Prospective study of intake of fruit, vegetables, and carotenoids and the risk of adult glioma

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ABSTRACT

Background: Nutrients in dietary fruit and vegetables have been hypothesized to lower the risk of glioma by reducing the endogenous formation of *N*-nitroso compounds. Studies examining fruit and vegetable consumption and brain tumors have relied on case-control study designs, with one exception, and results have been inconsistent.

Objective: We prospectively examined the relation between consumption of fruit and vegetables (and specifically carotenoids) and the risk of glioma among men and women in 3 large US cohort studies: the Health Professionals Follow-Up Study (HPFS), the Nurses' Health Study I (NHS I), and NHS II.

Design: Dietary intake was assessed by food-frequency questionnaires obtained at baseline and updated every 4 y through 2002 (HPFS and NHS I) or 2003 (NHS II). We identified 296 incident adult gliomas during 3 669 589 person-years of follow-up. Cox proportional hazard models were used to estimate incidence rate ratios (RR) and 95% CIs between intake of fruit, vegetables, and carotenoids and glioma risk, with adjustment for age and total caloric intake.

Results: Updated average consumption of total fruit and vegetables was not significantly associated with glioma risk in the men and women (pooled multivariate RR in a comparison of the highest with the lowest quintile: 1.12; 95% CI: 0.74, 1.69). Other fruit and vegetable subgroups, individual fruit and vegetables, and 5 major carotenoids were not significantly associated with risk of glioma.

Conclusion: Our findings suggest that fruit, vegetable, and carotenoid consumption is not likely associated strongly with the risk of adult glioma. *Am J Clin Nutr* 2007;85:877–86.

KEY WORDS Fruit, vegetables, glioma, prospective studies, epidemiology

INTRODUCTION

Age-adjusted incidence rates for primary malignant brain tumors range from 6.0 to 8.7 per 100 000 person-years across 16 of the US states participating in the Central Brain Tumor Registry of the United States (CBTRUS) (1). Although brain tumors are uncommon, they are associated with significant mortality and morbidity; the estimated 5-y survival rates are ≈30% for men and women (1). Gliomas represent the most common type of adult brain tumor (77% of malignant brain tumors) (1).

Established risk factors for glioma include increasing age, male sex, white race, and inherited factors (eg, Li-Fraumeni syndrome). Studies that have examined the association between fruit and vegetable intake and brain tumors have relied almost entirely on case-control study designs, with one exception (2), and results have been inconsistent (2–14). Limitations include the small number of glioma cases (2, 5, 8, 10), potential recall or selection bias (3, 5, 8–10, 12), and measurement error due to surrogate sources of information (3, 4, 6–8, 11, 13, 14). Nutrients and phytochemicals in fruit and vegetables have been hypothesized to lower the risk of glioma by reducing the endogenous formation of *N*-nitroso compounds (NOCs), which have been associated with elevated glioma risk (15). Vitamins C and E and phenolics have been shown to block endogenous nitrosation of nitrites (16–18). These dietary constituents and carotenoids may act as potent antioxidants and inhibit free radical generation and oxidative stress; through these mechanisms, fruit and vegetable consumption may reduce cancer risk, including the risk of gliomas (19–21). Given the limitations of previous studies that evaluated the role of fruit and vegetable intake and the risk of glioma, we conducted a prospective investigation in 3 large prospective studies of men and women with up to 22 y of follow-up, which represents the largest prospective study of this type.

SUBJECTS AND METHODS

Study populations

The Nurses' Health Study I (NHS I) was initiated in 1976, when 121 700 registered US female nurses aged 30–55 y returned a mailed questionnaire that assessed information on lifestyle factors and medical and smoking histories. Similarly, the Health Professionals Follow-Up Study (HPFS) is a cohort of 51 529 US male physicians, dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians who were 40–75 y of age at enrollment in 1986. The study design and methods of dietary assessment and follow-up for the Nurses' Health Study II (NHS II) are very similar to those of NHS I. In 1989, 116 686

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women aged 25–42 y and living in 14 US states were enrolled into the NHS II. Follow-up questionnaires are mailed biennially to all cohort members to update information on lifestyle factors and newly diagnosed medical conditions. The follow-up rate for the cohorts for incidence of cancer was >95% of the total possible person-years.

Dietary assessment

To assess dietary intake, food-frequency questionnaires (FFQs) were initially collected in 1986 for 49 935 men (HPFS), in 1980 for 92 468 women (NHS I), and in 1991 for 95 391 women (NHS II), and diet was generally updated every 4 y. For the NHS I, we used a 61-item semiquantitative FFQ at baseline in 1980 (22), which was expanded to ≈130 food items in 1984, 1986, and every 4 y thereafter. For the HPFS and NHS II cohorts, baseline dietary intake was assessed by using a 131-item FFQ (23). For each item, the participants were asked to report their average use over the preceding year. Serving sizes (eg, 1 banana or one-half cup broccoli) were specified for each food in the FFQ. Nine prespecified frequency responses were possible, ranging from never or almost never to ≥6 times per day. Specific fruit and vegetable items were used to derive total fruit and vegetable intakes as well as intake of composite fruit and vegetable groups, which included cruciferous vegetables, green leafy vegetables, yellow-orange vegetables, citrus fruit, and fruit and vegetables rich in vitamin C. For carotenoid values (α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, and lycopene; in μg/d), we used the US Department of Agriculture–National Cancer Institute database that was developed for fruit and vegetables and that includes data on the carotenoid content of tomato-based food products (24–26).

The reproducibility and validity of food intake have been described previously for the HPFS (23, 27) and the NHS I (22, 28, 29). In 127 men from the HPFS cohort, Pearson correlations between the average intake assessed by two 1-wk diet records completed 6 mo apart and the 1986 FFQ completed after the diet records ranged from 0.25 to 0.95 for specific fruit and vegetables, and the median correlations for fruit and vegetables were 0.77 and 0.46, respectively (27). In 173 members of the NHS I cohort, Pearson correlation coefficients, after correction for attenuation due to random error in diet records, between the 1980 FFQ and the means of four 1-wk diet records for fruit and vegetables averaged 0.54 (range: 0.17 for spinach to 0.84 for orange juice) (29). In nonsmoking men and women, intakes of carotenoids correlated reasonably well with specific carotenoid plasma concentrations in a subset of men and women (Pearson correlations ranged between 0.35 and 0.47 for the men and 0.21 and 0.48 for the women) (30).

Case ascertainment

On each biennial questionnaire, the participants were asked whether they had been diagnosed with any form of cancer, heart disease, or other medical conditions during the previous 2 y. When permission was received from the case subjects (or next of kin for decedents), medical records and pathology reports were obtained from hospitals and reviewed by study investigators, who were blinded to questionnaire exposure information. Nonrespondents were telephoned in an attempt to confirm the initial cancer report and date of diagnosis. Medical records were requested for reported and deceased glioma cases; ≈88% of glioma diagnoses were confirmed by medical records. When we were unable to obtain medical records, we attempted to corroborate diagnoses of glioma with additional information from the participant, next of kin, by death certificate, or by cross-linking with cancer registries. We only included case subjects for whom a medical record or other confirmation of the cancer was obtained. We included all glioma brain tumors; these included astrocytoma, glioblastoma, oligodendroglioma, ependymoma, and mixed glioma subtypes. Vital status was ascertained through next of kin and the National Death Index (NDI); both methods identify ≥98% of deaths in the cohorts (31). We identified 115 newly diagnosed gliomas between 1986 and 2002 among the men, 165 gliomas among the women in the NHS I between 1980 and 2002, and 16 gliomas among the women in the NHS II between 1991 and 2003.

Statistical analysis

Person-time of follow-up was calculated from the date for return of the baseline FFQ (1980 for NHS I, 1986 for HPFS, and 1991 for NHS II) until the date of glioma diagnosis, date of death from any cause, or the end of follow-up (31 December 2002 for HPFS, 31 May 2002 for NHS I, and 31 May 2003 for NHS II), whichever came first. After excluding the participants who reported a history of cancer other than nonmelanoma skin cancer and those with missing information on diet at baseline, the cohorts for analyses included 47 686 (93%) men in the HPFS who were followed for up to 16 y (709 701 person-years of follow-up), 87 662 (95%) women in the NHS I who were followed for up to 22 y, and 94 017 (99%) women in the NHS II who were followed for up to 12 y (2 959 888 total person-years of follow-up among the women). Over the period of follow-up, missing dietary data were carried forward from the previous follow-up cycle from which a participant had an available FFQ.

We estimated the power to detect trends across quartiles for specified incidence rate ratios (RR) in a comparison of the highest with the lowest quartile, assuming a linear relation and fixing the two-tailed $\alpha = 0.05$ (32). We found a 72% power to detect a RR of 1.5 between the highest and lowest quartiles, a 91% power to detect a RR of 1.7 between the highest and lowest quartiles, and a >99% power to detect a RR of 2.0 between the highest and lowest quartiles.

Baseline dietary intakes were determined by the 1986 FFQ for men in the HPFS, the 1980 FFQ for women in NHS I, and the 1991 FFQ for women in NHS II. For women in the NHS I, we also considered determining baseline dietary intakes with the expanded 1984 questionnaire; however, starting follow-up in 1984 resulted in a reduction in the total number of cases ($n = 124$ compared with 165), because only 88% of the cohort participants who responded to the 1980 FFQ responded to the 1984 FFQ and because we have 4 fewer years of follow-up. We divided the cohorts by quintiles of fruit, vegetable, and carotenoid intakes. Furthermore, we examined different fruit and vegetable subgroups to represent foods rich in certain nutrients and phytochemicals. Cox proportional hazards models for failure-time data were used to estimate the incidence RRs and 95% CIs for glioma risk and to simultaneously adjust for age (1 y) and total caloric intake, which minimizes extraneous variation introduced by underreporting or overreporting in the FFQ (33). Additional adjustment for potential risk factors, including total meat intake (which consisted of intakes of processed meats; bacon; hot dogs; hamburger; beef, pork, or lamb as a sandwich or mixed dish;

beef, pork, or lamb as a main dish; chicken with skin; and chicken without skin; in quintiles), alcohol consumption (0, 0.1–1.4, 1.5–4.9, 5.0–29.9, or ≥30.0 g/d), coffee consumption (0, ≤1, 2–3, or ≥4 cups/d), pack-years of cigarette smoking history (<10, 10–24, 25–44, or ≥45 pack-years), current smoking; processed meat intake (consisted of processed meats, bacon, and hot dogs; in quintiles), total intake of vitamins C or E (mg/d; energy-adjusted vitamin intake from diet and vitamin supplement; continuous), multivitamin supplement use (yes or no), state of residence in the United States (west, midwest, south, and northeast), body mass index (in kg/m^2; 18.0–22.9, 23.0–24.9, 25.0–26.9, 27.0–29.9, or ≥30.0), height (in inches and quintiles), type of profession [among men only; pharmacist, specialist (optometrist or podiatrist), physician, veterinarian, or dentist], and reproductive factors (status and age at menopause: premenopausal; postmenopausal, aged <45 y; postmenopausal, aged 45–49 y; postmenopausal, aged 50–55 y; or postmenopausal, aged >55 y), did not significantly change the associations of fruit and vegetable intake with glioma risk. Because of the relative homogeneity of the population of the male health professionals and female nurses, it was unnecessary to control for education or socioeconomic status.

In addition to evaluating the diet at baseline (1980 in NHS I, 1986 in HPFS, and 1991 in NHS II), we examined the relation between the intake of fruit, vegetables, and carotenoids and the risk of glioma by updating baseline dietary intakes with dietary intakes from subsequent questionnaires (in 1984, 1986, 1990, 1994, and 1998 in NHS I; 1990, 1994, and 1998 in HPFS; and 1995 and 1999 in NHS II). In these analyses, we assessed glioma risk in relation to the cumulative average of dietary intakes calculated from all of the preceding dietary questionnaires. For example, in the HPFS, dietary data from the 1986 FFQ was used for follow-up from 1986 to 1990; dietary data from the 1990 FFQ was used for follow-up from 1990 to 1994; data from the 1994 FFQ was used for follow-up from 1994 to 1998; and data from the 1998 FFQ was used for follow-up from 1998 to 2002. The use of cumulative averages may reduce within-person subject variation and better represent long-term average intake. We also examined the relation between recent intake of fruit and vegetables and the risk of glioma by updating diet with the most recent dietary questionnaire. In addition to dietary factors, all covariates were assessed at baseline and were repeatedly assessed from subsequent questionnaires and updated (similar to dietary data) in cumulative average, simple update, and lag analyses. Details of both of these methods are described elsewhere (33, 34). To minimize the possibility that baseline total fruit and vegetable intake may have been altered because of preclinical disease or for other reasons, an analysis excluding the first 2 y of follow-up was performed by using baseline total fruit and vegetable intake. To evaluate time from dietary exposure to glioma diagnosis, we also conducted a 2–6-y lag analysis (because diet was updated every 4 y) using fruit and vegetable intake over each 2-y follow-up cycle. For example, in the HPFS, dietary data from the 1986 FFQ was used for follow-up from 1988 to 1990; dietary data from the 1986 FFQ was used for follow-up from 1990 to 1992; data from the 1990 FFQ was used for follow-up from 1992 to 1994; data from the 1990 FFQ was used for follow-up from 1994 to 1996; data from the 1994 FFQ was used for follow-up from 1996 to 1998; data from the 1994 FFQ was used for follow-up from 1998 to 2000; and data from the 1998 FFQ was used for follow-up from 2000 to 2002.

Additional analyses were restricted by tumor histology [astrocytoma (ICD-O: 94003, 94013, 94113, 94103, 94203, 94213) or glioblastoma (ICD-O: 94403, 94413, 94423)] or tumor site [frontal (ICD-9: 191.1) or temporal (ICD-9: 191.2)]. Tests of linear trend for increasing categories of fruit, vegetable, and carotenoid intakes were conducted with the use of Cox proportional hazards regression by assigning the median values for each and treating those as a single continuous variable. Tests for (multiplicative) interaction were performed by examining stratum-specific estimates and formally with the use of likelihood ratio tests. The age-standardized expected number of cases was calculated by using the 5-y age-specific incidence rate of brain cancer obtained from the Surveillance, Epidemiology, and End Results registry, 1990–2003, and multiplying by the number of person-years in each 5-y age group in the cohort.

Because of the small number of glioma cases observed in the NHS II, the NHS I and NHS II cohorts were combined; the results in the women reflect the pooled estimates of the 2 cohorts. Before pooling with the use of a meta-analysis, tests of heterogeneity of the main exposures by cohort were performed by using the *Q* statistic, and data were pooled by using a random-effects model for the log of the RR (35); no statistically significant heterogeneity was observed. All reported *P* values are two-tailed. Statistical analyses were performed by using SAS software version 8.2 (SAS Institute Inc, Cary, NC).

RESULTS

At baseline, men and older women (from NHS I) with a high fruit and vegetable intake were less likely to smoke or drink alcohol or coffee than were individuals who consumed few fruit and vegetables (**Table 1**). In contrast, younger women (from NHS II) with a high fruit and vegetable intake were more likely to drink alcohol than were those with a low fruit and vegetable intake. Women with a high vegetable intake were more likely to eat meat than those with a low vegetable intake, but intake of meat did not vary across categories of fruit and vegetable intake for men. Intakes of vitamins C and E and multivitamin supplement use were higher among frequent consumers of fruit and vegetables.

The mean age of glioma cases was 65.5 y for men and 60.8 y and 40.6 y for women in the NHS I and NHS II, respectively (**Table 2**). We collected information on glioma histology or location for 76% (87 cases) of men and 82% (149 cases) of women. For both men (80%) and women (65%), glioblastoma was the most common histologic type [similar to CBTRUS data (1)]; anaplastic astrocytomas accounted for ≈8% and 23% of all cases in men and women, respectively. Glioma was most commonly found in the frontal (28%) and temporal lobe (22%).

After adjustment for age and total caloric intake, we observed no significant overall association between cumulative updated total fruit and vegetable intake and the risk of glioma (**Table 3**). Risk estimates did not change appreciably after additional inclusion of other covariates, including, but not limited to, total meat intake, alcohol consumption, coffee consumption, and smoking, and increasing age was the strongest risk factor for glioma risk among the men and women (RR for men: 4.7; RR in a comparison of 60–64-y-old women with those <40-y-old: 9.9). No significant relation was observed between updated intake of fruit, vegetables, cruciferous vegetables, yellow-orange vegetables,

TABLE 1
Age-standardized baseline characteristics by fruit and vegetable intake among men in the Health Professionals Follow-Up Study (HPFS, 1986) and women in the Nurses' Health Study I (NHS I, 1980) and NHS II (NHS II, 1991)[1]

	Fruit and vegetable intake (quintiles)			
Characteristic	1	3	5	*P* for trend
Men				
No. of participants	9206	9732	9421	
Age (y)	52.5 ± 9.4[2]	54.7 ± 9.7	55.8 ± 9.9	<0.001
Height (in)	69.9 ± 3.4	70.1 ± 3.2	70.1 ± 3.7	0.003
BMI (kg/m^2)	25.7 ± 3.2	25.6 ± 3.1	25.5 ± 3.2	<0.001
Past smoker (%)	42.7	43.8	44.4	<0.001
Current smoker (%)	16.2	8.9	6.0	<0.001
Cigarette smoking (pack-years)[3]	29.1 ± 20.3	24.6 ± 19.4	22.8 ± 18.3	<0.001
Daily dietary intakes				
Fruit and vegetables (servings)	2.4 ± 0.7	5.2 ± 0.4	10.6 ± 3.0	<0.001
Total meat (servings)[4]	1.3 ± 0.7	1.3 ± 0.7	1.3 ± 0.8	<0.001
Alcohol (g)	12.0 ± 16.5	11.5 ± 15.5	10.4 ± 14.4	<0.001
Coffee (cups)	1.5 ± 1.7	1.3 ± 1.5	1.1 ± 1.5	<0.001
Vitamin C (mg)[5]	309 ± 434	414 ± 457	576 ± 500	<0.001
Vitamin E (mg)[5]	43.4 ± 90.4	48.0 ± 89.4	61.4 ± 98.0	<0.001
Multivitamin use (%)	57.9	62.3	65.6	<0.001
Women, NHS I				
No. of participants	17 696	17 569	17 515	
Age (y)	45.2 ± 7.0	46.6 ± 7.1	48.0 ± 7.2	<0.001
Height (in)	64.3 ± 3.3	64.5 ± 3.1	64.5 ± 3.3	<0.001
BMI (kg/m^2)	24.4 ± 4.4	24.5 ± 4.4	24.6 ± 4.4	<0.001
Past smoker (%)	23.5	28.4	31.4	<0.001
Current smoker (%)	40.0	26.4	21.4	<0.001
Cigarette smoking (pack-years)[3]	24.2 ± 17.0	19.7 ± 16.0	17.9 ± 16.0	<0.001
Daily dietary intakes				
Fruit and vegetables (servings)	1.7 ± 0.5	3.7 ± 0.3	7.3 ± 2.0	<0.001
Total meat (servings)[4]	1.3 ± 0.7	1.4 ± 0.7	1.5 ± 0.8	<0.001
Alcohol (g)	7.3 ± 12.0	6.1 ± 10.0	5.7 ± 9.7	<0.001
Coffee (cups)	2.5 ± 2.1	2.3 ± 2.0	2.1 ± 2.0	<0.001
Vitamin C (mg)[5]	221 ± 456	294 ± 482	416 ± 588	<0.001
Vitamin E (mg)[5]	30.7 ± 96.8	33.2 ± 91.6	43.0 ± 99.1	<0.001
Multivitamin use (%)	28.6	34.4	39.2	<0.001
Women, NHS II				
No. of participants	18 712	19 121	18 068	<0.001
Age (y)	36.2 ± 4.8	36.6 ± 4.7	36.9 ± 4.6	<0.001
Height (in)	64.8 ± 2.6	64.9 ± 2.6	65.0 ± 2.7	<0.001
BMI (kg/m^2)	24.8 ± 5.7	24.5 ± 5.2	24.6 ± 5.2	<0.001
Past smoker (%)	19.3	22.5	24.4	<0.001
Current smoker (%)	16.9	11.1	10.1	<0.001
Cigarette smoking (pack-years)[3]	13.5 ± 9.4	11.7 ± 8.5	11.0 ± 8.1	<0.001
Daily dietary intakes				
Fruit and vegetables (servings)	2.0 ± 0.6	4.6 ± 0.4	9.6 ± 2.8	<0.001
Total meat (servings)[4]	1.1 ± 0.6	1.3 ± 0.6	1.4 ± 0.7	<0.001
Alcohol (g)	2.7 ± 6.1	3.2 ± 6.0	3.4 ± 6.2	<0.001
Coffee (cups)	1.2 ± 1.7	1.3 ± 1.6	1.3 ± 1.6	<0.001
Vitamin C (mg)[5]	199 ± 336	256 ± 318	333 ± 314	<0.001
Vitamin E (mg)[5]	23.8 ± 65.3	24.9 ± 58.0	29.1 ± 58.0	<0.001
Multivitamin use (%)	36.3	44.5	51.0	<0.001

[1] Baseline was 1986 for the men in the HPFS, 1980 for the women in the NHS I, and 1991 for the women in the NHS II. Of note, for the women, the 1980 food-frequency questionnaire included fewer fruit and vegetable items than did the 1991 food-frequency questionnaire.

[2] $\bar{x}$ ± SD (all such values).

[3] Pack-years are calculated for current and past smokers.

[4] Total meat consists of processed meats, bacon, hot dogs, hamburger, beef, pork, or lamb as a sandwich or mixed dish; beef, pork, or lamb as a main dish; chicken with skin; and chicken without skin.

[5] Energy-adjusted vitamin intake from the diet and vitamin supplements.

green-leafy vegetables, citrus fruit, or fruit and vegetables rich in vitamin C and glioma risk.

Results with the use of baseline or most recent total, fruit, vegetables, or composite fruit and vegetable groups were similar to those that used cumulative updated diet, with one exception: cruciferous vegetable intake (data not shown). At baseline, we observed a marginally statistically significant decrease in glioma risk in the women with the highest cruciferous vegetable intake compared with those with the lowest intake (RR in a comparison of the highest with the lowest intake quintile: 0.53; 95% CI: 0.33,

TABLE 2
Characteristics of glioma cases among men in the Health Professionals Follow-Up Study (HPFS: 1986 to 2002) and women in the Nurses' Health Study I (NHS I: 1980 to 2002) and NHS II (1991 to 2003)

	Cohort		
Glioma characteristic	Men, HPFS	Women, NHS I	Women, NHS II
Crude incidence rate (no. per 100 000 person-years)	16	9	1
Observed number of cases[1]	115	165	16
Expected number of cases[2]	111	170	45
Observed:expected	1.04 (0.86, 1.23)[3]	0.97 (0.83, 1.12)	0.36 (0.20, 0.55)
Age at diagnosis (y)			
$\bar{x} \pm$ SD	65.5 ± 9.9	60.8 ± 8.5	40.6 ± 6.5
Median	65.6	61.4	38.8
Range	43.5–85.1	38.8–78.5	28.1–49.1
Histology (%)			
Astrocytoma	94.3	89.1	75.0
Glioblastoma	80.5	66.4	50.0
Oligodendroglioma	0	3.6	16.7
Ependymoma	1.1	0.7	0
Mixed glioma	1.1	2.2	8.3
Unknown or missing	3.5	4.4	0
Anatomical site (%)			
Frontal	27.6	26.3	41.7
Temporal	20.7	23.4	0
Parietal	12.6	16.1	16.7
Occipital	5.7	0.7	0
Multilobar	6.9	11.7	25.0
Other[4]	10.3	3.7	0
Unknown or missing	16.2	18.1	16.6
Ethnicity			
White (%)	94.7	94.2	92.6

[1] Number of observed cases confirmed by pathology reports: HPFS = 87, NHS I = 137, NHS II = 12.
[2] Age-specific incidence rate of brain cancer obtained from Surveillance, Epidemiology, and End Results (SEER) registry, 1990 to 2003.
[3] 95% CI in parentheses (all such values).
[4] Cerebellum, brain stem, corpus callosum, tectum, or ventricle.

0.85; *P* for trend = 0.05); however, no significant association was observed in the men (RR in a comparison of the highest and lowest quintiles: 1.29; 95% CI: 0.73, 2.26; *P* for trend = 0.60).

To account for changes in diet because of preclinical manifestations of disease or for other reasons, we conducted additional analyses that excluded all cases of glioma diagnosed within the first 2 y of follow-up. On the basis of 268 glioma cases (2 190 548 person-years), no significant associations were observed for fruit, vegetables, or other fruit and vegetable subgroups (data not shown). Similarly, removing men or women who reported having changed their fruit or vegetable intake in the previous decade (on the baseline questionnaire) resulted in associations similar to those observed with the updated dietary intake (data not shown). To evaluate time from exposure to glioma diagnosis, we conducted a 2–6-y lag analysis (see Subjects and Methods); no significant association with glioma risk for fruit, vegetables, or other fruit and vegetable subgroups was observed (data not shown).

To further explore specific nutrients in fruit and vegetables, we examined the association between glioma risk and updated dietary intake of the major carotenoids for the men and women. No material relation was observed between the updated intake of α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, and lycopene and the risk of glioma (**Table 4**). For lycopene, baseline intake was associated with a suggestive increased glioma risk in the women; the RRs for glioma in the multivariate model were 1.23 for the 2nd quintile, 1.63 for the 3rd quintile, 1.11 for the 4th quintile, and 1.66 (95% CI: 1.04, 2.67) for the 5th quintile (*P* for trend = 0.04).

Intake of individual fruit and vegetables that constitute the composite fruit and vegetable food groups was not appreciably associated with glioma risk (data not shown). The only exception was intake of cabbage, cauliflower, or Brussels sprouts in the women: the RR of glioma in a comparison of the highest with the lowest tertile of intake was 0.60 (95% CI: 0.40, 0.91).

Additional analyses conducted among more homogeneous cancer subgroups showed similar null results between updated total fruit and vegetable intake for the other fruit and vegetable subgroups and the risk of astrocytoma or glioblastoma (data not shown). Furthermore, no significant relation between dietary intake and glioma risk was observed by anatomic site (frontal or temporal lobe, separately) or after excluding glioma cases that were not confirmed by pathology records (data not shown).

The association between updated total fruit and vegetable intake and glioma risk was examined across strata of cigarette smoking status (never or ever) and age (median) in the men and women (data not shown); there was no evidence that the association was significantly modified by either (*P* for interaction for smoking status: 0.67 and 0.87; *P* for interaction of age: 0.64 and 0.62, for men and women, respectively). Furthermore, there was no evidence that the association was significantly modified by meat intake (total or processed; data not shown).

TABLE 3
Cumulative updated intake of total fruit and vegetables, fruit, vegetables, and other food groupings and the risk of glioma among men in the Health Professionals Follow-Up Study (HPFS; 1986 to 2002) and women in the Nurses' Health Study I (NHS I; 1980 to 2002) and NHS II (1991 to 2003)[1]

	Quintile of intake					
	1	2	3	4	5	*P* for trend
Total fruit and vegetables						
Median intake (servings/d)						
Men	2.8	4.2	5.3	6.7	9.3	
Women	2.4	3.7	4.8	6.0	8.1	
Cases/person-years	50/727 303	59/743 188	54/738 487	60/737 502	73/723 109	
Pooled MV RR (95% CI)[2]	1.0 (reference)	1.05 (0.71–1.55)	0.92 (0.62–1.38)	0.97 (0.65–1.45)	1.19 (0.80–1.79)	0.32
Fruit						
Median intake (servings/d)						
Men	0.9	1.6	2.1	2.8	4.1	
Women	0.8	1.5	2.1	2.7	3.8	
Cases/person-years	46/732 241	55/734 546	65/741 203	52/737 621	78/723 978	
Pooled MV RR (95% CI)[2]	1.0 (reference)	1.10 (0.74–1.65)	1.19 (0.81–1.77)	0.95 (0.63–1.45)	1.41 (0.95–2.10)	0.12
Vegetables						
Median intake (servings/d)						
Men	1.9	2.4	3.1	3.8	5.0	
Women	1.2	2.0	2.6	3.4	4.8	
Cases/person-years	50/735 856	56/735 409	57/739 549	62/734 901	71/723 874	
Pooled MV RR (95% CI)[2]	1.0 (reference)	1.02 (0.69–1.52)	0.99 (0.66–1.48)	1.00 (0.67–1.49)	1.17 (0.78–1.75)	0.32
Cruciferous vegetables						
Median intake (servings/d)						
Men	0.9	1.8	2.6	3.9	6.5	
Women	0.9	1.7	2.4	3.3	5.3	
Cases/person-years	63/738 316	66/850 671	53/631 068	45/689 171	69/760 363	
Pooled MV RR (95% CI)[2]	1.0 (reference)	0.84 (0.59–1.19)	0.89 (0.61–1.31)	0.65 (0.43–0.96)	0.91 (0.64–1.31)	0.74
Yellow-orange vegetables						
Median intake (servings/d)						
Men	1.3	3.0	4.3	6.4	9.5	
Women	0.7	2.5	3.7	5.3	7.8	
Cases/person-years	53/723 282	74/800 777	44/678 309	65/741 892	60/725 329	
Pooled MV RR (95% CI)[2]	1.0 (reference)	1.04 (0.71–1.51)	0.77 (0.50–1.18)	0.90 (0.61–1.34)	0.91 (0.61–1.35)	0.63
Citrus fruit						
Median intake (servings/d)						
Men	0.9	3.4	6.0	8.2	13.0	
Women	1.0	3.4	6.0	8.0	11.6	
Cases/person-years	40/718 887	67/753 040	63/771 684	63/695 424	63/730 554	
Pooled MV RR (95% CI)[2]	1.0 (reference)	1.59 (1.07–2.38)	1.44 (0.96–2.15)	1.47 (0.98–2.22)	1.40 (0.93–2.13)	0.34
Fruit and vegetables rich in vitamin C						
Median intake (servings/d)						
Men	3.4	6.9	9.8	12.6	19.6	
Women	2.9	6.2	8.4	11.2	16.1	
Cases/person-years	52/731 267	53/749 597	58/724 752	63/737 844	70/726 129	
Pooled MV RR (95% CI)[2]	1.0 (reference)	0.92 (0.62–1.37)	1.03 (0.70–1.51)	1.03 (0.71–1.51)	1.14 (0.77–1.67)	0.32

[1] Glioma risk was assessed with relation to the cumulative average of dietary intakes, which was calculated from all dietary questionnaires (*see* Methods). Results were obtained from pooling the β coefficient and SEEs for the men and women by using the DerSimonian and Laird random-effects model. No evidence of heterogeneity by cohort was observed, $P = 0.9$. RR, rate ratio; MV, multivariate.

[2] Cox proportional hazards adjusted for age and total calorie intake.

DISCUSSION

In our study of fruit and vegetable consumption in well-defined, large prospective cohorts of men and women with a large number of glioma cases and validated, updated dietary information, we found no significant overall associations between consumption of total fruit or vegetables and glioma risk in men and women. Similarly, there was no evidence of any appreciable benefit from any of the specific subgroups of fruit and vegetables. Overall, no significant association was observed between dietary intakes of α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, and lycopene and the risk of glioma.

The biological rationale for the intake of fruit and vegetables in glioma prevention relates largely to the potential ability of several dietary constituents of fruit and vegetables to inhibit nitrosation. Nitrosation, the endogenous formation of NOC from dietary precursors, is a complex process that is dependent on the presence of dietary NOC precursors including nitrate and nitrite, the presence of bacteria and nitrosation catalysts or inhibitors, gastric pH, and other physiologic variables (36–39). The endogenous nitrosation reaction may be blocked by the presence of nitrogen scavengers, such as vitamins C and E, phenolics, or antioxidants in the diet (16–18). Both vitamins C and E are

TABLE 4
Cumulative updated intake of dietary carotenoids and the risk of glioma among men in the Health Professionals Follow-Up Study (HPFS; 1986 to 2002) and women in the Nurses' Health Study I (NHS I; 1980 to 2002) and NHS II (1991 to 2003)[1]

	Quintile of intake					*P* for trend
	1	2	3	4	5	
α-Carotene						
Median intake (μg/d)						
Men	304	502	691	1040	1782	
Women	280	456	618	911	1512	
Cases/person-years	55/728 000	55/733 917	55/738 476	55/739 749	76/729 447	
Pooled MV RR (95% CI)[2]	1.0 (reference)	0.91 (0.62–1.33)	0.85 (0.59–1.25)	0.84 (0.57–1.22)	1.10 (0.77–1.57)	0.43
β-Carotene						
Median intake (μg/d)						
Men	2130	5267	4359	5814	8950	
Women	1914	2945	3955	5267	7818	
Cases/person-years	56/726 837	52/736 862	55/737 997	68/735 870	65/732 023	
Pooled MV RR (95% CI)[2]	1.0 (reference)	0.84 (0.57–1.24)	0.87 (0.60–1.26)	0.99 (0.69–1.42)	0.92 (0.64–1.32)	0.27
β-Cryptoxanthin						
Median intake (μg/d)						
Men	74	134	190	254	368	
Women	88	150	202	256	360	
Cases/person-years	49/733 608	51/740 366	60/737 783	64/735 342	72/72 490	
Pooled MV RR (95% CI)[2]	1.0 (reference)	0.96 (0.64–1.44)	1.15 (0.78–1.70)	1.07 (0.73–1.58)	1.16 (0.79–1.69)	0.60
Lutein and zeaxanthin						
Median intake (μg/d)						
Men	1380	2194	2915	3814	5726	
Women	1494	2302	3050	4347	7033	
Cases/person-years	64/731 945	60/736 110	52/737 358	51/738 037	69/726 139	
Pooled MV RR (95% CI)[2]	1.0 (reference)	0.87 (0.61–1.25)	0.75 (0.52–1.08)	0.72 (0.50–1.04)	0.95 (0.67–1.35)	0.96
Lycopene						
Median intake (μg/d)						
Men	2997	4873	6437	8504	12 879	
Women	1429	3199	4229	5388	7362	
Cases/person-years	61/705 678	68/735 244	53/742 139	55/744 688	59/741 840	
Pooled MV RR (95% CI)[2]	1.0 (reference)	1.15 (0.81–1.64)	0.89 (0.61–1.30)	0.97 (0.66–1.41)	0.99 (0.68–1.45)	0.62

[1] Glioma risk was assessed with relation to the cumulative average of dietary intakes, which was calculated from all dietary questionnaires (*see* Methods). Results were obtained from pooling the β coefficient and SEEs for the men and women by using the DerSimonian and Laird random-effects model. No evidence of heterogeneity by cohort was observed, $P = 0.9$. RR, rate ratio; MV, multivariate.

[2] Cox proportional hazards adjusted for age and total calorie intake.

powerful reducing agents and are oxidized to reduce nitrous acid to nitric oxide (17). The presence of these vitamins and other nutrients, including carotenoids, from intake of fruit and vegetables in the diet may decrease or inhibit the endogenous formation of NOC or may act directly as antioxidants protecting against oxidative stress, free-radical reactions, DNA or cellular damage, and lipid peroxidation (19–21) and, consequentially, reduce the risk of glioma.

Observational studies of intakes of fruit and vegetables and their dietary constituents on the risk of glioma have been inconclusive (2–14) (**Table 5**). Several case-control studies report null results for intakes of fruit (4, 5, 7, 11), vegetables (4, 5, 7, 11), fruit or vegetable subgroups (5, 6, 11–13), and individual fruit and vegetable food items (3, 5, 6, 8). Two case-control studies by Hu et al (9, 10), which used the same study population, reported significant inverse associations between intakes of fruit and fresh vegetables and glioma or meningioma risk. Chen et al (6) reported a marginally statistically significantly reduced risk of glioma among those in the highest quartile of vegetable and dark yellow vegetable consumption compared with those in the lowest quartile and an ≈50% reduction in glioma risk with increased α-and β-carotene intake. In a recent case-control study, a reduced risk of glioma was observed for higher antioxidant index (*P* for trend = 0.002), carotenoids (α-and β-carotene combined; *P* for trend = 0.02), and β-carotene (*P* for trend = 0.04) (14). In the single prospective study, during 6 y of follow-up, 21 of 34 000 Seventh-Day Adventist participants developed glioma (2). No association was observed between the derived fruit index [canned or frozen fruit, dried fruit, fresh citrus fruit, and other fresh fruit (apples, bananas, and pears)], fresh citrus fruit, or unsweetened or sweetened real fruit juice and glioma risk.

The inverse association observed for cruciferous vegetables among the women may be due to chance, because it was not observed in the men and was marginally statistically significant only with the baseline analyses but not with other analytic approaches. Alternatively, it is possible that cruciferous vegetables, which include broccoli, cabbage, cauliflower, Brussels sprouts, and kale, play a role in glioma etiology, because experimental studies (40–42) have identified several compounds found in cruciferous vegetables, eg, isothiocyanate sulforaphane, that can induce phase 2 detoxifying enzymes in vitro and stimulate metabolism of drugs and other xenobiotics in humans (43–45).

TABLE 5
Summary of selected epidemiologic studies of the relation between intakes of dietary fruit, vegetables, and carotenoids and brain tumors[1]

Reference	Study location and time period	Subjects	Cases, proxy %	Risk estimate (95% CI)	Comments
Burch et al (1987)	Southern Ontario (Canada), 1979–1982	Glioma: 215 M and F 215 hospital control subjects	73	RR range: 0.13–0.58 RR = 1.33 (0.56, 3.16)	Various fruit items Tomatoes, once a month
Mills et al (1989)	California Seventh-Day Adventist cohort (USA), 1976–1982	Glioma: 21 M and F 6 y of follow-up		RR = 0.85 (0.28, 2.60) RR = 0.92 (0.37, 2.37) RR = 1.65 (0.59, 5.77) RR = 0.29 (0.01, 1.59)	Fruit index, ≥1 time/d Citrus fruit, ≥ 1 time/wk Unsweetened fruit juice, ≥ 1 time/wk Sweetened fruit juice, ≥1 time/wk
Preston-Martin and Mack (1991)	Los Angeles, CA (USA), 1980–1984	Glioma: 202 M 202 neighborhood control subjects	0	0.8 (0.4, 1.6)[2]	Citrus fruit, >5 times/wk
Ryan et al (1992)	Adelaide, Australia, 1987–1990	Glioma: 60 M, 50 F 417 population control subjects	25	NA	Citrus fruit or juice
Boeing et al (1993)	Germany, 1987–1988	Glioma: 115 M and F 418 population control subjects	23	1.1 (0.6, 1.9) 0.9 (0.5, 1.7)	Fruit, highest tertile Vegetables, highest tertile
Giles et al (1994)	Victoria Province, Australia, 1987–1991	Glioma: 166 M and F 409 population control subjects	>50	1.51 (0.95, 2.39) 0.69 (0.36, 1.31) 1.01 (0.62, 1.63) 0.53 (0.29, 0.95) 0.97 (0.60, 1.56) 0.70 (0.39, 1.26)	M, fruit, highest tertile F, fruit, highest tertile M, vegetables, highest tertile F, vegetables, highest tertile M, β-carotene, highest tertile F, β-carotene, highest tertile
Blowers et al (1996)	Los Angeles, CA (USA), 1986–1988	Glioma: 94 F 94 neighborhood control subjects	0	1.3 (0.5, 3.0) 1.3 (0.5, 3.2) 1.7 (0.7, 4.3) 0.2 (0.1, 0.7) 0.5 (0.2, 1.3)	Fruit, highest quartile Vegetables, highest quartile Citrus fruit or juice, highest quartile Bell peppers, highest quartile Carrots, highest quartile
Kaplan et al (1997)	Tel Hashomer, Israel, 1987–1991	Glioma (59) and meningioma; 139 M and F 278 friend and hospital control subjects	22	1.76 (1.03, 3.01)[3] 2.01 (1.17, 3.46)[3] 0.91 (0.55, 1.52)[3] 0.65 (0.37, 1.12)[3]	Fruit, ≥104.1 g/d Fruit, high vitamin C, ≥195.1 g/d Vegetables, ≥84.1 g/d Carrots, ≥23 g/d
Lee et al (1997)	San Francisco Bay, CA (USA), 1991–1994	Glioma: 434 M and F 434 population control subjects	46	NA	Fruit or vegetables high in vitamin A or C
Hu et al (1998)	Northeast China, 1989–1995	Glioma: 218 M and F 436 hospital control subjects	0	0.28 (0.16, 0.51) 0.51 (0.29, 0.89)	Fruit, ≥46 kg/y Vegetables, ≥125 kg/y
Hu et al (1999)	Northeast China, 1993–1995	Glioma (73) and meningioma; 129 M and F 258 hospital control subjects	0	0.15 (0.1, 0.4)[3] 0.18[4] 0.29 (0.1, 0.7)[3] 0.34[4] 2.54 (1.2, 5.6)	Fruit, highest quartile Fruit, highest quartile Fresh vegetables, highest quartile Fresh vegetables, highest quartile Salted vegetables, highest quartile
Chen et al (2002)	Nebraska (USA), 1988–1993	Glioma: 236 M and F 449 population control subjects	76	1.0 (0.6, 1.7) 0.5 (0.3, 1.0) 0.7 (0.4, 1.2) 0.6 (0.3, 1.0) 1.2 (0.7, 2.0) 0.7 (0.4, 1.3) 0.5 (0.3, 0.8) 0.5 (0.3, 0.9) 0.7 (0.4, 1.3) 1.2 (0.7, 2.1) 0.9 (0.5, 1.6)	Citrus fruit, highest quartile Vegetables, highest quartile Dark green vegetables, highest quartile Dark yellow vegetables, highest quartile Tomatoes, highest quartile High-nitrate vegetables, highest quartile α-Carotene, highest quartile α-Carotene, highest quartile β-Cryptoxanthin, highest quartile Lutein, highest quartile Lycopene, highest quartile

(*Continued*)

TABLE 5 (*Continued*)

Reference	Study location and time period	Subjects	Cases, proxy %	Risk estimate (95% CI)	Comments
Tedeschi Blok et al (2006)	San Francisco Bay, CA (USA), 1991–2000	Glioma: 802 M and F 846 population control subjects	46	0.57 (0.42, 0.78)	Antioxidant index, highest quartile
				0.65 (0.48, 0.88)	Carotenoids (α- and β-carotene combined, highest quartile
				0.76 (0.57, 1.02)	α-Carotene, highest quartile
				0.72 (0.54, 0.98)	β-Carotene, highest quartile
				0.92 (0.69, 1.21)	β-Cryptoxanthin, highest quartile
				0.79 (0.59, 1.05)	Lutein, highest quartile
				1.02 (0.76, 1.36)	Lycopene, highest quartile

[1] RR, relative risk; NA, no association (and risk estimate not reported).
[2] Odds ratio (OR; all such values).
[3] OR of glioma and meningioma cases combined.
[4] RR of glioma cases; CI not reported.

The suggestive elevation in risk of glioma for lycopene at baseline did not persist in the women when we examined cumulative, updated average intake, which took into account cooked and raw tomato-based sources of lycopene (the baseline NHS I questionnaire included intake of only fresh tomatoes). With the cumulatively updated data, we were able to enhance the precision of dietary assessments, account for changes in consumption over time, and reduce the potential for within-person misclassification of intake. Overall, we observed no significant relation between 5 major carotenoids and glioma risk.

The strengths of our study included its large sample size, the prospective design, long follow-up, and detailed and updated information on fruit and vegetable consumption, with up to 22 fruit items and 38 vegetable items. The prospective design precludes recall bias, and selection bias is minimized by the high rate of follow-up over a long period of time. No proxies were needed, because information on diet was obtained before the occurrence of disease. The availability of repeated dietary measures in the cohorts permitted a consideration of early (baseline), most recent (simple update), and long-term (cumulative updated and restricted analyses) dietary intake. We cannot exclude measurement error due to self-reported diet as a contributor to the lack of associations in the current study; however, we previously showed the accuracy of self-reported dietary intake in these cohorts, and the repeated assessment of intake may reduce within-person subject variation and better represent long-term average intake. Misclassification of disease status may occur because of undetected or underreported cancer due to misdiagnosis. We cannot exclude the possibility that some cancers may have been missed in the study; however, it is unlikely given the comprehensive method of disease surveillance in the cohorts. Furthermore, the results from the secondary analyses, which excluded glioma cases not confirmed by pathology records, were similar to those when all cases were considered.

In conclusion, our study provides limited support for the hypothesis that dietary intake of fruit, vegetables, and carotenoids with antioxidative properties and the potential ability to inhibit the endogenous formation of NOC may reduce the risk of adult glioma. High consumption of total fruit and vegetables, fruit and vegetable subgroups, and carotenoids were not significantly related to the risk of glioma in our large US cohorts.

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ELG, MJS, and DSM contributed to the study design. CNH was responsible for writing and revising the manuscript. ELG, BR, MJS, and DSM provided substantive editorial comments on manuscript drafts. None of the authors had any personal or financial conflicts of interest.

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