Consumption of Vegetables and Fruits and Urothelial Cancer Incidence: a Prospective Study[1]

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Abstract

Although most epidemiological studies concerning urothelial cancer support a possible protective effect of vegetable and fruit consumption, previous studies have been inconsistent with regard to which vegetables and fruits may be responsible for an inverse association. The association between the consumption of 21 vegetables and nine fruits and urothelial cancer risk was assessed in the Netherlands Cohort Study among 120,852 men and women aged 55–69 years at baseline in 1986. After 6.3 years of follow-up, 538 incident cases and 2,953 subcohort members with complete vegetable data and 569 cases and 3,123 subcohort members with complete fruit data were available for case-cohort analyses. In multivariable case-cohort analyses, the following age-, sex-, and smoking-adjusted incidence rate ratios for groups of vegetable and fruit consumption were observed (comparing highest *versus* lowest quintile of consumption): total vegetables, 0.91 [95% confidence interval (CI): 0.65–1.27]; cooked vegetables, 0.98 (CI: 0.71–1.35); raw vegetables, 1.10 (CI: 0.78–1.53); cooked leafy vegetables, 0.89 (CI: 0.65–1.23); raw leafy vegetables, 0.94 (CI: 0.73–1.22); pulses, 1.03 (CI: 0.74–1.44); brassicas, 0.75 (CI: 0.54–1.04); allium vegetables, 0.89 (CI: 0.67–1.19); total fruit, 0.74 (CI: 0.53–1.04); and citrus fruit, 0.85 (CI: 0.62–1.17). For three separate items (cauliflower, cooked carrots, and mandarins), a statistically significant inverse association was seen, whereas for other specific vegetables or fruit, no statistically significant association was observed. The data are suggestive of an inverse association between the consumption of brassicas, total fruit, and urothelial cancer risk, whereas total vegetable consumption did not appear to be associated with urothelial cancer risk.

Introduction

Urothelial cancer is the seventh most common cancer among men, accounting for ~200,000 new cases/year worldwide (1). Over the last 4 decades, many epidemiological studies and several reviews have been conducted to investigate determinants of urothelial cancer (2–6). These studies suggested that urothelial cancer is influenced by environmental factors, including cigarette smoking, fluid consumption, and exposure to industrial chemicals (*e.g.*, aromatic amines) and diet.

Among the dietary factors, most epidemiological studies support a possible protective effect of vegetable (7–12) and fruit consumption (7, 9, 12–14), although some studies have reported no association for vegetable (14, 15) or fruit consumption (8, 15). Previous studies also have been inconsistent with regard to which vegetables and fruits may be responsible for an inverse association.

Some possible anticarcinogenic mechanisms of substances in vegetables and fruits include antioxidant effects, effects on cell differentiation, increased activity of enzymes that detoxify carcinogens, blocked formation of nitrosamines, preserved integrity of intracellular matrixes, effects on DNA methylation, maintenance of normal DNA repair, increased apoptosis of cancer cells, and decreased cell proliferation (16). From a biological viewpoint, however, it is not yet clear whether the total amount of consumed vegetables and fruits is relevant for the possible protection against cancer or only the consumption of specific vegetables.

NLCS[3] with 6.3 years of follow-up and with substantially more incident urothelial cancer cases available than in previous studies provided the opportunity to study prospectively detailed relationships between the total amount of vegetable and fruit consumption, as well as the consumption frequency of specific vegetables and fruits and urothelial cancer incidence for men and women consuming a Dutch diet.

Materials and Methods

Cohort. This population-based prospective cohort study on diet and cancer started in the Netherlands in September 1986. In this year, 340,439 persons received a baseline questionnaire. The overall participation rate in the cohort was 35%, which led to 120,852 participants (58,279 men and 62,573 women aged 55–69 years). The study population originated from 204 municipal population registries throughout the country. The case cohort approach was used for data processing and analysis (17). Cases were enumerated from the entire cohort, whereas the accumulated person years in the cohort were estimated from a subcohort sample. After this approach, a subcohort of 3,500 subjects (1,688 men and 1,812 women) was randomly sampled from the cohort after baseline exposure measurement. The subcohort has been followed up for vital status information. No subcohort members were lost to follow-up during the follow-up

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[3] The abbreviations used are: NLCS, Netherlands Cohort Study; RR, rate ratio; CI, confidence interval.

period. The study design, including data collection strategies, has been described in detail previously (18).

Follow-Up. Follow-up for incident cancer was established by record linkage to cancer registries and the Dutch national database of pathology reports (19). The completeness of cancer follow-up was estimated to be >95% (20). The presented analysis is restricted to cancer incidence in 6.3 years of follow-up, from September 1986 to December 1992. After excluding prevalent cases with cancer other than skin cancer, a total of 3,346 subcohort members (1,630 men and 1,716 women) and 619 incident cases (532 men and 87 women) with microscopically confirmed, incident carcinomas of the urinary urothelial, ureters, renal pelvis, or urethra were identified. Of these cases, 584 (94.3%) were diagnosed with urothelial cancer, of which 559 (95.7%) were transitional cell carcinomas.

Exposure Status. At baseline, the cohort members completed a mailed, self-administered questionnaire on risk factors for cancer. Usual consumption of food and beverages during the year preceding the start of the study was assessed with a 150-item semiquantitative food frequency questionnaire. Participants were asked to report their consumption frequency of 21 vegetables (*i.e.,* string/French beans, broad beans, Brussels sprouts, cauliflower, cabbage (white/green), kale, spinach, cooked endive, raw endive, lettuce, leek, onions, cooked carrots, raw carrots, sweet peppers, tomatoes, red beets, sauerkraut, mushrooms, gherkins, and rhubarb) and nine fruits (*i.e.,* oranges, mandarins, grapefruits, orange/grapefruits juice, apples/pears, bananas, strawberries, grapes, and raisins/other dried fruits). Categories ranged from never or less then once per month to "three to seven times per week" for vegetable consumption and from never or less than once per month to "six to seven times per week" for fruit consumption. For onions, tomatoes, sweet peppers, and mushrooms, participants were asked to report the number they usually ate per week (onions and tomatoes) or month (sweet peppers and mushrooms, in boxes of 250 grams). Frequency of vegetable consumption was asked separately for summer and winter, from which a combined consumption frequency was calculated. For string beans and cooked endive, portion sizes were asked, which were used as indicators to calculate portion sizes of other vegetables, according to a vegetable-specific algorithm. To derive an individual serving size for each type of vegetable, the indicator serving size was multiplied with a type-specific factor calculated from the same pilot study data as the ratio of the means of the specific to the indicator serving sizes. The frequencies and amounts with regard to fruit consumption have been converted to consumption in grams per day using standard portion sizes. The choice of items for inclusion in the questionnaire was such that it could predict most of the interindividual variation in nutrient intakes. These items covered almost all vegetables and fruits eaten regularly in 1986, with the exception of chicory, red cabbage, cucumber, and broccoli, although the consumption of broccoli was not frequent at baseline. An open-ended question on other foods eaten on a regular basis was also included. Participants could write down how many times per week they ate such a food and how much they ate on each occasion. The questionnaire has been validated against a 9-day diet record. The Spearman correlation coefficient was 0.38 and 0.60 for total vegetable and total fruit consumption, respectively (21).

Statistical Analyses. Incidence RRs and corresponding 95% CIs for urothelial cancer were estimated using exponentially distributed failure time regression models (22) with the Stata statistical software package (23). SEs were estimated using the robust Hubert-White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator presented by Barlow (24) and Lin and Ying (25). We confirmed constancy of the baseline hazard visually by plotting the natural logarithm of the baseline survival function against failure time.

The following variables were subsequently considered as potential confounders based on earlier analyses (26–29): age (years); consumption of alcohol (grams/day); coffee (ml/day); tea (ml/day); total water (ml/day); current cigarette smoking (yes/no); cigarette smoking amount (cigarettes/day); cigarette smoking duration (years of cigarette smoking); occupational exposure to dye, rubber, leather, or vehicle fumes (ever/never); and first-degree family history of urothelial cancer (yes/no). Those variables that (combined) showed a >10% influence on the risk of urothelial cancer when considered in a multivariable model were included as covariates in multivariable analyses.

Subjects with incomplete or inconsistent dietary data were excluded from analyses (21). To check the quality and consistency of the responses on the several vegetable questions, a specific error index was computed. When the error index exceeded a certain value, *i.e.,* more than three errors, subjects were excluded from analyses on vegetable consumption. The error index was calculated as the sum of variables that indicated each the presence of an inconsistency or other response error. Finally, a total of 538 urothelial cancer cases (463 men and 75 women) and 2953 subcohort members (1456 men and 1497 women) remained for analyses on vegetable data, and 569 urothelial cancer cases (491 men and 78 women) and 3123 subcohort members (1525 men and 1598 women) remained for analyses on fruit data.

The RRs concerning vegetable and fruit consumption were similar for men and women. Therefore, we conducted regression analyses for men and women combined (*P*s for interaction >0.40) We first evaluated combined vegetable and fruit consumption, total vegetables, prepared and raw vegetables, vegetables categorized in botanical groups (*i.e.,* leafy vegetables, pulses, brassicas, and allium vegetables), total fruits, and citrus fruits after categorizing subjects into quintile or tertile levels of consumption depending on the distribution in the subcohort. Cases detected during the first and the first 2 years of follow-up were excluded from analyses to evaluate whether preclinical symptoms might have influenced the results. Specific vegetables and fruits were tested for linearity and evaluated as continuous variables (grams/day), and their multivariable RRs were expressed per increment of 25 grams/day, corresponding to a consumption frequency of a vegetable approximately once per week. The independent contribution of each specific vegetable or fruit was assessed by an analysis in which total vegetable and fruit consumption was included in the model simultaneously. Tests for trend in the RRs were based on likelihood ratio tests. RRs for quintiles of vegetable and fruits consumption were also computed separately in strata of smoking status and amount. For the same variables, subgroup analyses were performed after stratification by tumor morphology and invasiveness. To ensure that the results were not influenced by changes in exposure by subjects with preclinical urothelial cancer, we conducted analyses with and without cases diagnosed in the first 1 or 2 years of follow-up. The results from these analyses were essentially similar and, therefore, analyses with all cases were presented.

Results

Mean daily consumption of vegetables and fruits was calculated for categories of the most important potential confounders

Table 1 Mean (± SD) consumption of total vegetables and fruits in the subcohort stratified by potential risk factors of urothelial cancer; NLCS (1986–1992)

Characteristics	Vegetable consumption		Fruit consumption	
	n = 2953	grams/day (±SD)	n = 3123	grams/day (±SD)
Age (years)				
55–59	1132	188.7 (±76.6)	1198	170.8 (±119.8)
60–64	1025	191.3 (±74.4)	1082	176.1 (±114.8)
65–69	796	186.6 (±75.1)	843	182.3 (±116.8)
Alcohol consumption (grams/day)				
No consumption	676	181.4 (±80.5)	719	185.8 (±120.2)
<15	1484	187.7 (±71.9)	1567	180.3 (±116.2)
≥15	710	200.4 (±76.9)	741	153.9 (±111.9)
Coffee consumption (cups/day)				
No consumption	89	196.4 (±94.0)	98	206.9 (±134.8)
<5	1709	187.4 (±70.7)	1800	181.9 (±115.4)
≥5	1155	190.9 (±80.4)	1225	164.14 (±117.6)
Tea consumption (cups/day)				
No consumption	334	184.2 (±87.6)	363	151.2 (±119.5)
<4	1476	187.7 (±73.8)	1564	168.5 (±109.9)
≥4	1143	192.2 (±73.6)	1196	192.6 (±123.7)
Total water consumption (ml/day)				
Tertile 1 (<1847)	992	164.5 (±67.2)	1042	141.3 (±96.6)
Tertile 2 (≥1847–<2222)	1005	187.5 (±66.2)	1043	173.3 (±103.8)
Tertile 3 (≥2222)	956	216.2 (±83.1)	1038	212.8 (±136.7)
Current cigarette smoking				
No	2130	191.0 (±74.7)	2248	187.4 (±118.9)
Yes	823	184.0 (±77.1)	875	145.9 (±107.7)
Cigarette smoking amount				
Nonsmoker	1078	190.4 (±75.9)	1147	201.0 (±119.4)
<15 cig/day	875	187.3 (±71.5)	910	171.0 (±113.7)
≥15 cig/day	911	190.6 (±78.0)	950	154.5 (±114.8)
Cigarette smoking duration				
Nonsmoker	1078	190.4 (±75.9)	1147	201.0 (±119.4)
<35 years	941	192.8 (±74.8)	992	175.3 (±115.5)
≥35 years	897	183.3 (±74.9)	944	146.7 (±109.6)
High-risk occupation[a]				
No	2940	189.0 (±75.4)	3108	175.8 (±117.4)
Yes	13	203.2 (±83.7)	15	164.9 (±106.2)
Family history of urothelial cancer				
No	2896	189.1 (±75.2)	3064	175.5 (±117.2)
Yes	57	186.4 (±88.6)	59	185.8 (±124.0)
Vegetable consumption (grams/day)				
Tertile 1 (<150)	N/A[b]	N/A[b]	982	151.4 (±110.1)
Tertile 2 (≥150–<212)	N/A[b]	N/A[b]	1056	170.0 (±105.3)
Tertile 3 (≥212)	N/A[b]	N/A[b]	1085	203.4 (±128.6)
Fruit consumption (grams/day)				
Tertile 1 (<15)	970	173.9 (±72.2)	N/A[b]	N/A[b]
Tertile 2 (≥115–<205)	1022	188.5 (±72.2)	N/A[b]	N/A[b]
Tertile 3 (≥205)	961	204.9 (±78.7)	N/A[b]	N/A[b]

[a] Ever exposed to dye, rubber, leather, and vehicle fumes.
[b] Not applicable.

(Table 1). Age, coffee consumption, current cigarette smoking, cigarette smoking amount, cigarette smoking duration, high-risk occupation, and family history of urothelial cancer were not associated with vegetable consumption, whereas alcohol, tea, and total water consumption were positively associated. Concerning fruit consumption, we found no association with age, high-risk occupation, and family history of urothelial cancer; inverse associations were found with alcohol consumption, coffee consumption, current cigarette smoking, cigarette smoking amount, and cigarette smoking duration, and positive associations were found with tea consumption and total water consumption. Vegetable and fruit consumption were positively associated with each other (Table 1).

Incidence RR estimates for quintiles, quartiles, or tertiles of consumption are presented in Table 2 for total vegetable and fruit consumption and the consumption of groups of vegetables and fruits. Despite the distributions of vegetable and fruit intake across potential confounders, only age, sex, cigarette smoking amount, and cigarette smoking duration appeared to be the most influential confounders in multivariable analyses. The age- and sex-adjusted incidence RR showed an inverse association between the combined consumption of vegetables and fruits and urothelial cancer risk, which disappeared after additional correction for cigarette smoking amount and cigarette smoking duration. Of all vegetable groups, there was only suggestive evidence for an inverse association between the consumption of brassicas and urothelial cancer risk (RR: 0.75; CI: 0.54–1.04, comparing extreme consumption quintiles), although the linear dose response trend was borderline statistically significant (P trend = 0.06). Total fruit consumption was inversely associated

Table 2 Incidence RRs and 95% CI for urothelial cancer according to the consumption of vegetables and fruits, NLCS (1986–1992)

Vegetable/fruit consumption(grams/day in quintiles/categories)	Cases in cohort	Person years in subcohort	RR (95% CI)[a]	RR (95% CI)[b]
Vegetables and fruits combined				
1 (<241)	149	3523	1.00 (reference)	1.00 (reference)
2 (≥241–<311)	111	3606	0.76 (0.57–1.02)	0.81 (0.59–1.11)
3 (≥311–<379)	101	3591	0.72 (0.54–0.97)	0.80 (0.58–1.12)
4 (≥379–<471)	90	3614	0.71 (0.53–0.96)	0.76 (0.51–1.12)
5 (≥471)	87	3638	0.75 (0.56–1.03)	0.98 (0.60–1.61)
P for linear trend			0.02	0.39
Vegetable consumption				
Total vegetables				
1 (<126)	110	3535	1.00 (reference)	1.00 (reference)
2 (≥126–<163)	122	3607	1.07 (0.80–1.44)	1.09 (0.80–1.50)
3 (≥163–<196)	107	3598	1.01 (0.74–1.37)	1.04 (0.75–1.44)
4 (≥196–<242)	99	3575	0.95 (0.70–1.30)	0.99 (0.71–1.38)
5 (≥242)	100	3659	0.90 (0.66–1.23)	0.91 (0.65–1.27)
P for linear trend			0.29	0.38
Cooked vegetables				
1 (<16)	105	3508	1.00 (reference)	1.00 (reference)
2 (≥16–<28)	130	3601	1.22 (0.91–1.64)	1.14 (0.84–1.56)
3 (≥28–<40)	90	3601	0.84 (0.61–1.16)	0.83 (0.59–1.16)
4 (≥40–<58)	99	3603	0.95 (0.69–1.30)	0.86 (0.62–1.20)
5 (≥58)	114	3659	1.01 (0.75–1.37)	0.98 (0.71–1.35)
P for linear trend			0.42	0.28
Raw vegetables				
1 (<99)	122	3551	1.00 (reference)	1.00 (reference)
2 (≥99–<128)	108	3586	0.87 (0.65–1.17)	0.94 (0.69–1.29)
3 (≥128–<155)	117	3561	1.12 (0.83–1.50)	1.24 (0.91–1.70)
4 (≥155–<193)	95	3610	0.91 (0.67–1.24)	1.02 (0.73–1.43)
5 (≥193)	96	3665	0.94 (0.69–1.27)	1.10 (0.78–1.53)
P for linear trend			0.81	0.38
Leafy vegetables, cooked				
1 (<9)	117	3592	1.00 (reference)	1.00 (reference)
2 (≥9–<16)	106	3549	0.85 (0.63–1.15)	0.84 (0.61–1.15)
3 (≥16–<22)	100	3268	0.92 (0.67–1.25)	0.84 (0.61–1.17)
4 (≥22–<33)	106	3936	0.85 (0.63–1.14)	0.84 (0.61–1.16)
5 (≥33)	109	3628	0.90 (0.66–1.21)	0.89 (0.65–1.23)
P for linear trend			0.45	0.46
Leafy vegetables, raw				
1 (<6)	199	6453	1.00 (reference)	1.00 (reference)
2 (≥6–<12)	199	5942	1.17 (0.93–1.47)	1.24 (0.97–1.58)
3 (≥12)	140	5578	0.86 (0.67–1.10)	0.94 (0.73–1.22)
P for linear trend			0.24	0.75
Pulses				
1 (<15)	82	3273	1.00 (reference)	1.00 (reference)
2 (≥15–<24)	96	3694	1.05 (0.75–1.46)	0.98 (0.69–1.39)
3 (≥24–<33)	113	3650	1.06 (0.77–1.47)	0.95 (0.68–1.33)
4 (≥33–<47)	114	3769	1.06 (0.77–1.46)	0.94 (0.67–1.33)
5 (≥47)	133	3587	1.24 (0.90–1.70)	1.03 (0.74–1.44)
P for linear trend			0.14	0.87
Brassicas				
1 (<16)	108	3363	1.00 (reference)	1.00 (reference)
2 (≥16–<24)	106	3463	1.00 (0.74–1.36)	0.99 (0.71–1.37)
3 (≥24–<33)	112	3677	0.92 (0.68–1.24)	0.89 (0.64–1.22)
4 (≥33–<46)	119	3822	0.96 (0.71–1.29)	0.95 (0.69–1.30)
5 (≥46)	93	3648	0.75 (0.55–1.03)	0.75 (0.54–1.04)
P for linear trend			0.05	0.06
Allium vegetables				
1 (<12)	160	5064	1.00 (reference)	1.00 (reference)
2 (≥12–<26)	144	4399	1.08 (0.83–1.40)	1.08 (0.82–1.42)
3 (≥26–<43)	120	4271	0.99 (0.76–1.30)	1.01 (0.76–1.35)
4 (≥43)	114	4239	0.90 (0.69–1.18)	0.89 (0.67–1.19)
P for linear trend			0.35	0.35
Fruit consumption				
Total fruits				
1 (<83)	188	3832	1.00 (reference)	1.00 (reference)
2 (≥83–<131)	101	3734	0.64 (0.49–0.85)	0.69 (0.51–0.93)
3 (≥131–<183)	104	3845	0.65 (0.49–0.86)	0.72 (0.53–0.97)
4 (≥183–<256)	95	3758	0.64 (0.48–0.85)	0.70 (0.51–0.96)
5 (≥256)	81	3827	0.66 (0.48–0.89)	0.74 (0.53–1.04)
P for linear trend			<0.01	0.02
Citrus fruits				
1 (<15)	194	3835	1.00 (reference)	1.00 (reference)
2 (≥15–<41)	96	3829	0.55 (0.42–0.73)	0.60 (0.45–0.82)
3 (≥41–<84)	109	4276	0.62 (0.47–0.81)	0.71 (0.53–0.96)
4 (≥84–<128)	70	3257	0.61 (0.45–0.84)	0.69 (0.49–0.98)
5 (≥128)	100	3799	0.75 (0.56–1.00)	0.85 (0.62–1.17)
P for linear trend			0.01	0.22

[a] Adjusted for age (years) and sex.

[b] Adjusted for age (years), sex, number of cigarettes per day, years of cigarette smoking, and total vegetable consumption (grams/day, for fruits items) or total fruit consumption (grams/day, for vegetable consumption).

Table 3 Incidence RRs and 95% CIs for urothelial cancer for continuous variables of vegetables and fruit consumption, NLCS (1986–1992)

Vegetable/fruits (10th to 90th percentile, grams/day)	RR[a] (95% CI) per 25 grams	RR[b] (95% CI) per 25 grams
Vegetable and fruits combined (188–569)	0.99 (0.96–1.03)	N/A[c]
Total vegetables (105–297)	0.99 (0.96–1.03)	N/A[c]
Total fruits (41–324)	0.99 (0.96–1.01)	N/A[c]
Individual vegetable items		
String/French beans (6–39)	0.97 (0.81–1.15)	0.99 (0.80–1.21)
Broad beans (0–14)	1.14 (0.81–1.60)	1.20 (0.84–1.70)
Brussels sprouts (0–16)	1.03 (0.69–1.52)	1.09 (0.71–1.68)
Cauliflower (2–28)	0.77 (0.61–0.98)	0.75 (0.57–0.98)
Cabbage (white/green) (0–18)	0.99 (0.69–1.43)	1.04 (0.69–1.58)
Kale (0–8)	1.02 (0.48–2.21)	1.10 (0.50–2.44)
Spinach (0–22)	1.01 (0.75–1.37)	1.05 (0.77–1.44)
Endive, cooked (0–26)	0.96 (0.75–1.23)	0.99 (0.75–1.30)
Endive, raw (5-1)	0.70 (0.39–1.27)	0.71 (0.39–1.32)
Lettuce (0–18)	1.05 (0.71–1.55)	1.09 (0.72–1.65)
Leek (0–23)	1.00 (0.78–1.28)	1.03 (0.79–1.34)
Onions (0–44)	0.94 (0.82–1.07)	0.94 (0.80–1.10)
Carrots, cooked (0–20)	0.66 (0.47–0.92)	0.63 (0.43–0.92)
Carrots, raw (0–8)	1.16 (0.89–1.50)	1.19 (0.92–1.55)
Sweet peppers (0–8)	1.16 (0.62–2.16)	1.27 (0.66–2.44)
Tomatoes (0–47)	1.02 (0.91–1.15)	1.05 (0.92–1.21)
Red beets (0–20)	0.90 (0.66–1.22)	0.92 (0.66–1.27)
Sauerkraut (0–12)	0.87 (0.54–1.42)	0.91 (0.54–1.54)
Mushrooms (0–9)	0.68 (0.35–1.31)	0.69 (0.35–1.36)
Gherkins (0–5)	0.91 (0.68–1.22)	0.92 (0.68–1.25)
Rhubarb (0–6)	0.88 (0.52–1.50)	0.89 (0.52–1.51)
Individual fruit items		
Oranges (0–115)	1.00 (0.94–1.06)	1.02 (0.95–1.10)
Mandarins (0–15)	0.63 (0.42–0.96)	0.65 (0.43–1.00)
Grapefruits (0–36)	1.04 (0.94–1.16)	1.07 (0.96–1.21)
Orange/grapefruits juice (0–50)	1.00 (0.94–1.08)	1.00 (0.93–1.08)
Apples/pears (0–160)	0.97 (0.93–1.01)	0.97 (0.91–1.03)
Bananas (0–46)	1.05 (0.95–1.16)	1.08 (0.98–1.19)
Strawberries (0–18)	0.83 (0.59–1.16)	0.86 (0.61–1.22)
Grapes (0–14)	1.01 (0.71–1.44)	1.08 (0.75–1.54)
Raisins/other dried fruits (0–2)	0.37 (0.10–1.43)	0.40 (0.11–1.50)

[a] Adjusted for age (years), sex, number of cigarettes per day, years of cigarette smoking, total fruit consumption (grams/day, for vegetable items, or total vegetable consumption (grams/day, for fruits items).
[b] Adjusted for age (years), sex, number of cigarettes per day, years of cigarette smoking, total fruit consumption (grams/day), and total vegetable consumption (grams/day).
[c] Not applicable.

(RR: 0.74; CI: 0.53–1.04; P trend = 0.02), whereas the consumption of citrus fruits did not appear to be clearly associated with urothelial cancer risk (RR: 0.85; CI: 0.62–1.17; P trend = 0.22), comparing extreme consumption quintiles. Additional adjustment for cigarette smoking amount, cigarette smoking duration, total fruit consumption (for vegetable items), total vegetable consumption (for fruit items; Table 2), or other potential confounders did not change the results essentially.

For specific vegetables and fruits, RRs are presented for increments in mean daily consumption of 25 grams (Table 3). Statistically significant inverse associations were found for the consumption of cauliflower (RR: 0.77; CI: 0.61–0.98), cooked carrots (RR: 0.66; CI: 0.47–0.92), and mandarins (RR: 0.63; CI: 0.42–0.96), although the range in consumption was small (2–28, 0–20, and 0–15 grams/day, respectively). No statistically significant positive associations were observed. Table 3 also gives estimates for each vegetable or fruit after simultaneous adjustment for total vegetable and fruit consumption to assess their independent effects. The risk estimates remained practically stable (Table 3).

In Table 4, the age-, sex-, and smoking-adjusted RRs are presented for combinations of total vegetable and fruit consumption, both divided in tertiles of daily consumption. The protective effect of fruit consumption seemed largest among those in the lowest tertile of vegetable consumption. Subjects in the median tertiles of vegetable and fruit consumption had the lowest risk of urothelial cancer (RR: 0.66; CI: 0.43–0.95, compared with those in the lowest tertiles of vegetable and fruit consumption), although this could be attributable to chance (Table 4).

Table 4 Incidence RRs[a] and 95% CIs for urothelial cancer for combinations of tertiles of vegetables and fruit consumption, NLCS (1986–1992)

Fruit consumption	Vegetables consumption		
	≤150 grams/day	150–212 grams/day	≥212 grams/day
≤115 grams/day	1.00 (reference)	0.67 (0.45–1.01)	0.88 (0.56–1.39)
115–203 grams/day	0.93 (0.64–1.34)	0.64 (0.43–0.95)	0.66 (0.43–1.00)
≥203 grams/day	0.75 (0.51–1.12)	0.87 (0.59–1.28)	0.70 (0.47–1.05)

[a] Adjusted for age (years) and sex, number of cigarettes per day, and years of cigarette smoking.

Cigarette smoking is an important risk factor for urothelial cancer. Therefore, the analyses were stratified on smoking

Table 5 Incidence RR of urothelial cancer for vegetable and fruits consumption, according to cigarette smoking amount and cigarette smoking status categories; NLCS (1986–1992)

Smoking status and amount	No. of cases	Vegetable and fruit consumption (grams/day in tertiles)			
		1 (low)	2	3 (high)	P^a
Vegetable consumption					
Never[b]	59	1.00 (reference)	0.72 (0.37–1.39)	0.83 (0.43–1.59)	0.54
Ex					
<15 cig/day[c]	83	1.00 (reference)	1.16 (0.66–2.02)	0.90 (0.47–1.70)	0.78
≥15 cig/day[c]	151	1.00 (reference)	0.89 (0.55–1.44)	0.92 (0.57–1.48)	0.73
Current					
<15 cig/day[c]	73	1.00 (reference)	0.58 (0.29–1.16)	1.03 (0.53–2.00)	1.00
≥15 cig/day[c]	142	1.00 (reference)	0.97 (0.60–1.57)	0.78 (0.47–1.28)	0.34
Fruit consumption					
Never[b]	62	1.00 (reference)	1.08 (0.54–2.14)	0.98 (0.51–1.90)	0.93
Ex					
<15 cig/day[c]	85	1.00 (reference)	0.76 (0.40–1.43)	1.57 (0.89–2.77)	0.10
≥15 cig/day[c]	160	1.00 (reference)	0.77 (0.49–1.19)	0.68 (0.42–1.11)	0.11
Current					
<15 cig/day[c]	80	1.00 (reference)	0.75 (0.41–1.35)	0.50 (0.23–1.10)	0.08
≥15 cig/day[c]	150	1.00 (reference)	0.82 (0.51–1.33)	0.60 (0.35–1.03)	0.06

[a] *P* trend.
[b] Adjusted for age (years) and sex.
[c] Adjusted for age (years), sex, and years of cigarette smoking.

status and smoking amount (Table 5). Total vegetable consumption was not associated with urothelial cancer risk across different cigarette smoking categories. The protective effect of fruit consumption was most pronounced in ex-smokers who smoked >15 cigarettes/day and in current smokers, regardless of smoking amount (Table 5).

It appeared that a protective effect of vegetable and fruit consumption was mainly confined to invasive transitional cell carcinomas. The RRs for noninvasive tumors did not indicate a clear inverse association for vegetable or fruit consumption (data not shown).

Discussion

The results showed that total vegetable consumption was not associated with urothelial cancer risk, whereas the consumption of brassicas, especially cauliflower, was inversely associated with urothelial cancer risk. Although we did not find a clear inverse association between citrus fruits and urothelial cancer risk, the data suggested that an increased consumption of mandarins might protect against bladder cancer. The consumption of carrots might also decrease the risk of urothelial cancer.

The prospective nature of this cohort study together with completeness of follow-up, as has been achieved in this study, reduced the potential for selection bias to a minimum. Information bias is also largely avoided because dietary habits were reported before urothelial cancer was diagnosed. A change in dietary habits of subjects with latent urothelial cancer at the time of completing the baseline questionnaire remains, however, a possibility, although this is much less likely compared with subjects with, *e.g.,* gastrointestinal cancer.

A potentially more realistic threat to the interpretation of the observed inverse associations is residual confounding by risk factors for urothelial cancer. The most important risk factor in this respect is cigarette smoking (26). We modeled cigarette smoking habits such that they best explained urothelial cancer risk, resulting in a model including the habitual number of cigarettes smoked per day (smoking amount) and the number of years smoked (smoking duration), both as continuous variables. We were not able to explain our results on the basis of confounding by other factors.

Could the measurement of vegetable or fruit consumption itself have biased the results? Vegetables are generally considered as food items that are not very easy to assess in food-frequency questionnaires (as well as in other methods of dietary assessment), particularly if portion sizes have to be estimated. In the NLCS validation study, the correlation coefficient for total vegetable consumption was 0.38 (21), which is rather low, but comparable with the figure reported for many other prospective studies (31). One of the reasons for this low correlation may be the relative lack of true contrast in the frequency of vegetable consumption in a population such as the Dutch, because people are accustomed to a diet including one hot meal per day, which usually includes vegetables. A consequence of a relatively large measurement error would tend to attenuate the estimated RR. Because of individual preferences, contrast in consumption frequency of many specific vegetables is much higher. It was not possible to assess validity for specific vegetables in the NLCS validation study, because 9 days of dietary record are not sufficient to estimate consumption frequency of specific vegetables. To minimize the amount of uninformative data in addition to the general dietary exclusion criteria, we excluded subjects who appeared not to have understood how to fill in the questions on vegetable consumption; an extreme score on the vegetable error index defined those subjects.

To our knowledge, five case-control studies (7, 9, 11, 15, 32) and six cohort studies (8, 10, 12–14, 33) have evaluated the relationship between total vegetable or fruit consumption and urothelial cancer risk. Most studies found inverse associations for total fruit consumption (7, 9, 12–14), total vegetable consumption (7–12), and total consumption of fruits and vegetables combined (8, 14, 32), although one case-control study (15) and one cohort study (14) did not find an association between total vegetable consumption and urothelial cancer risk, and one case-control study (15) and one cohort study did not find an association between fruit consumption and urothelial cancer risk (8). Recently, a meta-analysis on epidemiological studies reported elevated urothelial cancer risks among subjects with low fruit consumption (RR = 1.40; CI: 1.08–1.83) and low vegetable consumption (RR = 1.16; CI: 1.01–1.34; Ref. 29).

One case-control study (34) and one cohort study (8) examined the association between brassica consumption and urothelial cancer risk. The results from both studies were in accordance with our results, indicating an inverse association. As in our study, the cohort study also presented an inverse association between cauliflower consumption and urothelial cancer risk (35). Brassicas have been widely studied for their anticarcinogenic properties in experimental studies and have been associated with reduced cancer risk (8, 16, 36, 37). They are unique in their high content of dithiolethiones and isothiocyanates. These organosulfur compounds have been shown to increase the activity of enzymes involved in the detoxification of carcinogens (8, 16).

Two case-control studies (34, 38) have reported a significant inverse association between carrot consumption and urothelial cancer risk, which is in agreement with the results of the present study, but three other cohort studies (8, 14, 39) have reported no association between orange-yellow vegetable consumption and urothelial cancer risk. These vegetables (*e.g.*, carrots) are sources of α- and β-carotene, which are antioxidants (16).

To date, no study estimated the association between the consumption of citrus fruit (*e.g.*, mandarins) and urothelial cancer risk. Citrus fruits are known for their high content of vitamin C, which, as an antioxidant, may protect cell membranes and DNA from oxidative damage. Vitamin C may additionally help prevent cancer via its ability to scavenge and reduce nitrite, thereby reducing substrate for the formation of nitrosamines.

The antioxidant mechanism has received most attention. The lack of effect of vegetable and fruit consumption among nonsmokers is consistent with this hypothesis because never smokers have not been exposed to smoking-induced oxidative stress.

Recently, Cohen (40) suggested that transitional cell carcinoma of the urinary urothelial might represent two distinct diseases: papillary transitional cell carcinoma, which is usually noninvasive, and the nonpapillary type, which tends to be invasive. In addition to different prognostic and pathogenic properties, these two diseases appear to involve different molecular events (40). From an etiological point of view, some distinction might be important. An inverse association between both vegetables and fruits was most pronounced in invasive transitional cell carcinomas of the urinary urothelial, irrespective of tumor morphology. Additional research is needed to evaluate this matter.

In conclusion, the data are suggestive of an inverse association between the consumption of brassicas, total fruit, and bladder cancer risk, which are unlikely to be attributable to residual confounding of smoking.

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