Human Papillomavirus-16 Modifies the Association between Fruit Consumption and Head and Neck Squamous Cell Carcinoma

Mara S. Meyer,[1] Katie M. Applebaum,[2,4] C. Sloane Furniss,[3] Edward S. Peters,[6] Brian G. Luckett,[6] Judith F. Smith,[7] Janine Bryan,[7] Michael D. McClean,[5] Carmen Marsit,[9] and Karl T. Kelsey[8,9]

Departments of [1]Epidemiology, [2]Environmental Health, and [3]Genetics and Complex Diseases, Harvard School of Public Health; Departments of [4]Epidemiology and [5]Environmental Health, Boston University School of Public Health, Boston, Massachusetts; [6]Epidemiology Program, Louisiana State University Health Sciences School of Public Health, New Orleans, Louisiana; [7]Department of Vaccine Biologics Research, Merck and Co., Inc., West Point, Pennsylvania; and Departments of [8]Community Health and [9]Pathology and Laboratory Medicine, Center for Environmental Health and Technology, Brown University, Providence, Rhode Island

Abstract

Human papillomavirus-16 (HPV-16) is a risk factor for head and neck squamous cell carcinoma (HNSCC). HPV-positive cancers have distinct disease cofactors and improved survival following treatment. There is conflicting evidence of a protective association of fruit consumption with HNSCC. As HPV-related disease is clinically distinct, we investigated whether the association between fruit consumption and HNSCC risk was modified by exposure to HPV-16. We studied 270 cases and 493 controls with fruit intake information and known HPV-16 antibody status. Cases were identified at nine Boston-area medical facilities between 1999 and 2003. Controls were randomly selected from the greater population and frequency matched to cases by age, gender, and town of residence. Controlling for age, gender, race, smoking, alcohol, total energy intake, body mass index, and education, the seronegative individuals had a significantly lower risk of HNSCC with increasing total fruit consumption [odds ratio (OR)$_{\text{tertile 2}}$, 0.60; 95% confidence interval (95% CI), 0.38-0.95; OR$_{\text{tertile 3}}$, 0.57; 95% CI, 0.35-0.95] and specifically increasing citrus fruit consumption (OR$_{\text{tertile 2}}$, 0.61; 95% CI, 0.39-0.97; OR$_{\text{tertile 3}}$, 0.59; 95% CI, 0.37-0.96). However, among the seropositive, risk increased with greater fruit consumption (OR$_{\text{tertile 2}}$, 2.27; 95% CI, 0.92-5.58; OR$_{\text{tertile 3}}$, 1.40; 95% CI, 0.55-3.59) and citrus fruit consumption (OR$_{\text{tertile 2}}$, 3.35; 95% CI, 1.36, 8.24; OR$_{\text{tertile 3}}$, 3.15; 95% CI, 1.23-8.08). This interaction was statistically significant ($P < 0.05$), showing that fruit consumption was associated with a reduced HNSCC risk among HPV-16-seronegative individuals but an increased HNSCC risk among the HPV-16-seropositive individuals. These findings suggest that dietary factors dramatically alter the pattern of occurrence of HPV-associated HNSCC and show that viral-related disease is clinically and etiologically distinct. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3419–26)

Introduction

In the United States, an estimated 47,000 incident cases of head and neck squamous cell carcinoma (HNSCC; cancer of the pharynx, larynx, and oral cavity), are expected to occur in 2008, resulting in 11,000 deaths (1). The major risk factors for HNSCC are tobacco and alcohol use, which are thought to account for ~75% of all cases (2). More recently, human papillomavirus (HPV) has been recognized as an important risk factor for HNSCC (3, 4).

HPV-16 is the most commonly identified HPV type in HNSCC tumors (5). In case-control studies of HNSCC, presence of antibodies against HPV-16 has been associated with an approximately 4-fold increased HNSCC risk (6-8). Consistent with the belief that HPV-16 is transmitted sexually, prevalence of antibodies against HPV-16 has been associated with the number of sexual partners and with the number of oral sex partners in studies of HNSCC (7, 9-11). Moreover, it has been suggested that HPV-16-related HNSCC has a distinct etiology as evidenced by recent analyses showing that alcohol consumption and tobacco use are not associated with HNSCC risk among HPV-16-positive individuals (12-14). Additionally, it has been shown that the clinical outcomes of patients with HPV-16-related HNSCC are significantly better than those with non-HPV-related disease (4, 7). In particular, the strongest evidence for a relationship with HPV-16 has been observed for pharyngeal cancer (14).

Diet is also thought to play a role in HNSCC risk; in particular, fruit consumption has been linked to decreased risk. Several case-control studies have reported that total fruit intake is inversely related to disease risk after adjustment for known risk factors (15-20). A meta-analysis of case-control studies found that high fruit intake significantly decreased the risk of oral and pharyngeal cancer [odds ratio (OR), 0.53; 95% confidence

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Requests for reprints: Karl T. Kelsey, Departments of Community Health and Pathology and Laboratory Medicine, Center for Environmental Health and Technology, Brown University, Providence, RI 02912. Phone: 401-863-6420; Fax: 401-863-9008. E-mail: Karl_Kelsey@brown.edu

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interval (95% CI), 0.37-0.76] and laryngeal cancer (OR, 0.73; 95% CI, 0.64-0.84) after adjusting for smoking (21). A more recent meta-analysis of 16 studies also reported an overall protective effect of fruit consumption on oral cancer risk, although not all comparisons were statistically significant (22). Importantly, results have been somewhat inconsistent with several studies reporting no association between total fruit intake and risk of HNSCC (23) or oral and pharyngeal cancer (24, 25).

There are fewer prospective studies investigating the effect of diet on HNSCC. The European Investigation into Cancer and Nutrition reported that the highest quintile of fruit intake was associated with lower risk of upper aerodigestive squamous cell cancers (relative risk, 0.63; 95% CI, 0.42-0.96; ref. 26). A recent prospective analysis of the NIH-American Association of Retired Persons Diet and Health Study detected a modest inverse association between head and neck cancers and total fruit and vegetable intake (hazard ratio, 0.94; 95% CI, 0.89-0.99), although the relationships with fruit intake alone (hazard ratio, 0.87; 95% CI, 0.86-1.11) and citrus fruit (hazard ratio, 0.90; 95% CI, 0.75-1.08) were not statistically significant (27). In an analysis of the Health Professionals' Follow-up Study, Maserejian et al. (28) did not find a significant reduction in risk of oral premalignant lesions with total fruit intake in multivariate models (highest quintile relative risk, 0.77; 95% CI, 0.47-1.27).

Consumption of citrus fruit, specifically, has been shown to have a strong inverse relationship with HNSCC and oral premalignant lesions (24, 28). In multivariate analyses, De Stefani et al. reported an OR of 0.3 (95% CI, 0.2-0.7) for oral and pharyngeal cancer comparing the highest quartile of citrus fruit consumption with the lowest quintile (20). Levi et al. found similar results with an OR of 0.38 (95% CI, 0.20-0.73) for the highest tertile of citrus fruit intake (16). A case-control study from the early 1990s also reported an inverse association with fruits, particularly oranges and tangerines (29). However, this study did not find an association with dietary vitamin C intake. Not all studies show significant reductions with citrus fruit consumption, as Garrote et al. reported an OR of 0.78 (95% CI, 0.40-1.51) for the highest tertile of intake (30). It has been suggested that the higher vitamin C concentration of such foods may be responsible for the apparent protective association, whereas other components such as flavonoids and polyphenols may also play a anticarcinogenic role (29, 31).

In addition, a review of epidemiologic studies regarding the influence of diet on cervical carcinogenesis reported a protective association of fruits and vegetables against persistent HPV infection (32). However, there are little data assessing the role of fruit consumption in negating risk of cervical neoplasia (32). A separate study of cervical cancer suggested a weak inverse relationship with plasma vitamin C levels after careful adjustment for HPV status (33). Goodman et al. also observed that high plasma levels of antioxidants may be protective against cervical squamous intraepithelial lesions regardless of HPV status (33). Although this research suggests that dietary modification of cancer risk may differ by HPV status, the extent to which the effect of diet on HNSCC is modified by the presence of HPV-16 remains unclear.

Accordingly, here, we have examined whether the association between fruit consumption and HNSCC differs in the presence of HPV-16. To our knowledge, there are little data that address whether diet and its association with HNSCC risk varies by HPV status, but as HPV status so profoundly influences the etiology of the disease and its outcome, we hypothesized that it may also modify the effect of fruit consumption on risk.

Materials and Methods

Study Population. The study design has been described in detail previously (34). Briefly, incident cases of HNSCC were identified at nine Boston-area medical facilities between December 1999 and December 2003. Eligible cases were at least 18 years old, resided in the Greater Boston Metropolitan Area, were alive at time of initial contact, and were diagnosed within the previous 6 months. The Greater Boston Metropolitan Area is a region consisting of 249 cities and towns within 1 hour drive from Boston. For this study, HNSCC was defined as carcinoma of the tongue, gum, floor of mouth, other location in mouth, oropharynx, hypopharynx, ill-defined site within lip oral cavity and pharynx, and larynx corresponding to the *International Classification of Disease-Ninth Revision* codes 141, 143 to 146, 148, 149, and 161, respectively. Patients with carcinoma *in situ*, lip, salivary gland, or nasopharyngeal cancers were excluded. Prevalent and recurring cancers were also excluded. Cases were histologically confirmed by a study pathologist. The Massachusetts town book methodology was used to identify potential controls (35). Controls were frequency matched (1:1) to cases by age (±3 years), gender, and town of residence. The institutional review boards at the nine participating medical facilities and the Harvard School of Public Health approved all study protocol and materials. All study participants provided informed consent.

Questionnaire. Baseline characteristics, including information on sociodemographics, alcohol consumption, and smoking habits, were collected from a comprehensive, self-administered questionnaire. Decade-specific data on alcohol consumption and tobacco use were collected. These data were used to estimate pack-years (packs smoked per day multiplied by years smoked) and average alcoholic drinks consumed per week (14). Diet information was collected using a semiquantitative food frequency questionnaire (FFQ) assessing food consumption 5 years before diagnosis for cases and date of enrollment for controls. The FFQ has been validated previously (36, 37). Fruit intake was calculated as the amount consumed in 1 week. Total fruit intake included fresh apples/pears, apple juice, bananas, blueberries, cantaloupe, grapefruit, grapefruit juice, oranges, orange juice, other fruit juice, prunes, raisins, strawberries, and peaches/apricots/plums. Citrus fruit intake consisted of grapefruit, grapefruit juice, oranges, and orange juice. Vegetable intake was also calculated as the amount consumed in 1 week. Total vegetable intake included tomatoes, tomato juice, tomato sauce, string beans, broccoli, cabbage, cauliflower, Brussels sprouts, raw and cooked carrots, corn, peas, beans, winter squash, yams/sweet potatoes, zucchini/summer squash, eggplant, raw and cooked spinach, kale, iceberg and romaine lettuce, celery, green peppers, and onions. Green vegetable intake consisted of raw and cooked spinach, kale, and romaine

Table 1. Selected characteristics for HNSCC cases and controls

	Cases (n = 270)	Controls (n = 493)	P*
Age, mean ± SD	59.9 ± 11.7	61.0 ± 11.5	
Sex			
Male	190 (70.4)	359 (72.8)	
Female	80 (29.6)	134 (27.2)	
Race			
White	250 (92.6)	457 (92.7)	0.98
Other	20 (7.4)	36 (7.3)	
Highest education level			
High school or less	107 (39.6)	150 (30.4)	0.01
More than high school	163 (60.4)	343 (69.6)	
BMI			
<25	103 (38.2)	162 (32.9)	0.08
25 to <30	115 (42.6)	210 (42.6)	
≥30	52 (19.3)	121 (24.5)	
Tobacco, pack-years			
Never	53 (19.6)	164 (33.3)	<0.01
>0 to <16	50 (18.5)	114 (23.1)	
16-41	67 (24.8)	125 (25.4)	
>41	100 (37.0)	90 (18.3)	
Alcohol consumption, drinks/wk			
≤3.1	48 (17.8)	138 (28.0)	<0.01
>3.1 to 7.3	48 (17.8)	139 (28.2)	
>7.3 to 22.1	63 (23.3)	135 (27.4)	
>22.1	111 (41.1)	81 (16.4)	
HPV-16 antibody titer			
Positive	82 (30.4)	53 (10.8)	<0.01
Negative	188 (69.6)	440 (89.3)	
Total calories			
≤1,532.4	69 (25.6)	123 (25.0)	0.18
1,532.5-1,920.5	57 (21.1)	123 (25.0)	
1,920.6-2,404.9	58 (21.5)	124 (25.0)	
>2,404.9	86 (31.1)	123 (25.0)	
Total fruit consumption, servings/wk			
≤9.4	123 (45.6)	165 (33.5)	<0.01
>9.4 to 16.7	81 (30.0)	164 (33.3)	
>16.7	66 (24.4)	164 (33.3)	
Citrus fruit consumption, servings/wk			
≤2.2	116 (43.0)	159 (32.3)	0.02
>2.2 to 7.2	82 (30.4)	167 (33.9)	
>7.2	72 (26.7)	167 (33.9)	
Vitamin C intake			
Tertile 1	108 (40.0)	146 (29.6)	0.02
Tertile 2	79 (29.3)	175 (35.5)	
Tertile 3	83 (30.7)	172 (34.9)	

*Tests controlled for age and gender.

lettuce. Subjects who completed less than half of the FFQ were eliminated from the analysis.

HPV-16 Serology. Beginning with subjects enrolled in 2001 or later, venous blood samples were collected. Serum was separated within 24 h of blood drawing and all samples were frozen at −80°C. Frozen samples were shipped to Merck & Co. laboratory in West Point, PA, for testing. HPV-16 serologic titer was determined by HPV competitive Luminex immunoassay (cLIA) (38). A cutoff point of 12 milli-Merck units/mL was used to classify a positive result. The methods have been described elsewhere (7).

HPV-16 Viral DNA in Tumor Samples. The methods for the collection of HNSCC tumor samples and extraction of DNA have been described previously (7, 39). The assays were conducted by investigators blinded to patients' HPV-16 serology, sexual history, and other risk factors. The short fragment PCR (SPF) assay was used to detect HPV-16 in tumor DNA (7). The β-actin locus was amplified for use as a control, modifying the previously published methods of Kleter et al. (40). In addition, DNA from Siha and Ca33 cells was used for positive and negative controls, respectively.

Statistical Analysis. Subjects were excluded if their nonalcohol caloric intake was outside the plausible range of 800 to 4,200 kcal/d for males and 500 to 3,500 kcal/d for females (23). Subjects missing body mass index (BMI) were also excluded. We used unconditional logistic regression to calculate ORs and 95% CIs using SAS version 9.1 (SAS Institute). Total fruit and citrus fruit consumption was analyzed using tertiles of intake as determined by the distribution in the controls. We used the nutrient residual method to assess the effect of dietary vitamin C intake (excluding nutritional supplements; ref. 41). Total and green vegetable intake was also assessed in a similar manner following reports of a possible association with HNSCC (16, 18).

All estimates were adjusted for age, gender, race (Caucasian versus other), pack-years (linear), average

alcoholic drinks per week, total energy intake, BMI, and education (high school diploma or less versus higher education). Total energy intake excluded calories from alcohol and was divided into quartiles based on the distribution in controls. Alcohol was divided into quartiles determined by the joint distribution in cases and controls. BMI was calculated using self-reported height and weight 5 years before enrollment. BMI categories were assigned based on the current guidelines (underweight, <18.5; normal, 18.5-24.9; overweight, 25.0-29.9; obese, ≥30.0) as determined by the Centers for Disease Control and Prevention (42). Due to the low number of underweight participants (n = 7), the underweight and normal BMI categories were combined as the reference group. Spearman correlation coefficients were calculated to assess possible collinearity between risk factors.

We used joint effects models and models stratified by HPV-16 serologic status to analyze the relationship between fruit consumption and risk of HNSCC using those who are in the lowest quartile of fruit consumption in each stratum as the reference group. Statistical interaction was evaluated using a likelihood ratio test comparing a model containing the fruit categories and HPV-16 serology, and their cross-products, to a model without the cross products. This was a two-sided test with 2 *df.* P values < 0.05 were considered statistically significant. In the joint effects models, those who were HPV-16 seronegative and were in the lowest tertile of fruit consumption served as the reference group. These analyses were also carried out restricting to cases of pharyngeal cancer, because the literature points to a particularly strong relationship between the virus and this subtype of HNSCC. In addition, a case-only analysis of 151 HNSCC cases with tumor DNA data was conducted to determine if presence of HPV-16 DNA in tumors differed by fruit consumption.

Results

Of the 823 eligible cases identified for this study, 57 refused to participate and 44 did not complete the questionnaire. The final enrollment of 722 cases resulted in an 88% case participation rate. For the controls, 1,643 eligible individuals were contacted to participate in the study, although 828 refused. Of the 815 subjects who consented, 771 were finally enrolled, although 6 controls were later withdrawn when matched to an ineligible case. The completed enrollment of 765 control subjects represents an overall control participation rate of 47%. Following the initiation of blood collection in 2001, blood was successfully obtained from 81% of cases and 80% of controls enrolled after that time. There were 298 cases and 498 controls who completed the FFQ and whose HPV-16 serologic status was determined. After excluding 55 subjects not meeting the caloric intake and 36 missing BMI, there were 270 cases and 493 controls in the analysis, including 122 cases of pharyngeal cancer, 101 cancers of the oral cavity, and 46 of the larynx. DNA was obtained from paraffin-embedded tumors from 151 eligible case patients with dietary data.

Baseline characteristics for cases and controls are shown in Table 1. There was no difference between cases and controls by race, energy intake, or BMI while controlling for age and gender. However, cases were significantly less likely to have achieved a higher education and more likely to smoke and consume more alcohol compared with controls. Cases also consumed less citrus fruit and less fruit overall than did controls. Furthermore, whereas only 10.8% of controls tested positive for HPV-16 antibodies, 30.4% of cases had serologically detectable antibodies against the virus. The Spearman correlation coefficients revealed no significant correlation between smoking and fruit intake among controls (r = −0.07) or the total study population (r = −0.15). Similar results were found for alcohol and fruit intake among controls (r = −0.04) and the total study population (r = −0.12).

Baseline characteristics were also compared by HPV-16 serologic status among controls only (data not shown). There were no differences between the HPV-16 seropositive compared with the seronegative with respect to BMI, alcohol intake, total fruit, or citrus fruit intake. However, HPV-16-seropositive individuals smoked less compared with the HPV-16-seronegative individuals.

Results from an analysis of total fruit consumption and HNSCC stratified by HPV-16 serology are presented in Table 2. Among the HPV-16 seronegative, those with greater fruit consumption had a significantly lower frequency of HNSCC ($OR_{tertile\ 2}$, 0.60; 95% CI, 0.38-0.95;

Table 2. Association between fruit consumption and risk of HNSCC by HPV-16 serology

Fruit consumption, servings/wk	HPV-16 serology	Cases, n (%)	Controls, n (%)	Stratified model, OR* (95% CI)	Joint effects model, OR* (95% CI)
Total fruit					
≤9.4	Negative	91 (33.7)	140 (28.4)	Reference	Reference
>9.4 to 16.7		52 (19.3)	151 (30.6)	0.60 (0.38-0.95)	0.60 (0.38-0.95)
>16.7		45 (16.7)	149 (30.2)	0.57 (0.35-0.95)	0.57 (0.35-0.95)
≤9.4	Positive	32 (11.9)	25 (5.1)	Reference	2.31 (1.21-4.40)
>9.4 to 16.7		29 (10.7)	13 (2.6)	2.27 (0.92-5.58)	5.24 (2.44-11.27)
>16.7		21 (7.8)	15 (3.0)	1.40 (0.55-3.59)	3.23 (1.45-7.21)
					$P_{interaction}$ = 0.03
Citrus fruit					
≤2.2	Negative	89 (33.0)	133 (27.0)	Reference	Reference
>2.2 to 7.2		51 (18.9)	153 (31.0)	0.61 (0.39-0.97)	0.61 (0.39-0.97)
>7.2		48 (17.8)	154 (31.2)	0.59 (0.37-0.96)	0.59 (0.37-0.96)
≤2.2	Positive	27 (10.0)	26 (5.3)	Reference	1.51 (0.79-2.87)
>2.2 to 7.2		31 (11.5)	14 (2.8)	3.35 (1.36-8.24)	6.61 (3.01-14.55)
>7.2		24 (8.9)	13 (2.6)	3.15 (1.23-8.08)	5.19 (2.34-11.54)
					$P_{interaction}$ < 0.001

*ORs adjusted for age, gender, race, pack-years of smoking, alcohol, total energy intake, BMI, and education.

Table 3. Association between vitamin C intake and risk of HNSCC stratified by HPV-16 serology

HPV-16 serology	Vitamin C	Cases, *n* (%)	Controls, *n* (%)	OR* (95% CI)
Negative	Tertile 1	83 (30.7)	128 (26.0)	Reference
	Tertile 2	45 (16.7)	154 (31.2)	0.62 (0.38-0.99)
	Tertile 3	60 (22.2)	158 (32.1)	0.81 (0.51-1.28)
Positive	Tertile 1	25 (9.3)	18 (3.7)	Reference
	Tertile 2	34 (12.6)	21 (4.3)	1.35 (0.56-3.25)
	Tertile 3	23 (8.5)	14 (2.8)	1.83 (0.69-4.84)
				$P_{interaction}$ = 0.21

*ORs adjusted for age, gender, race, pack-years of smoking, alcohol, total energy intake, BMI, and education.

$OR_{tertile\ 3}$, 0.57; 95% CI, 0.35-0.95). However, among the HPV-16 seropositive, risk of HNSCC was elevated with increasing fruit consumption ($OR_{tertile\ 2}$, 2.27; 95% CI, 0.92-5.58; $OR_{tertile\ 3}$, 1.40; 95% CI, 0.55-3.59). The interaction between HPV-16 serology and fruit consumption indicated that these differences were statistically significant ($P < 0.05$). Looking within the lowest tertile of fruit consumption in the joint effects model, HNSCC risk was greater for the HPV-16 seropositive relative to the HPV-16 seronegative (OR, 2.31; 95% CI, 1.21-4.40). The upper tertiles of fruit consumption were similarly related to an increased risk of HNSCC in the HPV-16 seropositive compared with the lowest tertile of fruit consumption in the seronegative ($OR_{tertile\ 2}$, 5.24; 95% CI, 2.44-11.27; $OR_{tertile\ 3}$, 3.23; 95% CI, 1.45-7.21).

The same differences in HNSCC risk were observed by looking at citrus fruit consumption alone (Table 2). Greater citrus fruit consumption was associated with a reduced risk of HNSCC among the HPV-16 seronegative ($OR_{tertile\ 2}$, 0.61; 95% CI, 0.39-0.97; $OR_{tertile\ 3}$, 0.59; 95% CI, 0.37-0.96), although among the HPV-16 seropositive, citrus fruit consumption increased HNSCC risk ($OR_{tertile\ 2}$, 3.35; 95% CI, 1.36-8.24; $OR_{tertile\ 3}$, 3.15; 95% CI, 1.23-8.08). The interaction between HPV-16 serology and citrus fruit was statistically significant ($P < 0.05$). An increased risk of HNSCC with citrus fruit consumption was also observed in the joint effects analysis, with each tertile of citrus fruit intake in the HPV-16 seropositive compared with the lowest tertile of citrus fruit intake in the seronegative ($OR_{tertile\ 1}$, 1.51; 95% CI, 0.79-2.87; $OR_{tertile\ 2}$, 6.61; 95% CI, 3.01-14.55; $OR_{tertile\ 3}$, 5.19; 95% CI, 2.34-11.54).

We further examined this relationship by focusing on the dietary component of vitamin C (Table 3). Increasing vitamin C intake was associated with a reduced risk of HNSCC among the HPV-16 seronegative, but among the HPV-16 seropositive greater vitamin C consumption was associated with an increasing HNSCC risk. However, these differences were not statistically significant ($P_{interaction}$ = 0.21). Vitamin C intake including that from nutritional supplements, was also assessed, but the conclusions were the same (data not shown).

When restricted to cases of pharyngeal cancer, the relationship between HNSCC and total fruit intake was similar to that reported for total HNSCC cases (Table 4). The protective effect observed among HPV-16-seronegative participants was slightly stronger in cases of pharyngeal cancer ($OR_{tertile\ 2}$, 0.47; 95% CI, 0.24-0.93; $OR_{tertile\ 2}$, 0.39; 95% CI, 0.17-0.87), whereas the association in the HPV-16 seropositive was of similar magnitude ($OR_{tertile\ 2}$, 2.32; 95% CI, 0.87-6.19; $OR_{tertile\ 3}$, 1.69; 95% CI, 0.59-4.82). An increased risk of pharyngeal cancer for the HPV-16 seropositive was also observed in the joint effects analysis when compared with the lowest tertile of fruit intake in the seronegative ($OR_{tertile\ 1}$, 3.85; 95% CI, 1.81-8.19; $OR_{tertile\ 2}$, 8.92; 95% CI, 3.80-20.95; $OR_{tertile\ 3}$, 6.49; 95% CI, 2.62-16.05). Results from the stratified analysis for citrus fruit revealed a strong inverse relationship among the HPV-16 seronegative ($OR_{tertile\ 2}$, 0.31; 95% CI, 0.15-0.65; $OR_{tertile\ 3}$, 0.36; 95% CI, 0.17-0.76) and a statistically significant increased risk of pharyngeal cancer in the HPV-16 seropositive ($OR_{tertile\ 2}$, 3.55; 95%

Table 4. Association between fruit consumption and risk of pharyngeal cancer by HPV-16 serology

Fruit consumption, servings/wk	HPV-16 serology	Cases, *n* (%)	Controls, *n* (%)	Stratified model, OR* (95% CI)	Joint effects model, OR* (95% CI)
Total fruit					
≤9.4	Negative	36 (29.5)	140 (28.4)	Reference	Reference
>9.4 to 16.7		16 (13.1)	151 (30.6)	0.47 (0.24-0.93)	0.47 (0.24-0.93)
>16.7		11 (9.0)	149 (30.2)	0.39 (0.17-0.87)	0.39 (0.17-0.87)
≤9.4	Positive	22 (18.0)	25 (5.1)	Reference	3.85 (1.81-8.19)
>9.4 to 16.7		21 (17.2)	13 (2.6)	2.32 (0.87-6.19)	8.92 (3.80-20.95)
>16.7		16 (13.1)	15 (3.0)	1.69 (0.59-4.82)	6.49 (2.62-16.05)
					$P_{interaction}$ = 0.01
Citrus fruit					
≤2.2	Negative	39 (32.0)	139 (28.2)	Reference	Reference
>2.2 to 7.2		11 (9.0)	147 (29.8)	0.31 (0.15-0.65)	0.31 (0.15-0.65)
>7.2		13 (10.7)	154 (31.2)	0.36 (0.17-0.76)	0.36 (0.17-0.76)
≤2.2	Positive	19 (15.6)	28 (5.7)	Reference	2.37 (1.13-5.0)
>2.2 to 7.2		19 (15.6)	12 (2.4)	3.55 (1.28-9.86)	8.41 (3.43-20.62)
>7.2		21 (17.2)	13 (2.6)	4.20 (1.53-11.51)	9.96 (4.16-23.83)
					$P_{interaction}$ < 0.001

*ORs adjusted for age, gender, race, pack-years of smoking, alcohol, total energy intake, BMI, and education.

Table 5. Association between presence of HPV DNA in tumors and fruit consumption

	HPV-16 DNA-positive tumors (n = 48), n (%)	HPV-16 DNA-negative tumors (n = 103), n (%)	OR (95% CI)*
Total fruit, servings/wk			
≤9.4	18 (37.5)	46 (44.7)	Reference
>9.4 to 16.7	17 (35.4)	28 (27.2)	1.01 (0.41-2.53)
>16.7	13 (27.1)	29 (28.2)	0.74 (0.26-2.05)
Citrus fruit, servings/wk			
≤2.2	19 (39.6)	43 (41.8)	Reference
>2.2 to 7.2	12 (25.0)	35 (34.0)	0.47 (0.18-1.25)
>7.2	17 (35.4)	25 (24.3)	0.97 (0.38-2.48)
Vitamin C			
Tertile 1	18 (37.5)	32 (31.1)	Reference
Tertile 2	14 (29.2)	37 (35.9)	0.43 (0.16-1.14)
Tertile 3	16 (33.3)	34 (33.0)	0.50 (0.19-1.31)

*ORs adjusted for age, gender, race, pack-years of smoking, alcohol, total energy intake, BMI, and education.

CI, 1.28-9.86; $OR_{tertile\ 3}$, 4.20; 95% CI, 1.53-11.51). Results of the joint effects analysis can be found in Table 4.

To further explore the interaction of HPV-16 and diet in HNSCC risk, we conducted a case-only analysis using data on detectable HPV-16 DNA in the tumors (Table 5). Of the 151 cases in the analysis, 48 (32%) had detectable HPV-16 DNA in their tumors and 103 (68%) did not. Overall, cases that had detectable HPV-16 DNA in tumors consumed less fruit, citrus fruit, and vitamin C. However, there were no statistical differences between fruit intake and having HPV-16 DNA in the tumors.

Analyses looking at total vegetable intake (P = 0.46) and green vegetable intake (P = 0.48) on HNSCC risk did not reveal a significant interaction with HPV-16 status (data not shown).

Discussion

HPV-16-seronegative individuals with greater fruit or greater citrus consumption had a significantly lower frequency of HNSCC than those with low fruit or low citrus intake; however, fruit intake appeared to increase risk of HNSCC among the HPV-16-seropositive. These findings remained significant when we analyzed the interactions among pharyngeal cancer cases, the HNSCC site most strongly associated with HPV-16. The divergence by HPV-16 serologic status was evident for vitamin C intake as well, but the interaction was not statistically significant, suggesting that vitamin C alone may not be responsible for this relationship.

In analyzing how dietary factors influenced HNSCC risk in our study population, we previously found no significant association between fruit consumption and HNSCC after adjustment for potential confounders (23). The results of the present analysis suggest that this observation was due, in part, to effect modification by HPV-16 serologic status (which might bias the effect of fruit toward the null). Given the existing literature on the relationship between fruit consumption and HNSCC, the observed interaction with HPV-16 was unexpected. However, it raises the issue that the inconsistent findings between overall fruit or citrus fruit and HNSCC risk in the literature may be partially explained by differences in the prevalence of HPV-16 exposure across populations. That is, populations with a lower prevalence of HPV-16 exposure may be more likely to observe a protective association between fruit and HNSCC, whereas populations with a higher prevalence of HPV-16 would be less likely to observe a reduced risk of HNSCC with fruit consumption.

Although we are unaware of similar studies conducted in HNSCC populations, the relationship between HPV-16 and fruit consumption has been investigated in subjects with HPV-related cervical cancer. One study reported a possible protective effect of increased fruit intake against persistent HPV infection (32). Fruits were also considered protective against invasive cervical cancer by an expert committee in 1998 (43). Interestingly, a recent case-control study reported an insignificant trend of increasing risk of *in situ* cervical cancer (P = 0.06) and invasive cancer (P = 0.89) with increasing intake of foods high in vitamin C for cases compared with controls (44). However, these investigators found no significant association between intake of foods high in vitamin C and incidence of *in situ* or invasive cervical cancer after adjustment for HPV. Overall, evidence relating dietary factors to development of cervical cancer has been inconsistent, and few studies have considered the relationship within strata of HPV status, because almost all cervical cancers are HPV-positive (32).

The relationship of nutrient intake with viral infections and immune system status has been well studied. The association of nutrition and vitamin intake with disease status in patients with HIV suggests that the behavior of the virus may be mediated at least in part by the nutritional status of its host (45, 46). One cross-sectional study reported that HIV-positive subjects on highly active antiretroviral therapy with high serum zinc levels had lower log viral load levels than subjects in the lowest quartile of serum zinc (47). Micronutrient deficiency has been linked to faster progression of the disease, particularly in HIV-positive women. A randomized, double-blind, placebo-controlled trial assessed the effect of giving vitamin supplements to pregnant women in Tanzania on the risk of vertical transmission and disease progression (48). The study found benefits from multivitamins on adverse pregnancy outcomes, lowered viral loads, and increased CD cell counts (46, 48).

Our data show a significant interaction between HPV-16 and fruit intake and the risk of HNSCC, although the mechanism is unclear. However, it appears that the observed association is not explained by correlation between fruit intake and other HNSCC risk factors. It is possible that the ingestion of acidic foods including citrus fruit may erode the mucosal lining of the gastrointestinal

tract, enhancing the likelihood of infection upon exposure to HPV-16. It is known that a higher proportion of inflammatory cells are observed in the buccal mucosa of smokers compared with nonsmokers (49) and that inflammation caused by tobacco and alcohol use could increase permeability of mucosal surfaces to virus particles. High fruit intake and acidic fruit in particular may combine with these established risk factors to cause mucosal wounding and allow the virus to better access the bloodstream.

It is also possible that nutrient intake modifies the immune response. The development of persistent infection with an oncogenic strain of HPV is associated with higher risk of cancer (50). Consequently, consumption of fruits might enhance the efficacy of the immune response to DNA damage-associated carcinogenic clonal expansion while paradoxically promoting a persistent serologically detectable response to HPV. Overall, it is unlikely that fruit consumption patterns are related to the risk of acquiring HPV, as fruit intake in our study did not differ by HPV status.

In addition, clinical research has shown that the consumption of grapefruit, grapefruit juice, or Seville orange juice may alter disease-associated metabolism (51-53). Consumption of these products impairs the metabolism of many prescription drugs, including components of highly active antiretroviral therapy (54); this is thought to be due primarily to interactions between the citrus fruits and drug-metabolizing enzyme cytochrome *P*4503A (53). The grapefruit flavonoid naringenin has also been reported to reduce secretion of hepatitis C virus in infected cells (55). These findings suggest that citrus fruit consumption may affect the body's response to viral infections and their treatment. However, a direct link between these modulations and cancer risk has not been established.

There are several methodologic issues that were taken into consideration for this population-based case-control analysis. Low control participation in this study (47% overall) could result in a control population that does not accurately represent the study base that gave rise to the cases, resulting in selection bias. Furthermore, 378 (35.1%) cases from the initial study population did not complete the FFQ compared with only 50 (6.3%) controls. It is possible that participating controls were more health-conscious than nonparticipants and may adhere to a healthier diet and lifestyle. Low control participation would not explain the observed associations unless participation was differential by HPV-16 status. Although we are unable to determine the extent of such differences, we do not believe that participation rates differed based on HPV-16 antibody status. The prevalence of HPV-16 among controls included in the final analysis is 10.8%. A similar estimate for the seroprevalence of HPV-16 in the general U.S. population ages 50 to 59 years (10.6%; 95% CI, 8.1-13.8%) was reported in a study using data from the third National Health and Nutrition Examination Surveys (NHANES III), suggesting that control participation rates were not determined differentially by HPV-16 status (56).

To further investigate the possible effect of selection bias, we compared the distribution of risk factors (age, gender, race, education, smoking status, and alcohol intake) between those cases included in the analysis and those who were not included. We found no significant differences in age, pack-years of smoking, alcoholic drinks per week, race, or education between the two groups, indicating that the potential for selection bias to account for these findings is small. The same analysis comparing the distribution of these risk factors between controls included in the analysis and those not included yielded the same conclusions.

There is always the possibility that unmeasured confounders or residual confounding could explain the observations reported in this paper. We further adjusted for potential confounding by socioeconomic status by including household income in the multivariate models. However, this addition did not change the effect estimates, and the variable was not retained in final models. Smoking status and alcohol consumption were assessed through self-reported questionnaire data and as such are vulnerable to bias despite detailed measurement. In order for such bias to affect our findings, our study population would have had to report smoking and alcohol consumption differentially by HPV-16 serologic status. Because participants did not initially know that HPV-16 serology would be determined, it is unlikely to have affected their reporting accuracy. Future analyses on this topic could explore the relationship between fruit consumption, HPV-16, and HNSCC in never-smokers to further reduce residual confounding by smoking and elucidate the etiologic importance of HPV-16. We were unable to restrict our analysis to nonsmokers and non-drinkers due to small sample sizes in our population.

In this analysis, dietary data were self-reported using a validated FFQ. Cases and controls were asked to report their diet 5 years before date of diagnosis or date of enrollment, respectively. This measure reduced the influence of recent diet changes due to progression of HNSCC. It is highly unlikely that HPV-16 status influenced a participant's ability to accurately recall fruit consumption; thus, we do not believe that recall bias could wholly account for our findings.

The results of our analysis underscore the importance of measuring HPV-16 status in studies of HNSCC. Our findings of an increased risk of HNSCC among HPV-positive individuals with increasing total fruit and citrus intake require confirmation in different populations. In particular, using nonsmokers or low drinkers as the reference group for future analyses would further reduce the influence of residual confounding and help elucidate the relationship with HPV-16-related HNSCC. Our data highlight the fact that it is important to account for HPV-16 status when studying HNSCC, as HPV-16-related HNSCC represents a distinct disease, and failure to do so may affect study results.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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