American Journal of Epidemiology

Vol. 152, No. 12
Printed in U.S.A.

Prospective Study of Dietary Supplements, Macronutrients, Micronutrients, and Risk of Bladder Cancer in US Men

Dominique S. Michaud,[1] Donna Spiegelman,[2,3] Steven K. Clinton,[4] Eric B. Rimm,[1,2,5] Walter C. Willett,[1,2,5] and Edward Giovannucci[1,2,5]

Data derived from laboratory investigations suggest that a number of dietary variables may contribute to bladder carcinogenesis. Although bladder cancer is the fourth leading cause of cancer in men in the United States, dietary studies are few. The authors examined the relations between intakes of macro- and micronutrients and the risk of bladder cancer among men in the prospective Health Professionals Follow-Up Study. Each participant completed a 131-item food frequency questionnaire in 1986 and in 1990, from which nutrient intakes were calculated. During 12 years of follow-up, 320 cases of bladder cancer were diagnosed. No association was observed for total caloric or macronutrient intake and bladder cancer risk. Similarly, we found no relation for dietary intake of potassium, sodium, calcium, magnesium, phosphorus, iron, or water-soluble vitamins and bladder cancer risk. Total vitamin E intake and vitamin E supplements were inversely associated with risk. In addition, a dose-response relation was observed for duration of vitamin E supplement use. A suggestive inverse association was seen with dose of vitamin C supplement use. More studies are needed to determine the role of vitamins E and C supplement intake in bladder carcinogenesis. *Am J Epidemiol* 2000;152:1145–53.

bladder neoplasms; dietary fat; dietary supplements; prospective studies; vitamins

Cigarette smoking is responsible for nearly half of male bladder cancer deaths in the United States (1), but a large portion of bladder cancer incidence remains unexplained. Bladder cancer incidence and death rates display substantial geographic variation (2), suggesting a role for environmental factors such as diet. Although low fluid intake (3) and diets limited in cruciferous vegetables (4) may contribute to bladder cancer risk, the role of other dietary factors in bladder cancer etiology remains poorly understood. A number of nutrients, including dietary fat; energy intake; folic acid; calcium; and vitamins A, C, and E, have been hypothesized to influence bladder cancer risk based upon descriptive epidemiology and laboratory studies in cell culture and animal models (5, 6).

Received for publication November 17, 1999, and accepted for publication March 31, 2000.

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaire; RR, relative risk.

[1] Department of Nutrition, Harvard School of Public Health, Boston, MA.
[2] Department of Epidemiology, Harvard School of Public Health, Boston, MA.
[3] Department of Biostatistics, Harvard School of Public Health, Boston, MA.
[4] The Arthur G. James Cancer Hospital and Research Institute, The Ohio State University, Columbus, OH.
[5] Channing Laboratory, Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA.
Reprint requests to Dr. Edward Giovannucci, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115 (e-mail: edward.giovannucci@channing.harvard.edu).

Several case-control and a few cohort studies have examined various dietary hypotheses in relation to bladder cancer risk, but results have thus far been inconclusive. Retrospective assessment of diet, incomplete dietary assessment, inadequate adjustment of potentially strong confounders, and the small number of cases in prospective studies have limited many of these investigations. Few studies on diet and bladder cancer risk have collected detailed dietary exposure data (7, 8), and only one validated the dietary method within the target population (8). Consequently, most studies measured a select group of foods and were unable to evaluate intake of individual nutrients or total energy intake adequately.

The Health Professionals Follow-Up Study is a prospective study in which dietary information on each participant was obtained in 1986 and subsequently every 4 years by using a food frequency questionnaire. This dietary instrument was previously validated in a subset of the cohort. Because the relation between various nutrients and bladder cancer risk is unclear, we examined the association between intake of macronutrients and micronutrients (minerals and vitamins) and bladder cancer risk in this prospective cohort study.

MATERIALS AND METHODS

Study population

The Health Professionals Follow-Up Study is a prospective cohort initiated in 1986, when 51,529 predominately White men aged 40–75 years answered a detailed mailed questionnaire on diet and medical history. This cohort con-

sists of dentists (57.6 percent), veterinarians (19.6 percent), pharmacists (8.1 percent), optometrists (7.3 percent), osteopathic physicians (4.3 percent), and podiatrists (3.1 percent). All 50 states were represented, and no exclusions were made by race. Every 2 years, follow-up questionnaires were mailed to all surviving cohort members, up to six times per follow-up cycle for nonrespondents, to update data on exposures and newly diagnosed medical conditions. This investigation was approved by the Human Subjects Committee of the Harvard School of Public Health.

To form the cohort for analysis, we excluded 1,596 men with implausibly high or low scores for total food intake (outside the range of 800–4,200 kcal/day) or with 70 items left blank on the baseline dietary questionnaire and 18 men with missing date of birth. In addition, 2,006 men with cancers (excluding nonmelanoma skin cancer) diagnosed before 1986 were excluded because these men may have changed their diets as a result of their disease. The remaining 47,909 men were eligible for follow-up. Follow-up rate for this cohort averaged 94 percent per follow-up cycle during the first 12 years of the study. The National Death Index was used to determine vital status for nonrespondents; the remaining nonrespondents were assumed to be alive and at risk for bladder cancer.

Dietary assessment

To assess dietary intake, we used a 131-item semiquantitative food frequency questionnaire (FFQ) (9), which is an expanded version of a previously validated questionnaire (10). The baseline dietary questionnaire was administered in 1986, and dietary intake information was updated in 1990. The questionnaire assesses average frequency of intake over the previous year. For each man, we calculated caloric and nutrient intake by multiplying the frequency that each food item was reported by the caloric or nutrient content for the specified portion size. Food composition data were primarily based on data from the US Department of Agriculture (11). Reproducibility and validity of the questionnaire in this study have previously been examined by comparing nutrient intakes from two 1-week diet records with that of the FFQ among a subset of 127 men from the Health Professionals Follow-up Study cohort (9). For most nutrients examined here, correlation coefficients between the energy-adjusted nutrient intakes measured by diet records and the FFQ were between 0.5 and 0.7 (9).

Participants self-reported their intakes of vitamin and mineral supplements on the biennial questionnaires, which included questions on multivitamins; vitamins A, C, and E; iron; zinc; and calcium. On the baseline questionnaire, detailed information was requested on use (never, past, current) and, for current users, amounts (four categories with varying ranges of amount per day) or frequency for multivitamins (<3, 3–5, 6–9, and ≥10/day), and duration (0–1, 2–4, 5–9, and ≥10 years). Subsequent questionnaires included all of the same questions, except those on duration (because these can be derived from the baseline questionnaires).

Assessment of nondietary factors

At baseline and biennially thereafter, men provided information on their state of residence, current smoking status, exercise habits, weight, height, and medication use. The baseline questionnaire provided detailed information on past smoking habits, time since quitting, and average number of cigarettes smoked per day before age 15 years and at ages 15–19, 20–29, 30–39, 40–49, 50–59, and 60 years and older. To control for smoking, total pack-years of smoking was derived to incorporate all past smoking experience. One cigarette pack-year is equivalent to having smoked one pack (20 cigarettes) per day for an entire year.

Case ascertainment

On each questionnaire, participants indicated whether they had been diagnosed with any cancer, heart disease, or other medical conditions. We confirmed the self-reported diagnosis of bladder cancer by review of medical records. When permission to obtain medical records was denied, we attempted to confirm the initial cancer report and date of diagnosis with an additional letter or telephone call. If the primary cause of death, as reported by the National Death Index, was a previously unreported bladder cancer, we contacted family members to obtain permission to retrieve medical records or at least to confirm the diagnosis of bladder cancer. For three bladder cases, we used death certificates to confirm a previous, unconfirmed, self-reported diagnosis of bladder cancer.

The endpoints in this study were 320 bladder cancer cases newly diagnosed between 1986 and January 31, 1998, of which 86 percent were confirmed with medical records. On the basis of review of pathology reports, more than 90 percent of bladder cancer cases were transitional cell carcinomas.

Statistical analysis

We computed person-time of follow-up for each participant from the return date of the 1986 questionnaire to the date of bladder cancer diagnosis, death from any cause, or January 31, 1998, whichever came first. In the main analysis, exposures were determined by the 1986 questionnaire (except for age and current smoking status, which were updated every 2 years in all analyses). To adjust for age (5-year categories), pack-years of smoking, and updated current smoking status, we used pooled logistic regression with 2-year time increment (12). This approach has been shown to be asymptotically equivalent to the Cox regression model with time-dependent covariates, given short time intervals and low probability of the outcome within the interval (12). In multivariate analyses, in addition to age and smoking, we adjusted for total fluid intake (3), cruciferous vegetable intake (4), and geographic region because these variables influenced risk for bladder cancer in this population. Total energy intake is largely determined by body size, metabolic efficiency, and energy expenditures. The associations between intakes of specific nutrients and disease cannot be considered primary effects of diet if the nutrient intake is not adjusted for these differences (13). We

therefore adjusted for total energy intake by using the residual method (13). Adjustment for total energy intake also minimizes extraneous variation introduced by under- or overreporting on the FFQ (13). Tests for trend were obtained by assigning the median value for each category and modeling this variable as a continuous variable, using the pooled logistic regression model.

Analysis of minerals were restricted to "macrominerals" (minerals required in amounts greater than 100 mg/day), which include sodium, potassium, calcium, phosphorus, and magnesium (data on chloride were not available).

Although we do not know the latency or induction period for specific dietary exposures, we decided a priori not to update exposure in the main analyses because most occupational risk factors for bladder cancer have been shown to have long latency periods (14). However, because cumulative averaging may reduce within-person subject variation and better represent long-term average intake, we also performed multivariate analyses by using cumulative averages of 1986 and 1990 dietary data. In these analyses, dietary data from the 1986 questionnaire were used for follow-up between 1986 and 1990, and the average of 1986 and 1990 dietary intakes was used subsequently (i.e., 1990–1996). Duration of current vitamin supplement use was updated biennially. Although the dose of current vitamin supplements was not updated in the main analysis, we did update dose biennially in a secondary analysis to examine whether recent supplement use plays a role in bladder carcinogenesis.

RESULTS

Age and smoking were both strongly associated with bladder cancer incidence in this cohort. Men with a history of 65 or more pack-years of cigarette smoking were 3.6 times (95 percent confidence interval (CI): 2.2, 4.6) more likely to develop bladder cancer than were men who had never smoked cigarettes, pipes, or cigars. Relative risks of bladder cancer incidence were 4.6, 5.3, and 5.7 for the age groups 70–74, 75–79, and 80 or more years, respectively, compared with those aged 50–54 years (controlling for pack-years of cigarette smoked).

No relation was observed between total energy intake and bladder cancer risk in an age-adjusted model or in a multivariate analysis controlling for age in 5-year categories, smoking history (pack-years), current smoking status, geographic region of the United States, quintiles of cruciferous vegetables, and total fluid intake (table 1). Examined separately, energy-adjusted total fat, carbohydrate, and protein intakes were not associated with incidence of bladder cancer (table 1).

TABLE 1. Relation between total calorie or macronutrient intake in 1986 and incident bladder cancer, Health Professionals Follow-up Study, 1986–1998*

	Quintile of intake†					*p* value trend test‡
	1	2	3	4	5	
Total calories (kcal/day)	1,250	1,606	1,903	2,245	2,830	
Cases	72	77	60	54	57	
RR	1.0	1.09	0.86	0.80	0.88	0.18
MV RR§ (95% CI)¶	1.0 (Reference)	1.17 (0.84, 1.62)	0.97 (0.68, 1.37)	0.97 (0.68, 1.40)	1.13 (0.78, 1.65)	0.81
Total fat (g/day)	53.4	64.5	71.6	78.3	88.5	
Cases	65	63	66	66	60	
RR	1.0	1.03	1.12	1.15	1.04	0.64
MV RR§ (95% CI)	1.0 (Reference)	1.01 (0.71, 1.43)	1.08 (0.77, 1.53)	1.08 (0.76, 1.53)	0.90 (0.63, 1.29)	0.71
Saturated fat (g/day)	16.7	21.3	24.3	27.3	32.1	
Cases	68	60	65	68	59	
RR	1.0	0.94	1.09	1.17	1.02	0.57
MV RR§ (95% CI)	1.0 (Reference)	0.91 (0.64, 1.29)	1.02 (0.72, 1.43)	1.07 (0.76, 1.51)	0.86 (0.60, 1.24)	0.67
Protein (g/day)	72.6	83.7	91.6	100	113.4	
Cases	78	57	54	60	71	
RR	1.0	0.75	0.69	0.76	0.84	0.35
MV RR§ (95% CI)	1.0 (Reference)	0.79 (0.56, 1.11)	0.76 (0.53, 1.07)	0.83 (0.59, 1.16)	0.92 (0.67, 1.28)	0.76
Animal protein (g/day)	46.6	58.3	66.8	75.6	89.9	
Cases	74	60	51	62	73	
RR	1.0	0.84	0.71	0.83	0.94	0.79
MV RR§ (95% CI)	1.0 (Reference)	0.86 (0.61, 1.20)	0.73 (0.51, 1.04)	0.86 (0.61, 1.21)	0.97 (0.70, 1.35)	0.95
Carbohydrate (g/day)	182	213	234	256	288	
Cases	74	65	62	51	68	
RR	1.0	0.91	0.87	0.70	0.88	0.22
MV RR§ (95% CI)	1.0 (Reference)	1.00 (0.71, 1.39)	1.02 (0.73, 1.44)	0.86 (0.60, 1.24)	1.12 (0.80, 1.57)	0.76

* Relative risks (RRs) are age adjusted, and all macronutrients are also energy adjusted.
† Values for intake are medians for each quintile.
‡ The median values were assigned to each category and modeled as a continuous variable for the tests of trend.
§ Multivariate relative risks (MV RRs) are additionally adjusted for pack-years of smoking history, current smoking status, geographic region of the United States, total fluid intake, and cruciferous vegetable intake.
¶ CI, confidence interval.

When modeled simultaneously to adjust mutually for each other, total fat, carbohydrate, and protein intakes were not associated with bladder cancer risk. Associations were also null when using the values of each macronutrient unadjusted for energy. Similarly, *trans* fatty acid, saturated, monounsaturated, and polyunsaturated fat intakes were not related to bladder cancer risk (all *p* values were greater than 0.3, for tests of trend), whether modeled separately or together simultaneously (data not shown, except for saturated fat in table 1).

Intakes of magnesium and phosphorus were each inversely associated with the incidence of bladder cancer before adjustment for potential confounders (with significant *p* values for trend tests), but the inverse associations were attenuated in multivariate analyses (table 2). Smoking and total fluid intake both contributed to the differences observed between the age-adjusted and multivariate models. Intakes of calcium, sodium, potassium, and iron were not associated with bladder cancer incidence.

No association was observed when comparing the risk of bladder cancer by quintile of water-soluble vitamin intake (based on both supplement use and dietary intake) in age-adjusted or multivariate analyses (table 3). In table 4, relative risks and confidence intervals are shown for intake of fat-soluble vitamins and incidence of bladder cancer. No apparent relation was observed between total intake of vitamins A and D and bladder cancer risk. While both vitamins E and K were inversely associated with bladder cancer risk in age-adjusted models, the association for vitamin K was attenuated after controlling for cruciferous vegetable intake. The inverse association between vitamin E and bladder cancer risk remained statistically significant in the multivariate model. Results were similar to those reported in tables 3 and 4 when using cumulative updated averages based on dietary information from the 1986 and 1990 questionnaires (data not shown).

Because dietary vitamin E is an overall measure of various tocopherol subtypes, which include α-, δ-, and γ-tocopherol, we examined each of these separately. Of these subtypes, only α-tocopherol (including supplement intake) was inversely associated with risk of bladder cancer (multivariate relative risk (RR) = 0.67, 95 percent CI: 0.46, 0.96 for top-to-bottom quintile comparison).

To examine the association of intake from diet alone, we created quintiles of vitamins A, C, and E after excluding multivitamin and individual supplement users. In these analyses, dietary intakes of vitamins A and C were not associated with bladder cancer risk, but a nonsignificantly lower risk was observed for higher intakes of dietary vitamin E (multivariate analyses, top-to-bottom quintile comparison: for vitamin A, RR = 0.97, 95 percent CI: 0.60, 1.58 (178 cases); for vitamin C, RR = 1.03, 95 percent CI: 0.63, 1.78 (170 cases), and for vitamin E, RR = 0.71, 95 percent CI: 0.43, 1.46 (175 cases).

TABLE 2. Relation between intake of minerals in 1986 and incident bladder cancer, Health Professionals Follow-up Study, 1986–1998*

Nutrient	Quintile of intake†					*p* value trend test‡
	1	2	3	4	5	
Calcium (mg/day)	501	649	788	992	1,416	
Cases	64	70	64	47	75	
RR	1.0	1.08	0.96	0.68	1.01	0.63
MV RR§ (95% CI)¶	1.0 (Reference)	1.19 (0.85, 1.67)	1.09 (0.77, 1.55)	0.82 (0.56, 1.21)	1.28 (0.91, 1.79)	0.40
Sodium (mg/day)	2,086	2,608	3,068	3,593	4,624	
Cases	60	72	69	66	53	
RR	1.0	1.26	1.20	1.13	0.92	0.40
MV RR§ (95% CI)	1.0 (Reference)	1.27 (0.90, 1.79)	1.19 (0.84, 1.69)	1.13 (0.80, 1.61)	0.89 (0.61, 1.30)	0.30
Potassium (mg/day)	2,611	3,041	3,367	3,732	4,385	
Cases	58	53	73	77	59	
RR	1.0	0.84	1.07	1.04	0.70	0.16
MV RR§ (95% CI)	1.0 (Reference)	0.94 (0.64, 1.36)	1.28 (0.90, 1.82)	1.31 (0.92, 1.86)	0.93 (0.63, 1.37)	0.74
Magnesium (mg/day)	263	307	343	384	457	
Cases	74	62	68	58	58	
RR	1.0	0.79	0.83	0.66	0.63	0.007
MV RR§ (95% CI)	1.0 (Reference)	0.88 (0.63, 1.24)	0.99 (0.70, 1.38)	0.83 (0.58, 1.18)	0.84 (0.58, 1.20)	0.32
Phosphorus (mg/day)	1,101	1,250	1,364	1,495	1,728	
Cases	80	56	75	53	56	
RR	1.0	0.72	0.95	0.66	0.65	0.01
MV RR§ (95% CI)	1.0 (Reference)	0.82 (0.57, 1.14)	1.11 (0.80, 1.52)	0.80 (0.57, 1.14)	0.85 (0.57, 1.21)	0.40
Iron (mg/day)	10.8	12.6	14.4	17.8	35.9	
Cases	72	65	62	62	59	
RR	1.0	0.87	0.84	0.80	0.71	0.09
MV RR§ (95% CI)	1.0 (Reference)	0.93 (0.67, 1.31)	0.97 (0.69, 1.37)	0.97 (0.68, 1.37)	0.83 (0.59, 1.18)	0.29

* Relative risks (RRs) are age and energy adjusted.
† Values for intake are medians for each quintile.
‡ The median values were assigned to each category and modeled as a continuous variable for the tests of trend.
§ Multivariate relative risks (MV RRs) are additionally adjusted for pack-years of smoking history, current smoking status, geographic region of the United States, cruciferous vegetable intake, and total fluid intake.
¶ CI, confidence interval.

TABLE 3. Relation between total intake of water-soluble vitamins in 1986 and incident bladder cancer, Health Professionals Follow-up Study, 1986–1998*

	Quintile of intake†					*p* value trend test‡
	1	2	3	4	5	
Vitamin C (mg/day)	95	157	228	403	1,159	
Cases	60	65	74	65	56	
RR	1.0	0.92	1.02	0.89	0.76	0.10
MV RR§ (95% CI)¶	1.0 (Reference)	1.05 (0.74, 1.50)	1.23 (0.87, 1.74)	1.10 (0.77, 1.58)	0.90 (0.62, 1.31)	0.26
B_1 (mg/day)	1.12	1.41	1.73	2.94	12.98	
Cases	69	55	68	69	59	
RR	1.0	0.73	0.85	0.83	0.72	0.23
MV RR§ (95% CI)	1.0 (Reference)	0.83 (0.58, 1.19)	1.06 (0.75, 1.49)	0.98 (0.70, 1.38)	0.84 (0.59, 1.20)	0.36
B_2 (mg/day)	1.5	1.9	2.4	3.6	13.0	
Cases	63	78	50	67	62	
RR	1.0	1.11	0.68	0.86	0.81	0.33
MV RR§ (95% CI)	1.0 (Reference)	1.25 (0.89, 1.75)	0.82 (0.56, 1.19)	1.01 (0.71, 1.43)	0.94 (0.66, 1.34)	0.52
Niacin (mg/day)	20.5	25.1	30.0	42.6	99.7	
Cases	71	52	66	70	61	
RR	1.0	0.74	0.91	0.88	0.78	0.37
MV RR§ (95% CI)	1.0 (Reference)	0.79 (0.55, 1.13)	1.00 (0.71, 1.41)	0.96 (0.69, 1.34)	0.86 (0.61, 1.21)	0.58
B_6 (mg/day)	1.7	2.1	2.6	4.1	9.9	
Cases	63	60	68	72	57	
RR	1.0	0.84	0.83	0.87	0.69	0.09
MV RR§ (95% CI)	1.0 (Reference)	0.95 (0.66, 1.36)	1.02 (0.72, 1.46)	1.04 (0.73, 1.47)	0.83 (0.58, 1.20)	0.25
Folate (μg/day)	244	317	388	517	841	
Cases	61	72	61	58	68	
RR	1.0	1.07	0.87	0.83	0.91	0.43
MV RR§ (95% CI)	1.0 (Reference)	1.21 (0.86, 1.71)	1.03 (0.72, 1.48)	1.00 (0.69, 1.45)	1.09 (0.76, 1.55)	0.99
B_{12} (μg/day)	5	7	9	13	22	
Cases	66	55	62	75	62	
RR	1.0	0.84	0.90	0.98	0.83	0.52
MV RR§ (95% CI)	1.0 (Reference)	0.87 (0.61, 1.25)	0.94 (0.66, 1.33)	1.04 (0.75, 1.46)	0.85 (0.60, 1.20)	0.53

* Total vitamin intakes include dietary and supplement intakes; relative risks (RRs) are age and energy adjusted.
† Values for intake are medians for each quintile.
‡ The median values were assigned to each category and modeled as a continuous variable for the tests of trend.
§ Multivariate relative risks (MV RRs) are additionally adjusted for pack-years of smoking history, current smoking status, geographic region of the United States, cruciferous vegetable intake, and total fluid intake.
¶ CI, confidence interval.

In multivariate models, current use of E supplements (at baseline) was inversely associated with bladder cancer risk when compared with never users (current vs. never users, RR = 0.70, 95 percent CI: 0.52, 0.96). Vitamin A and C supplement users experienced a slight, but not statistically significant, reduction in risk of bladder cancer (current vs. never users, RR = 0.85, 95 percent CI: 0.58, 1.26 for vitamin A and RR = 0.83, 95 percent CI: 0.54, 1.26 for vitamin C). Updating current use of these vitamins biennially weakened the associations observed when using baseline supplement use only (data not shown).

We examined the relation between dose and duration of multiple vitamins; vitamins A, C, and E supplement use; and bladder cancer risk (tables 5 and 6). Neither duration nor dose of multivitamin use or vitamin A intake was associated with bladder cancer risk (tables 5 and 6). Duration of vitamin E supplement intake was inversely associated with risk of bladder cancer ($p = 0.03$ for test of trend); men with 10 or more years of vitamin E had a multivariate relative risk of 0.68 compared with never users (table 5). While duration of use of vitamin C supplements was also inversely associated with bladder cancer risk, this association was no longer statistically significant after controlling for smoking history (table 5). Nonstatistically significant inverse associations were observed for supplemental dose of vitamins C and E (table 6).

The relations between vitamins E and C and bladder cancer risk were examined by stratifying on smoking status (at baseline); models were adjusted for pack-years of smoking in current and past smokers. Uses of vitamins E and C supplements were each inversely associated with bladder cancer risk in past smokers (182 cases; compared with never users, multivariate RR = 0.49, 95 percent CI: 0.30, 0.79 for current use of vitamin E; multivariate RR = 0.52, 95 percent CI: 0.34, 0.80, and RR = 0.77, 95 percent CI: 0.45, 1.32 for seasonal and current users of vitamin C, respectively). Current vitamin C supplement use was also associated with a lower risk of bladder cancer in never smokers (multivariate RR = 0.48, 95 percent CI: 0.17, 1.37, 66 cases), but no associations were observed for seasonal vitamin C users or current vitamin E users. No associations were observed for supplement users of vitamins E or C in current smokers, although confidence intervals were wide due to small number of cases in these analyses (56 cases).

TABLE 4. Relation between intake of fat-soluble vitamins in 1986 and incident bladder cancer, Health Professionals Follow-up Study, 1986–1998*

	Quintile of intake†					*p* value trend test‡
	1	2	3	4	5	
Vitamin A (IU/day)	6,256	9,243	12,712	17,368	27,216	
Cases	54	79	50	70	67	
RR	1.0	1.24	0.73	0.95	0.85	0.15
MV RR§ (95% CI)¶	1.0	1.36 (0.96, 1.93)	0.84 (0.57, 1.24)	1.14 (0.79, 1.64)	1.05 (0.72, 1.53)	0.78
Vitamin E (mg/day)	5.4	7.1	9.5	25.1	415	
Cases	74	56	58	79	53	
RR	1.0	0.72	0.67	0.92	0.54	0.007
MV RR§ (95% CI)	1.0	0.79 (0.56, 1.12)	0.79 (0.56, 1.13)	1.03 (0.75, 1.42)	0.64 (0.45, 0.92)	0.03
Vitamin D (IU/day)	99	174	262	429	750	
Cases	59	74	53	61	73	
RR	1.0	1.11	0.74	0.84	0.91	0.48
MV RR§ (95% CI)	1.0	1.22 (0.87, 1.72)	0.85 (0.59, 1.24)	1.00 (0.70, 1.44)	1.06 (0.75, 1.50)	0.98
Vitamin K (μg/day)	85	127	165	214	313	
Cases	75	66	64	55	60	
RR	1.0	0.85	0.81	0.69	0.72	0.04
MV RR§ (95% CI)	1.0	0.92 (0.65, 1.30)	0.91 (0.63, 1.30)	0.80 (0.54, 1.19)	0.85 (0.55, 1.29)	0.41

* Total vitamin intakes include dietary and supplement intakes; relative risks (RRs) are age adjusted and energy adjusted.
† Values for intake are medians for each quintile.
‡ The median values were assigned to each category and modeled as a continuous variable for the tests of trend.
§ Multivariate relative risks (MV RRs) are additionally adjusted for pack-years of smoking history, current smoking status, geographic region of the United States, total fluid intake, and cruciferous vegetable intake.
¶ CI, confidence interval.

TABLE 5. Relative risks and 95% confidence intervals of bladder cancer risk according to the duration of current supplement use, Health Professionals Follow-up Study, 1986–1998

Vitamin	Years of supplement use*				*p* value trend test†
	0	≤5	6–9	≥10	
Vitamin A					
Cases	237	32	10	9	
Person-years	407,119	66,872	15,301	18,571	
RR‡	1.0	0.80	1.03	0.65	0.20
MV RR§ (95% CI)‡	1.0 (Reference)	0.85 (0.59, 1.23)	1.13 (0.60, 2.13)	0.72 (0.37, 1.41)	0.41
Vitamin E					
Cases	184	58	19	27	
Person-years	304,237	114,890	35,451	53,087	
RR	1.0	0.82	0.79	0.63	0.01
MV RR§ (95% CI)	1.0 (Reference)	0.85 (0.63, 1.14)	0.84 (0.52, 1.35)	0.68 (0.45, 1.03)	0.03
Vitamin C (mg/day)					
Cases	141	74	29	44	
Person-years	218,245	136,721	55,685	97,213	
RR	1.0	0.94	0.89	0.65	0.02
MV RR§ (95% CI)	1.0 (Reference)	1.00 (0.75, 1.32)	0.96 (0.64, 1.43)	0.73 (0.52, 1.03)	0.08
Multivitamin					
Cases	119	52	42	75	
Person-years	241,634	89,647	57,581	118,804	
RR	1.0	1.16	1.45	1.11	0.57
MV RR§ (95% CI)	1.0 (Reference)	1.17 (0.85, 1.63)	1.49 (1.05, 2.13)	1.17 (0.87, 1.57)	0.35

* Duration of supplement use was updated biennially.
† Tests for trend include nonusers. The median values were assigned to each category and modeled as a continuous variable for the tests of trend.
‡ RR, relative risk; CI, confidence interval.
§ Multivariate relative risks (MV RRs) are additionally adjusted for pack-years of smoking history, current smoking status, geographic region of the United States, cruciferous vegetable intake, and total fluid intake.

TABLE 6. Relative risks and 95% confidence intervals of bladder cancer risk according to the dose of current supplement use at baseline, Health Professionals Follow-up Study, 1986–1998*

Vitamin categories	Dose					*p* value trend test†
	1	2	3	4	5	
Vitamin A (IU/day)	0	<1,000	1,000–4,999	5,000–9,999	≥10,000	
Cases	188	16	20	50	46	
Person-years	320,284	31,935	35,237	69,197	70,070	
RR‡	1.0	0.83	1.00	1.10	0.98	0.88
MV RR§ (95% CI)‡	1.0	0.86 (0.52, 1.44)	1.05 (0.66, 1.67)	1.14 (0.83, 1.56)	1.03 (0.75, 1.43)	0.63
Vitamin E (mg/day)	0	<25	25–249	250–499	>500	
Cases	205	38	39	27	11	
Person-years	331,058	55,937	66,796	54,540	18,392	
RR	1.0	1.08	0.88	0.63	0.80	0.05
MV RR§ (95% CI)	1.0	1.12 (0.79, 1.59)	0.91 (0.65, 1.28)	0.69 (0.46, 1.03)	0.83 (0.45, 1.52)	0.10
Vitamin C (mg/day)	0	<100	100–499	500–999	≥1,000	
Cases	164	43	52	35	26	
Person-years	265,590	55,263	88,460	56,538	60,871	
RR	1.0	1.16	0.97	0.87	0.69	0.05
MV RR§ (95% CI)	1.0	1.20 (0.86, 1.68)	1.05 (0.77, 1.44)	0.94 (0.65, 1.35)	0.73 (0.48, 1.10)	0.10
Multivitamin (no./week)	0	≤2	3–5	6–9	≥10	
Cases	163	3	41	66	15	
Person-years	296,422	16,925	70,997	95,757	27,565	
RR	1.0	0.37	1.00	1.08	0.93	0.74
MV RR§ (95% CI)	1.0	0.39 (0.12, 1.22)	1.03 (0.73, 1.46)	1.10 (0.82, 1.46)	1.00 (0.59, 1.70)	0.55

* For individual vitamins, estimated dose includes vitamin supplement contribution from multivitamin use.
† Tests for trend include nonusers.
‡ RR, relative risk; CI, confidence interval.
§ Multivariate relative risks (MV RRs) are additionally adjusted for pack-years of smoking history, current smoking status, geographic region of the United States, cruciferous vegetable intake, and total fluid intake.

DISCUSSION

In this prospective study of male health professionals, total intake of vitamin E was inversely associated with bladder cancer risk; taking vitamin E supplements for 10 or more years decreased risk by more than 30 percent. A suggestive, although not significant, dose-response relation was noted between bladder cancer risk and dose of vitamins E and C supplements. We observed no association between total energy intake, fats, proteins, or carbohydrate and the risk of bladder cancer. In addition, micronutrients reported here were not related to bladder cancer risk in multivariate models.

Four case-control studies that examined total fat intake and bladder cancer risk have reported a direct relation (7, 8, 15, 16), with relative risks ranging from 1.3 to 1.8 with higher intake of total fat, but none of these were statistically significant. Range of intake for total fat was similar in two of the studies (approximately 60 g/day difference between top and bottom categories), but was not reported in the other two studies (8, 15). Saturated fat intake, when reported, was largely responsible for the observed association between total fat intake and bladder cancer risk (7, 16). In one study, a statistically significant relative risk of 2.3 for the top quartile of saturated fat intake compared with the bottom quartile was reported (7). In a cohort study, no association was reported between bladder cancer risk and total fat intake estimated with a single 24-hour dietary recall (17). Although possible biologic hypotheses relating fat intake to bladder cancer have been proposed (18), the lack of experimental data (19) and overall inconsistent observations suggest that the relation between total fat intake, or saturated fat intake, and bladder cancer risk is weak, if it exists at all.

Our results for carbohydrate and protein intake were consistent with prior reports in which no association was observed with bladder cancer risk (7, 8, 15, 17). The same studies reported a range of relative risks for bladder cancer and protein intake (0.6–1.5 for high vs. low intake) that are consistent with a null association.

In heterozygous p53-deficient mice, energy restriction inhibits bladder carcinogenesis (20). Previously, to our knowledge, only five epidemiologic studies have had sufficient dietary information to estimate total energy intake (7, 8, 15–17). In one case-control study, those in the top quartile of caloric intake had a relative risk of 2.72 for bladder cancer compared with the bottom quartile, but only among males less than age 65 years (8). The same study also reported increase risk with total fat intake. No relation has been observed between caloric intake and bladder cancer in other studies (7, 15–17). As total energy intake is largely determined by differences in physical activity and body size, this variable must be interpreted with caution (13).

In this study, we observed no association between sodium, calcium, magnesium, or potassium intake and bladder cancer risk in the multivariate analyses, although inverse associations were observed in age-adjusted analyses. Calcium intake was directly associated with bladder cancer risk in one study (7), and high sodium intake resulted in elevated risks of bladder cancer in another (8). Although neither of these associations was observed in this study, it is

unclear whether or not previous studies with complete dietary data have examined these minerals (because null results are not always reported), and thus, more studies are needed to confirm these results.

Animal studies indicate that diets deficient in methyl groups result in DNA hypomethylation by producing imbalances in deoxynucleotide pools (21). Since folate is a component of methyl-group metabolism, it may play a role in carcinogenesis. Previously, dietary and supplement intake of folate was reported in one case-control study (16), which showed that folate was inversely associated with bladder cancer risk (RR = 0.5, 95 percent CI: 0.3, 0.9 comparing top with bottom quartile of total diet and supplement intake, after controlling for smoking). We did not find an association between total folate intake (diet and supplement intake) and bladder cancer incidence in this study.

Because vitamin E has been shown to have strong antioxidant properties in lipid-soluble environments, has the capacity to keep selenium in the reduced state, and inhibits the formation of nitrosamines at low pH, this vitamin could potentially participate in bladder carcinogenesis (22). Only two observational studies have examined the intake of vitamin E supplements in relation to bladder cancer risk, and although both reported an inverse association (RR = 0.51 for top vs. bottom quartile comparison of supplement intake (16) and RR = 0.74 for supplement users vs. nonusers, (23)), only one was statistically significant (16). Dietary vitamin E intake was also inversely, but not statistically significantly, associated with bladder cancer risk in one study (7). In the Alpha-Tocopherol, Beta-Carotene Prevention Trial, a randomized, double-blind, placebo-controlled trial among smokers, 9.5 percent more bladder cancers were diagnosed in the participants who received alpha-tocopherol supplementation (24). However, the dose of vitamin E was relatively low (50 mg/day) in the Alpha-Tocopherol, Beta-Carotene Prevention Trial. Similarly, we did not find that smokers who were taking vitamin E supplements had lower risk of bladder cancer, although numbers for that analysis were small. More studies are needed to determine whether smoking modifies the association between vitamin E supplement use and bladder cancer risk.

Both experimental data and results from epidemiologic studies have been inconsistent with regard to the effect of vitamin C on bladder carcinogenesis. In mice, ascorbic acid inhibited carcinogen-induced bladder tumors in one study (25), but had no effect on the promotion of bladder cancer in rats (26, 27). Although vitamin C supplement use has been inversely related to bladder cancer risk in three case-control studies (16, 23, 28) and one cohort study (15), only two of these associations were statistically significant (16, 23). In contrast, dietary vitamin C has not been associated with bladder cancer risk (7, 8, 15, 23, 28–30), with the exception of one case-control study that detected a statistically significant inverse association (16). We observed no association for dietary intake of vitamin C without supplementation, but found a marginally significant association between duration of vitamin C use and bladder cancer risk. More data will determine whether duration of vitamin C supplementation use is truly associated with bladder cancer risk.

With the exception of vitamin E and our earlier findings showing beneficial effects of fluid intake and cruciferous vegetables (3, 4), our evaluations of other dietary factors have been generally unproductive. Since previous associations have been observed between intake of certain micronutrients and other diseases in this cohort study (31–33), it is unlikely that the null associations observed here were the result of substantial random misclassification in dietary intake assessment or restricted range of intake. One possible reason for some of the null associations observed may be that diet in early life is more critical than that later in life. Alternatively, some of the more important chemicals found in foods might not be macronutrients or micronutrients, but other agents, such as isothiocyanates, which are not available in food composition databases.

In this cohort, we observed a statistically significant inverse association between vitamin E intake and bladder cancer risk, which was strongest among those who had been taking vitamin E supplements for many years. A suggestive inverse association was noted for intake of vitamin C supplement dose and bladder cancer risk. No associations were observed between intake of total energy, macronutrients, or other micronutrients and bladder cancer risk. Few other prospective studies have been able to examine dietary factors and bladder cancer risk, and none had collected sufficient dietary data to measure total caloric intake. Multivitamin or vitamin A supplements were not associated with bladder cancer. Additional studies will be necessary to determine the role of vitamins E and C supplement intake in bladder carcinogenesis and to investigate the possible interaction between vitamin E supplement use and smoking status.

ACKNOWLEDGMENTS

Supported by research grants CA 55075 and HL 35464 from the National Institutes of Health.

REFERENCES

1. Shopland DR. Tobacco use and its contribution to early cancer mortality with a special emphasis on cigarette smoking. Environ Health Perspect 1995;103 (Suppl. 8):131–41.
2. Silverman DT, Hartge P, Morrison AS, et al. Epidemiology of bladder cancer. Hematol Oncol Clin North Am 1992;6:1–30.
3. Michaud DS, Spiegelman D, Clinton SK, et al. Intake of total fluid and specific beverages and risk of bladder cancer in U.S. men. N Engl J Med 1999;340:1390–7.
4. Michaud DS, Spiegelman D, Clinton SK, et al. Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. J Natl Cancer Inst 1999;91:605–13.
5. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practice. Int J Cancer 1975;15:617–31.
6. Clinton SK, Michaud D, Giovannucci E. Nutrition and bladder cancer. In: Heber D, Blackburn GL, Go VLW, eds. Nutritional oncology. San Diego, CA: Academic Press, Inc., 1999:463–75.
7. Riboli E, Gonzalez CA, Lopez-Abente G, et al. Diet and bladder cancer in Spain: a multi-centre case-control study. Int J Cancer 1991;49:214–19.

8. Vena JE, Graham S, Freudenheim J, et al. Diet in the epidemiology of bladder cancer in western New York. Nutr Cancer 1992;18:255–64.
9. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26.
10. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
11. United States Department of Agriculture, Agricultural Research Service. US Department of Agriculture, Nutrient Database for Standard Reference, release 10. Beltsville, MD: United States Department of Agriculture, 1995.
12. D'Agostino RB, Lee ML, Belanger AJ, et al. Relation of pooled logistic regression to time dependent Cox regression analysis: the Framingham Heart Study. Stat Med 1990;9:1501–5.
13. Willett W. Nutritional epidemiology. New York, NY: Oxford University Press, 1990.
14. Bryan GT. Pathogenesis of human urinary bladder cancer. Environ Health Perspect 1983;49:201–7.
15. Steineck G, Hagman V, Gerhardsson M, et al. Vitamin A supplements, fried foods, fat and urothelial cancer. A case-referent study in Stockholm in 1985–87. Int J Cancer 1990;45:1006–11.
16. Bruemmer B, White-E, Vaughan TL, et al. Nutrient intake in relation to bladder cancer among middle-aged men and women. Am J Epidemiol 1996;144:485–95.
17. Chyou PH, Nomura A, Stemmermann GN. A prospective study of diet, smoking, and lower urinary tract cancer. Ann Epidemiol 1993;3:211–16.
18. Hebert JR, Miller DR. A cross-national investigation of diet and bladder cancer. Eur J Cancer 1994;6:778–84.
19. Kitano M, Mori S, Chen T, et al. Lack of promoting effect of linoleic, linoleic or palmitic acid on urinary bladder carcinogenesis in rats. Jpn J Cancer Res 1995;86:530–4.
20. Dunn SE, Kari FW, French J, et al. Dietary restriction reduces insulin-like growth factor I levels which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. Cancer Res 1997;57:4667–72.
21. Christman JK, Sheikhnejad G, Dizik M, et al. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. Carcinogenesis 1993;14:551–7.
22. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control 1991;2:427–42.
23. Shibata A, Paganini-Hill A, Ross RK, et al. Intake of vegetables, fruits, beta-carotene, vitamin C and vitamin supplements and cancer incidence among the elderly: a prospective study. Br J Cancer 1992;66:673–9.
24. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35.
25. Pipkin GE, Schlegel JU, Nishimura R, et al. Inhibitory effect of L-ascorbate on tumor formation in urinary bladders implanted with 3-hydroxyanthranilic acid. Proc Soc Exp Biol Med 1969;131:522–4.
26. Fukushima S, Kurata Y, Shibata MA, et al. Promotion by ascorbic acid sodium erythrobate and ethoxyquin of neoplastic lesions in rats initiated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. Cancer Lett 1984;23:29–37.
27. Fukushima S, Imaida K, Sakata T, et al. Promoting effects of sodium L-ascorbate on two-stage urinary bladder carcinogenesis. Cancer Res 1983;43:4454–7.
28. Nomura AMY, Kolonel LN, Hankin JH, et al. Dietary factors in cancer of the lower urinary tract. Int J Cancer 1991;48:199–205.
29. Risch HA, Burch JD, Miller AB, et al. Dietary factors and the incidence of cancer of the urinary bladder. Am J Epidemiol 1988;127:1179–91.
30. Kolonel LN, Hinds MW, Nomura AMY, et al. Relationship of dietary vitamin A and ascorbic acid intake to the risk for cancers of the lung, bladder, and prostate in Hawaii. Natl Cancer Inst Monogr 1985;69:137–42.
31. Rimm EB, Stampfer MJ, Ascherio A, et al. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993;328:1450–6.
32. Kearney J, Giovannucci E, Rimm EB, et al. Calcium, vitamin D, and dairy foods and the occurrence of colon cancer in men. Am J Epidemiol 1996;143:907–17.
33. Giovannucci E, Stampfer MJ, Colditz GA, et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 1993;85:875–84.