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Intake of fruits and vegetables, carotenoids, folate, and vitamins A, C, E and risk of bladder cancer among women (United States)

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Abstract

Objective: To examine the relation between fruits and vegetables, carotenoids, folate, and vitamins A, C, E and the risk of bladder cancer in a prospective study of women.
Methods: A total of 237 incident bladder cancer cases were documented during 20 years of follow-up among 88,796 women enrolled in the Nurses' Health Study. Dietary intake was assessed by food-frequency questionnaires every two to four years and incident diagnosis of bladder cancer was ascertained every two years. Cox proportional hazard models were used to estimate incidence rate ratios (RR) and 95% confidence intervals (CI) for bladder cancer risk, adjusting for age, pack-years of smoking, current smoking, and total caloric intake.
Results: Consumption of total fruits and vegetables was not associated with bladder cancer risk (RR = 1.08, 95% CI = 0.70–1.65, for >5.5 compared to <2.5 servings per day). Similarly, dietary intakes of carotenoids, folate, and vitamins A, C, E, were not related to bladder cancer risk. No association was observed between supplemental intake of multivitamins, vitamins A, C, E and bladder cancer risk.
Conclusions: We did not observe any association for fruit and vegetable consumption or vitamin intake and bladder cancer risk among women.

Introduction

Cancer of the bladder is the 11th most common cancer in the world, with the highest rates reported in North America, Europe, northern Africa, and China [1]. In the United States (US), approximately 60,250 new cases of bladder cancer are expected in 2004 [2]. Tobacco smoking is the best established behavioral risk factor for bladder cancer, with smokers experiencing two- to four-fold higher risk than nonsmokers [3]. In addition, occupational exposure to aromatic amines [4] and schistosomal infections [5] are known to cause bladder cancer, but prevalence of exposure to these factors has been greatly minimized in the US.

Dietary fruits and vegetables may modify the risk of bladder cancer given that these foods are a rich source of nutrients and phytochemicals that may have anticancer properties. Proposed mechanisms for their anticarcinogenic activity include antioxidant properties, activation of carcinogen-detoxifying enzymes, and inhibition of tumor cell proliferation [6–8]. Observational studies of fruits and vegetables and their dietary constituents on risk of bladder cancer are inconclusive. The majority of studies of fruit and vegetable intake and risk of bladder cancer suggest a decreased risk of bladder cancer among those with higher consumption of fruits or vegetables [9–18], though some found no relationship [19–22]. Several of these studies focused mainly on men [14, 19, 22] or included few bladder cancer cases among women [10, 15–17]. In one

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meta-analysis, diets low in fruit intake were associated with a statistically significant 40% increased risk of bladder cancer, and diets low in vegetable intake were associated with a statistically significant 16% increased risk of bladder cancer [23]. In a more recent meta-analysis, an inverse association was observed for fruit consumption but vegetable intake was not related to bladder cancer risk, regardless of study design [24]. There may be several reasons why previous studies have not consistently supported the hypothesis that higher consumption of fruits or vegetables reduces the risk of bladder cancer; limitations include residual confounding by cigarette smoking or potential recall or selection bias in case–control studies.

Several candidate anticarcinogenic nutrients and phytochemicals found in fruits and vegetables may lower the risk of bladder cancer. Previous studies suggest that high dietary carotenoid intake [10], dietary and supplemental vitamin C intake [12], and long-duration vitamin E supplement use [25, 26] may decrease the risk of bladder cancer; although some studies have reported no association for carotenoids [12, 22, 27], and vitamins A, C, E [11, 20, 27–29].

Given the limitations of previous observational studies evaluating the role of fruits and vegetables and constituent nutrients on the risk of bladder cancer, including limited data on women, we examined the association between intakes of fruits and vegetables, carotenoids, folate, and vitamins A, C, E, and risk of bladder cancer in a large prospective cohort of US women with up to 20 years of follow-up.

Materials and methods

Study population

The Nurses' Health Study (NHS) is an ongoing prospective study of 121,700 registered US female nurses, aged 30 to 55 years. The cohort was established in 1976 and has been followed to evaluate diet and lifestyle factors and risk of cancer, heart disease, and other major chronic diseases in women. At baseline, enrollees returned a mailed questionnaire that assessed information on lifestyle factors, medical, and smoking histories. The first dietary questionnaire was sent out to all cohort members in 1980. Follow-up questionnaires are mailed biennially to the entire cohort to update information on exposures and newly diagnosed medical conditions. This investigation was approved by the Institutional Review Board (IRB) of the Brigham and Women's Hospital, Boston, MA.

In 1980, we excluded 29,236 women who did not respond to the food-frequency questionnaire (FFQ) and 45 women who were not eligible for the cohort (*e.g.* male). In addition, 3,623 women who reported a history of cancer other than nonmelanoma skin cancer by 1980 were excluded. The analytic cohort consisted of 88,796 women at baseline followed for up to 20 years (1,710,006 person-years of follow-up). Follow-up rates averaged 98% over each two-year follow-up cycle from 1980 through 2000. Vital status was ascertained through next of kin and the National Death Index; both methods identify at least 98% of deaths in the cohort [30].

Dietary assessment

To assess dietary intake, we used a 61-item semi-quantitative FFQ in 1980 [31], which was expanded to approximately 130 food items in 1984, 1986, and every four years thereafter. For each item, participants were asked to report their average use over the preceding year. Serving sizes (*e.g.* one banana or half-a-cup of broccoli) were specified for each food in the FFQ. Nine prespecified frequency responses were possible, ranging from never or almost never, to six or more times per day. Nutrient intakes were calculated by multiplying the frequency that each food item was reported by the nutrient content for the specified portion size. Participants also reported their current use and dose of vitamins A, C, E supplements and brand and type of multivitamins biennially. For current users of multivitamins or vitamin supplements at baseline, duration of use was also ascertained. To calculate vitamin intake from food and supplements combined, the contributions from multivitamins and other supplements were added to vitamin intakes from food only. Food composition data were primarily based on the nutrient database of the US Department of Agriculture [32]. For carotenoids values (α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene), we used the US Department of Agriculture-National Cancer Institute database that was developed for fruits and vegetables and that includes data on the carotenoids content of tomato-based food products [33–35].

The baseline (1980) total vegetable group consists of the following items: tomatoes, tomato juice, sweet potatoes, winter squash, peas, lima beans, spinach or other greens, corn, carrots, cabbage, cauliflower, brussel sprouts, broccoli, string beans, beans, and lentils. The baseline total fruit group consists of the following items: bananas, peaches, apricots, plums, oranges, orange juice, grapefruit juice, apples, pears, and other fruits.

We also updated baseline food intakes with diet from subsequent questionnaires (in 1984, 1986, 1990, 1994, and 1998). The cumulative average total vegetable group consists of the baseline total vegetable group (excluding

beans and lentils) and the following items: tomato sauce, yams, coleslaw, mixed vegetables, alfalfa sprouts, celery, mushrooms, eggplant, zucchini or other summer squash, kale, iceberg or head lettuce, romaine or leaf lettuce, green pepper, garlic, and red chili sauce. The cumulative average total fruit group consists of the baseline total fruit group and the following items: raisins, avocados, cantaloupe, watermelon, apple juice or cider, other fruit juice, grapefruit, strawberries, and blueberries.

In addition to combining all fruits and vegetables for the baseline analysis, we created three other groups: cruciferous vegetables (broccoli, cabbage, cauliflower, and brussel sprouts), yellow vegetables (winter squash, sweet potatoes, and carrots), and citrus fruits (orange, orange juice, and grapefruit juice). Spinach was the only frequently consumed green-leafy vegetable assessed on the 1980 FFQ. For the cumulative average diet analysis the following items were added: kale and coleslaw to the cruciferous vegetable group; yams to the yellow vegetable group; kale and romaine or leaf lettuce to the green-leafy vegetable group; and grapefruit to the citrus fruit group.

The reproducibility and validity of food intake have been described previously [31, 36]. After correction for attenuation due to random error in diet records, the correlation coefficients between the 1980 FFQ and diet records for fruits and vegetables averaged 0.54 (range, 0.17 for spinach to 0.84 for orange juice). The reported mean intakes from the FFQ were higher than those from the diet records. Pearson correlation coefficients between estimates from the 1980 FFQ and the average of four, one-week diet records were 0.49 for total vitamin A (including contributions from food and supplements) and 0.75 for total vitamin C in women. Vitamin E intake estimated from the FFQ was positively correlated with plasma levels of α-tocopherol in women ($r = 0.41$) [37]. In nonsmoking women, the Pearson correlation coefficient between dietary carotenoid intake and plasma concentrations of carotenoids were 0.21–0.48 [38].

Case ascertainment

On each biennial questionnaire participants were asked whether they had been diagnosed with any cancer, heart disease, or other medical conditions during the previous two years. Self-reported diagnosis of bladder cancer was confirmed by review of medical records. After receiving permission from identified cases (or next of kin for decedents), hospital records and pathology reports were obtained and reviewed by trained physicians, blinded to questionnaire exposure information. Nonrespondents were telephoned in an attempt to confirm the initial cancer report and date of diagnosis.

A total of 237 bladder cancer cases newly diagnosed between 1980 and 31 December 2000 were included in this report, of which approximately 87% were confirmed by medical record review. Diagnoses of bladder cancer not confirmed by medical records were corroborated with additional information from the participant, next of kin, or by death certificate.

Statistical analysis

Person-time of follow-up was calculated from the date for return of the 1980 FFQ until the date of bladder cancer diagnosis, date of death from any cause, or 31 December 2000, whichever came first. Participants who reported cancers other than nonmelanoma skin cancer were excluded at baseline and when cancer was diagnosed after baseline.

Using the baseline diet questionnaire, fruit and vegetable intakes were categorized by dividing each into quintiles and then finding the closest whole (or half) frequency of use for each cutoff to ease interpretation. Total vitamin intake (*i.e.* food and supplement use) and dietary carotenoid intake were classified into quintiles based on the distribution of the entire analytical cohort. Baseline dietary intakes and supplement use status were determined by the 1980 questionnaire; age and smoking status were updated every two years in all analyses. We also considered determining baseline dietary intakes by the expanded 1984 questionnaire; however, starting follow-up in 1984 resulted in a reduction in the number of cases (n = 130) as only 88% of cohort participants who responded to the 1980 FFQ responded to the 1984 FFQ, and because we have four fewer years of follow-up. Cox proportional hazards models were used to estimate incidence RR and 95% CI for bladder cancer risk and simultaneously adjust for age, pack-years of cigarette smoking history (*i.e.* number of packs smoked per day multiplied by the number of years of smoking; <10, 10–24, 25–44, 45–64, and 65 or more pack-years), and current smoking status. In addition, all analyses were adjusted for quintile of total caloric intake to minimize extraneous variation introduced by under-reporting or overreporting in the FFQ [39]. Additional adjustment for potential confounders, including quintile of total fluid intake (from milk, coffee, juice, soda, alcohol, *etc.*, but not including water as it was not assessed in 1980); geographic region in the US (*i.e.* West, Midwest, South, and Northeast); body mass index (BMI, kg/m^2); height (in); and reproductive factors (*i.e.* status and age at menopause: premenopausal; postmenopausal, age <45; postmenopausal, age 45 to 49; postmenopausal, age 50 to 55; postmenopausal, age >55) did not change the associations of the food groups

and nutrients with bladder cancer risk. Because of the relative homogeneity of this population of female nurses, it was unnecessary to control for education or socioeconomic status. Tests of linear trend for increasing categories of fruit, vegetable, and nutrient intakes were conducted by assigning the median values for each and treating those as a single continuous variable, using Cox proportional hazards regression. All reported *p*-values are two-tailed. Tests for (multiplicative) interaction were performed by examining stratum-specific estimates and formally by use of likelihood ratio tests. Quartiles for total fruit and vegetable intake were created for stratified analyses due to limited statistical power to use quintiles (of cutpoints).

We also examined the relationship between total fruit and vegetable, fruit, and vegetable intakes and risk of bladder cancer by updating baseline food intakes with diet from subsequent questionnaires (in 1984, 1986, 1990, 1994, and 1998). In these analyses bladder cancer risk was related to the cumulative average of diet calculated from all of the preceding dietary questionnaires; details of this method are described elsewhere [39]. Using cumulative averages may reduce within-person subject variation and better represent long-term average intake. To represent past diet but reduce some measurement error, we also examined the relationship between total fruit and vegetable intake and bladder cancer risk using dietary data from the 1980 questionnaire for follow-up from 1980 to 1984 and the average of 1980 and 1984 dietary intakes for follow-up from 1984 to 2000. If a women did not respond to the 1984 questionnaire fruit and vegetable intake was determined by the 1980 questionnaire. Duration of current vitamin supplement use (categorized as a combination of never, past, current less than five years, current five to nine years, and current ten years or more) was updated biennially after taking into account years at baseline use.

Results

At baseline in 1980, approximately 20% of women reported consuming greater than 5.5 servings of fruits and vegetables per day; less than ten percent reported consuming seven or more servings of fruits and vegetables daily. Women with a high fruit and vegetable intake were less likely to smoke than women who consumed few fruits and vegetables (Table 1). Intakes of vitamins A, C, E, and folate were higher among frequent consumers of fruits and vegetables. BMI did not vary across categories

Table 1. Baseline characteristics[a] by total fruit and vegetable intake, Nurses' Health Study 1980–2000

Characteristic	Category of fruit and vegetable intake (servings/day)				
	< 2.5	2.5–3.5	3.6–4.5	4.6–5.5	> 5.5
Number of women	19,888	20,225	18,186	12,431	18,066
Age (years)	45.3	46.2	46.9	47.4	48.1
Height (in)	64.3	64.4	64.5	64.5	64.5
Body mass index (kg/m^2)	24.1	24.3	24.3	24.3	24.4
Age at menopause[b] (years)	49.0	49.3	49.5	49.6	49.6
Smoking history					
Smoking status (%)					
Never	36.7	42.8	45.1	47.9	47.1
Past	23.7	26.8	29.0	28.3	31.4
Current (cigarettes/day)					
⩽14	8.6	8.1	7.4	7.4	7.6
15–34	25.3	19.2	16.1	14.2	12.3
⩾35	5.7	3.1	2.3	2.2	1.6
Pack-years of smoking[c]	29.8	26.0	24.7	24.0	22.8
Age started smoking[c] (years)	19.5	19.8	19.7	19.7	19.7
Dietary intakes (daily)					
Fluid (ml)	4,240	4,328	4,246	4,132	3,858
Vitamin A (μg)[d]	1,727	2,003	2,270	2,511	3,041
Vitamin C (mg)[d]	225	261	304	346	417
Vitamin E (mg)[d]	31	33	34	37	43
Folate (mg)[d]	279	334	373	402	465
Multivitamin use (%)	28.9	32.4	35.1	37.1	39.4

[a] Standardized to the age distribution of the study population.
[b] Among women with natural menopause.
[c] Values are for past and current smokers only.
[d] Energy-adjusted vitamin intake from diet and vitamin supplement.

of fruit and vegetable intake. A strong predictor of bladder cancer risk was pack-years of smoking; women with history of heavy smoking had a substantially higher risk of bladder cancer (age-adjusted RR = 5.04, 95% CI = 3.27–7.77, for 65 or more pack-years of cigarette smoking compared with never smokers).

Overall, we observed no association between fruit and vegetable intake at baseline and risk of bladder cancer (Table 2). An inverse association was observed between total fruit and vegetable intake and bladder cancer risk in the age-adjusted model (*p*-value, test for trend = 0.04); however, point estimates were attenuated after adjustment for pack-years of cigarette smoking, current smoking, and total caloric intake. Risk estimates did not change appreciably after additional inclusion of other covariates as pack-years of smoking accounted for most of the difference between the age and multivariate (MV) adjusted analyses. No association was observed between cruciferous vegetables, yellow vegetables, or citrus fruits and risk of bladder cancer (Table 2). Spinach (the only green-leafy vegetable) was not associated with bladder cancer risk (*p*-value, test for trend = 0.57). Furthermore, no association was observed for the other specific fruits and vegetables.

Table 2. Rate ratios (RR) and 95% confidence intervals (CI) for incident bladder cancer by categories of total fruits and vegetables, fruits, vegetables, and other food groupings at baseline (1980)[a]

	Categories of intake					*p*-Value, test for trend
	1	2	3	4	5	
Fruits and vegetables, servings/day	< 2.5	2.5–3.5	3.6–4.5	4.6–5.5	> 5.5	
Median	1.9	3.0	4.0	5.0	6.8	
No. of cases	56	56	55	25	45	
Person-years	382,702	390,186	350,745	239,280	347,092	
RR	1.00	0.91	0.96	0.61	0.72	0.04
MV RR (95% CI)	1.00 (referent)	1.10 (0.75–1.60)	1.24 (0.84–1.82)	0.83 (0.51–1.35)	1.08 (0.70–1.65)	0.99
Fruits, servings/day	⩽1.0	1.1–1.5	1.6–2.0	2.1–3.0	> 3.0	
Median	0.6	1.3	1.7	2.4	3.8	
No. of cases	58	38	48	51	42	
Person-years	367,962	306,808	268,684	422,074	344,478	
RR	1.00	0.72	1.02	0.65	0.61	0.01
MV RR (95% CI)	1.00 (referent)	0.87 (0.57–1.32)	1.35 (0.91–1.99)	0.93 (0.63–1.38)	0.95 (0.62–1.46)	0.77
Vegetables, servings/day	< 1.5	1.5–2.0	2.1–2.5	2.6–3.0	> 3.0	
Median	1.1	1.8	2.3	2.7	3.7	
No. of cases	89	54	37	16	41	
Person-years	679,191	339,652	260,824	165,959	264,379	
RR	1.00	1.17	1.03	0.66	1.07	0.85
MV RR (95% CI)	1.00 (referent)	1.27 (0.90–1.79)	1.15 (0.78–1.70)	0.77 (0.45–1.32)	1.29 (0.87–1.91)	0.40
Cruciferous vegetables, servings/week	< 1.0	1.0–2.0	2.1–3.0	3.1–4.0	> 4.0	
Median	0.9	1.5	2.5	3.5	6.0	
No. of cases	78	42	61	12	44	
Person-years	572,416	317,967	426,158	105,120	288,345	
RR	1.00	0.95	0.99	0.79	1.03	0.90
MV RR (95% CI)	1.00 (referent)	0.98 (0.67–1.43)	0.98 (0.70–1.38)	0.84 (0.46–1.55)	1.10 (0.76–1.60)	0.64
Yellow vegetables, servings/week	⩽1.0	1.1–2.0	2.1–3.0	3.1–4.0	> 4.0	
Median	0.9	1.5	2.5	3.5	6.0	
No. of cases	92	54	11	47	33	
Person-years	680,816	385,355	116,246	291,690	235,900	
RR	1.00	0.95	0.61	1.10	0.91	0.85
MV RR (95% CI)	1.00 (referent)	1.06 (0.76–1.49)	0.68 (0.36–1.28)	1.33 (0.93–1.90)	1.16 (0.77–1.75)	0.30
Citrus fruits, servings/week	< 3.0	3.0–5.0	5.1–7.0	7.1–9.0	> 9.0	
Median	0.9	3.5	6.5	8.0	14.0	
No. of cases	70	28	53	38	48	
Person-years	420,021	274,250	364,107	295,459	356,170	
RR	1.00	0.60	0.79	0.74	0.71	0.16
MV RR (95% CI)	1.00 (referent)	0.70 (0.45–1.09)	0.95 (0.66–1.37)	0.98 (0.65–1.46)	0.96 (0.66–1.42)	0.82

[a] RR are adjusted for age; multivariate RR (MV RR) are adjusted for age, pack-years of cigarette smoking, current smoking, and total caloric intake.

The association between total fruit and vegetable intake and bladder cancer risk was examined across strata of cigarette smoking status. After controlling for age, pack-years of cigarette smoking (except in never smoker model), and total caloric intake the RR of bladder cancer for the highest quartile of total fruit and vegetable intake compared to the lowest quartile of intake among never smokers was 1.83 (95% CI = 0.70–4.78), for past smokers the RR was 0.89 (95% CI = 0.48–1.66), and for current smokers the RR was 0.95 (95% CI = 0.49–1.86) (p-value for interaction = 0.54). There was no evidence that the association between cruciferous vegetables, yellow-vegetables, or citrus fruits and risk of bladder cancer was modified by smoking status (data not shown).

Results using both 1980 and 1984 FFQ were similar to the main analyses (data not shown). Similarly, results using cumulatively updated diet (see Methods) were similar to those using the baseline questionnaire only. For example, using the same categories of intake at baseline, the RR for bladder cancer obtained by updating fruit and vegetable intake in the MV model were 0.77 for 2.5–3.5 servings/day, 1.24 for 3.6–4.5 servings/day, 1.05 for 4.6–5.5 servings/day, and 1.18 (95% CI = 0.71–1.94) for >5.5 servings/day.

To further explore specific nutrients in fruits and vegetables, we examined the association between bladder cancer risk and dietary intake of the major carotenoids at baseline. For the age-adjusted and MV models, no material relation was observed between intake of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, or lycopene and risk of bladder cancer (Table 3).

Total dietary intake (from diet and supplement use) of vitamins A, C, E, and folate also were not associated

Table 3. Rate ratios (RR) and 95% confidence intervals (CI) for incident bladder cancer by quintiles of baseline (1980) carotenoid intake[a]

	Quintiles of intake					p-Value, test for trend
	1	2	3	4	5	
α-Carotene, μg/day						
Median	192	313	441	648	1,561	
No. of cases	41	44	46	57	49	
Person-years	339,715	343,003	343,423	341,542	342,323	
RR	1.00	1.06	1.08	1.25	1.10	0.72
MV RR (95% CI)	1.00 (referent)	1.22 (0.78–1.92)	1.18 (0.76–1.82)	1.52 (0.98–2.34)	1.34 (0.88–2.06)	0.25
β-Carotene, μg/day						
Median	1,358	2,236	3,353	5,281	8,545	
No. of cases	40	48	51	44	54	
Person-years	342,133	341,823	342,990	341,746	341,315	
RR	1.00	1.15	1.19	1.00	1.19	0.67
MV RR (95% CI)	1.00 (referent)	1.25 (0.82–1.92)	1.29 (0.85–1.96)	1.16 (0.75–1.78)	1.40 (0.93–2.13)	0.23
β-Cryptoxanthin, μg/day						
Median	19.5	49.3	79.5	125	220	
No. of cases	52	46	52	37	50	
Person-years	340,708	342,933	345,262	342,524	338,578	
RR	1.00	0.87	0.94	0.64	0.80	0.19
MV RR (95% CI)	1.00 (referent)	1.05 (0.70–1.58)	1.20 (0.81–1.77)	0.85 (0.55–1.30)	1.09 (0.73–1.63)	0.97
Lutein/zeaxanthin, μg/day						
Median	1,172	2,064	2,814	6,047	11,689	
No. of cases	35	55	53	47	47	
Person-years	341,378	341,815	342,313	343,105	341,395	
RR	1.00	1.58	1.45	1.35	1.24	0.82
MV RR (95% CI)	1.00 (referent)	1.63 (1.06–2.50)	1.45 (0.93–2.24)	1.41(0.91–2.20)	1.33 (0.85–2.07)	0.89
Lycopene, μg/day						
Median	844	1,758	4,102	6,422	11,179	
No. of cases	40	44	61	36	56	
Person-years	343,915	343,363	342,434	341,426	338,869	
RR	1.00	1.10	1.49	0.85	1.25	0.62
MV RR (95% CI)	1.00 (referent)	1.03 (0.66–1.60)	1.57 (1.04–2.37)	0.84 (0.53–1.33)	1.18 (0.78–1.79)	0.74

[a] RR are adjusted for age; multivariate RR (MV RR) are adjusted for age, pack-years of cigarette smoking, current smoking, and total caloric intake.

Table 4. Rate ratios (RR) and 95% confidence intervals (CI) for incident bladder cancer by quintiles of baseline (1980) vitamins A, C, E, and folate intake[a]

	Quintiles of intake					*p*-Value, test for trend
	1	2	3	4	5	
Vitamin A, μg/day						
Median	806	1,232	1,768	2,592	4,153	
No. of cases	38	54	58	44	43	
Person-years	343,555	343,907	343,086	342,314	337,144	
RR	1.00	1.31	1.36	1.02	0.93	0.19
MV RR (95% CI)	1.00 (referent)	1.45 (0.96–2.21)	1.61 (1.06–2.44)	1.20 (0.77–1.86)	1.08 (0.69–1.68)	0.44
Vitamin C, mg/day						
Median	70	113	155	228	704	
No. of cases	50	43	52	41	51	
Person-years	344,836	346,985	338,720	342,513	336,951	
RR	1.00	0.82	0.96	0.73	0.87	0.79
MV RR (95% CI)	1.00 (referent)	0.94 (0.62–1.42)	1.20 (0.81–1.78)	0.90 (0.59–1.38)	1.03 (0.69–1.53)	0.96
Vitamin E, mg/day						
Median	5.1	6.5	8.0	12.7	99.3	
No. of cases	46	51	42	44	54	
Person-years	335,871	345,664	352,760	338,779	336,932	
RR	1.00	1.09	0.85	0.94	1.03	0.71
MV RR (95% CI)	1.00 (referent)	1.30 (0.87–1.95)	1.10 (0.72–1.69)	1.18 (0.77–1.80)	1.26 (0.85–1.89)	0.49
Folate, mg/day						
Median	158	217	277	392	698	
No. of cases	41	54	57	47	38	
Person-years	341,237	344,629	343,415	341,876	338,848	
RR	1.00	1.24	1.22	1.02	0.81	0.07
MV RR (95% CI)	1.00 (referent)	1.43 (0.95–2.15)	1.47 (0.98–2.22)	1.29(0.84–1.98)	0.98 (0.62–1.53)	0.27

[a] RR are adjusted for age; multivariate RR (MV RR) are adjusted for age, pack-years of cigarette smoking, current smoking, and total caloric intake.

with bladder cancer risk (Table 4). Updating vitamin intake showed similar results to those in which the baseline questionnaire only was used. For example, the RR for bladder cancer obtained by updating vitamin E intake in the MV model were 0.96 for the second quintile, 1.43 for the 3rd quintile, 0.73 for the 4th quintile, and 1.16 for the 5th quintile.

We also evaluated the association between bladder cancer risk and baseline status (yes/no) and baseline dose of multivitamins and vitamins A, C, E supplement intake and duration of supplement intake, updating information biennially. No statistically significant association was observed between status or dose of baseline vitamin supplement use and risk of bladder cancer (data not shown). No overall association was observed between duration of multivitamins and single vitamin supplements and the risk of bladder cancer; however, there was a suggestive decrease in bladder cancer risk among current users of vitamin E supplements for ten or more years (Table 5). Furthermore, there was no evidence that the association between duration of current vitamin E supplement use and risk of bladder cancer was modified by smoking status (data not shown).

Discussion

In this prospective study of women, no association was observed between consumption of fruits or vegetables and bladder cancer risk in the MV analysis. Similarly, various food groupings (e.g. cruciferous vegetables) were not related to bladder cancer risk. Dietary intakes of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, or lycopene and total intake (from diet and supplement use) of vitamins A, C, E, and folate were not associated with risk of bladder cancer. Overall, supplement intake of multivitamins and vitamins A, C, E were not related to bladder cancer risk.

Epidemiologic studies of fruit or vegetable intake and bladder cancer risk have yielded inconsistent results. Some studies have observed inverse associations among specific food groups. For example, intake of cruciferous vegetables was inversely associated with bladder cancer risk among men in the Health Professionals Follow-up Study (HPFS) cohort (RR = 0.49, 95% CI = 0.32–0.75, for the highest category compared to the lowest) [19]; however, this result has yet to be confirmed in other prospective studies [15, 22], including the current study.

Table 5. Rate ratios (RR) and 95% confidence intervals (CI) for incident bladder cancer by duration of current vitamin supplement use[a]

	Years of supplement use[b]					*p*-Value, test for trend[c]
	Never	Past	Current, < 5	Current, 5–9	Current, > 10	
Vitamin A[d]						
No. of cases	217	13	6			
Person-years[e]	1,513,409	116,362	52,148			
RR	1.0	0.59	0.74			0.15
MV RR (95% CI)	1.0 (referent)	0.61 (0.35–1.08)	0.79 (0.35–1.78)			0.20
Vitamin C						
No. of cases	132	37	26	16	24	
Person-years[e]	989,537	237,260	204,896	120,631	132,279	
RR	1.0	0.88	0.87	0.86	1.00	0.94
MV RR (95% CI)	1.0 (referent)	0.92 (0.63–1.33)	0.93 (0.61–1.42)	0.90 (0.54–1.53)	1.09 (0.70–1.69)	0.70
Vitamin E						
No. of cases	155	27	30	16	9	
Person-years[e]	1,178,849	171,198	186,595	90,647	70,497	
RR	1.0	0.93	1.02	1.08	0.66	0.32
MV RR (95% CI)	1.0 (referent)	0.95 (0.63–1.44)	1.09 (0.73–1.63)	1.15 (0.68–1.94)	0.72 (0.37–1.42)	0.48
Multivitamins						
No. of cases	88	47	32	31	34	
Person-years[e]	674,644	312,262	295,618	174,005	213,175	
RR	1.0	0.91	0.72	1.13	0.90	0.87
MV RR (95% CI)	1.0 (referent)	0.93 (0.65–1.34)	0.76 (0.50–1.14)	1.18 (0.78–1.80)	0.96 (0.64–1.44)	0.65

[a] RR are adjusted for age; multivariate RR (MV RR) are adjusted for age, pack-years of cigarette smoking, current smoking, and total caloric intake.
[b] Duration of supplement use was updated biennially.
[c] Test for trend includes nonusers. Median values were modeled as a continuous variable for the test for trend.
[d] Top category is for less than five years of current use.
[e] Value does not add up to 1,710,006 due to person-years in the category for current users with unknown duration (data not shown).

Most cohort studies have observed no association for α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, or lycopene [18, 19, 22], although an inverse association was observed for β-cryptoxanthin in one study [27]. Our null results are consistent with findings from other cohort studies.

The epidemiologic evidence relating folate intake and risk of bladder cancer is limited. In a case–control study an inverse association was reported for folate intake from diet and supplements combined (RR = 0.50, 95% CI = 0.30–0.90, for the highest compared to the lowest quartile) after adjustment for cigarette smoking and total caloric intake among other potential confounders [12]. In accordance with the current study results, three additional prospective studies did not observe an association between folate intake and bladder cancer risk [22, 26, 27].

Vitamin A has been shown to inhibit bladder carcinogenesis at various stages of carcinogen treatment in early experimental studies [40, 41]. Some epidemiological studies have reported a moderate to strong protective association between the highest compared to the lowest category of vitamin A intake and bladder cancer risk [9, 12, 29, 42]; although other studies, including a meta-analysis [23], have reported no statistically significant associations [11, 20, 27, 28, 43]. Our overall findings are consistent with the notion that vitamin A intake is not associated with the risk of bladder cancer.

Most studies, including the current study, have observed no association between vitamin C intake and bladder cancer risk [11, 18, 20, 28, 29, 43]; some studies reported a decreased risk [10, 12, 27]. Two [18, 26] of three prospective studies [18, 25, 26] show a decreased risk of bladder cancer among vitamin C supplement users. Of the three case–control studies of incident bladder cancer [10, 12, 28], only one reported statistically significant lower risks with use of vitamin C supplements compared to never users (RR = 0.40, 95% CI = 0.20–0.80) [12].

Few studies have examined the relation between vitamin E supplement use and bladder cancer risk, and only three have included data on duration of use [25, 26, 44]. After a median 6.1 years of follow-up, no associa-

tion between vitamin E supplement use and risk of bladder cancer was observed among men enrolled in the Alpha-Tocopherol Beta-Carotene (ATBC) chemoprevention trial (RR = 1.10, 95% CI = 0.80–1.50) [44]. In the Cancer Prevention Study II (CPS-II) cohort, regular use of vitamin E supplements ($\geqslant$15 times per month) ten or more years was associated with a lower bladder cancer mortality (among women, RR = 0.52, 95% CI = 0.19–1.40; overall, RR = 0.60, 95% CI = 0.37–0.96) [25]. The greatest reduction in mortality was observed among current smokers at enrollment (RR = 0.31, 95% CI = 0.10–0.89) compared to past or never smokers, who experienced weaker, nonstatistically significant risks of bladder cancer mortality. Among men in the HPFS, current use of vitamin E supplements for ten or more years was associated with a statistically significant trend of lower bladder cancer risk with increasing duration of use (RR = 0.68, 95% CI = 0.45–1.03; *p*-value, test for trend = 0.03) [26]. Furthermore, a statistically significant inverse association with bladder cancer risk was observed for current users of vitamin E, compared to never users of vitamin E, among past smokers (RR = 0.49, 95% CI = 0.30–0.79), but not among current or never smokers [26]. Similar to the result found in the HPFS, the current study among women cannot exclude a possible reduced risk of bladder cancer among current users of vitamin E supplements for ten years or more. Discrepancies between existing studies could be explained by inadequate statistical power to examine long duration of vitamin E supplement use.

Cigarette smoking is strongly related to patterns of nutrient intake [45], including fruit and vegetable intake, with cigarette smokers having lower circulating antioxidant micronutrient concentrations due to increased cumulative exposure to reactive oxygen species [46]. It is possible that the beneficial effect of high fruit and vegetable intake may be more pronounced in current smokers. One study reported a stronger protective effect between cruciferous vegetables and bladder cancer risk among never smokers compared to past or current smokers; however, the test for statistical multiplicative interaction was not statistically significant [19]. Our data do not suggest that smoking modifies the association between fruits and vegetables and bladder cancer risk in women.

The current study has several strengths. The majority of the previous cohort studies on diet and bladder cancer have focused on men only [14, 19, 22] or included few bladder cancer cases (less than 35) among women [15, 17]. Bladder cancer incidence is two to five times higher in men than women [3], which may suggest major etiologic differences, even after accounting for smoking history. This study included over 230 women with bladder cancer. The prospective design precludes recall bias and selection bias is minimized by the very high rate of follow-up over a long period of time. In addition, we were able to control tightly for history of smoking and update smoking status.

Dietary information was obtained using a detailed, validated semi-quantitative FFQ at baseline and in 1984, 1986 and every four years thereafter. With cumulatively updated dietary data, we were able to enhance the precision of dietary assessments and account for changes in fruit and vegetable and nutrient consumption over time and reduce the potential for misclassification of intake. The lack of an association observed in the current study between fruit and vegetable consumption and bladder cancer risk might reflect nondifferential measurement error in intake, which results in a bias towards the null. While we cannot exclude this possibility, it is unlikely that there would be more misclassification in this study than in previous studies that have observed lower risks of bladder cancer using similar dietary methods [12, 17–19, 22] since we had up to six measurements of diet.

The most relevant dietary exposure period for bladder cancer is unknown. Given that the best established behavioral risk factor for bladder cancer, smoking history, takes a decade or longer to exert a carcinogenic effect on the bladder, exposure to dietary constituents in the distant past may be more important than recent diet. The NHS spans 20 years of follow-up since the first diet assessment, and the availability of repeated dietary measures permits a consideration of both early and long-term dietary intake. Analyses using only baseline dietary information were similar to analyses that used cumulative, updated fruit and vegetable intake. Furthermore, given the long period of follow-up, it is unlikely that our null results were simply due to inadequate follow-up time.

This prospective study among women does not provide support of an inverse association for total fruits and vegetable intake and risk of bladder cancer. No associations were observed between dietary or supplemental intakes of carotenoids, vitamins A, C, and folate and bladder cancer risk. A suggestive inverse association was noted for women who had been taking vitamin E supplements for many years. Additional studies are needed to clarify the role of long-duration vitamin E supplement use on bladder cancer risk and to investigate the possible modification of risk by smoking status. Until future studies can better elucidate the role of diet in bladder carcinogenesis, prevention of bladder cancer should be practiced primarily through smoking prevention and cessation.

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