in prospective studies compared with case–control studies [7]. Lack of association between some micronutrients and bladder cancer might also be due to collinearity among nutrients as a result of shared food sources [51]. For instance, many fruits and vegetables rich in carotenoids are also good sources of vitamin C, potassium, and folate.

To our knowledge, phosphorus has been investigated in relation to bladder cancer in only one other epidemiological study [20] that reported no association following multivariate analyses. Although we observed an approximate 50% reduction in the odds of bladder cancer associated with higher dietary intake of phosphorus, it was not statistically significant. Given this ubiquitous micronutrient is an important physiological component of DNA, RNA, ATP, and cell membranes, it may be worthy of further consideration [52].

In our study, a higher intake of vitamin E related to a reduced risk of bladder cancer among heaviest smokers, a finding consistent with those reported in another American case–control study [27]. The reduced odds of bladder cancer we observed for the highest quartile of total carotenoids among the heaviest smokers also agrees with results presented in another case–control study conducted in the USA [8]. Two additional American case–control studies [22, 48] also reported increased risk of bladder cancer among smokers with the lower intakes of total [22] and specific carotenoids, lutein, and zeaxanthin [48]. Castelao et al. [8] suggested that carotenoids provide protection against bladder cancer for smokers by detoxifying arylamines. Borderline statistically significant interactions between the number of cigarettes smoked per day and intakes of thiamin and niacin may warrant further investigation.

Our findings indicate that higher intakes of vitamin D, thiamin, niacin, carotenoids, and possibly vitamin E may be beneficial to participants in the older age group. Older individuals may have limited access to regular sunshine (e.g., living in institutions) and therefore have to rely strongly on dietary sources of vitamin D and supplementation. Bladder cancer is a disease that typically affects older people [53], and bioavailability of B-group vitamins may be compromised in this demographic by certain drugs (e.g., acid lowering agents) [54]. Additionally, vitamin E, like carotenoids acts as an antioxidant and, as suggested by

our results, could be more beneficial under conditions of the greatest oxidative stress such as smoking and aging [27].

Strengths and limitations

There were limitations associated with our study. Like most case–control studies, potential for recall bias is a consideration in dietary estimation. However, as dietary factors are not commonly associated with bladder cancer etiology recall of dietary intake is likely to be non-differential between cases and controls. Other potential limitations relating to measurement of dietary exposures include only one dietary collection period and the possibility of preclinical disease altering dietary intake. Although there was only one collection period, dietary data were collected using a detailed and validated FFQ [43]. As for dietary change, a majority of the cases in our study were diagnosed with superficial bladder cancer, and it is considered unlikely that preclinical disease would alter dietary behavior [28]. Although we cannot totally exclude the possibility of recall bias, dietary intake for major dietary components; calories, total fat, alcohol, and coffee were comparable between cases and controls suggesting that it should be minimal.

Finally, controls were drawn from two sources—Department of Transportation (for those under 65 years) and Medicare (for those 65 years and older). These are considered nearly complete sources of records in our population. Among cases, over 95% report having a driver's license (for those under 65 years) as do over 95% (of those 65 years and older) report being enrolled in Medicare (data not shown). We examined the characteristics of controls younger than 65 years and over 65 years and found them to be comparable with respect to sex, smoking, and medical access variables (i.e., visit to a doctor in the past 2 years); however, older subjects had less than a college education (40% of those under 65 years and 50% of those 65 years and older). However, level of education is not a strong risk factor for bladder cancer (including in our own data) and thus was not considered a potential confounder in our analysis.

While multiple comparisons were made in this study, we attempted to address this issue by utilizing the FPRP [46]. Statistically significant associations between higher intakes of carotenoids, vitamin D, thiamin, and niacin and risk of bladder cancer among the older individuals remained robust following this test. Although it is worth noting that statistical power was low and determining appropriate prior probabilities is difficult [55]. Biologically plausible mechanisms of action and similar results reported from other studies provide additional support for some of our findings [8, 20, 27].

Conclusion

In conclusion, the effects of vitamin E, carotenoids, vitamin D, thiamin, and niacin in relation to the risk of developing bladder cancer may warrant further investigation. Future studies should focus on optimal doses and combinations of these micronutrients particularly for high risk groups such as heavy smokers and older individuals.

Conflict of Interest The authors declare no conflict of interest.

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