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MAJOR ARTICLE

Dietary Intake and Risk of Persistent Human Papillomavirus (HPV) Infection: The Ludwig-McGill HPV Natural History Study

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The association between dietary intake and persistence of type-specific human papillomavirus (HPV) infection, during a 12-month period, among 433 women participating in the Ludwig-McGill HPV Natural History Study was evaluated by use of a nested case-control design. Dietary intake was assessed by a food-frequency questionnaire at the month-4 visit. HPV status was assessed at months 0, 4, 8, and 12 by polymerase chain reaction (MY09/11). Only women who ever tested positive for HPV were included in the present study: 248 had transient HPV infections (1 of 4 positive tests or nonconsecutively positive), and 185 had persistent HPV infections (≥2 consecutive tests positive for the same HPV type). Risk of type-specific, persistent HPV infection was lower among women reporting intake values of β-cryptoxanthin and lutein/zeaxanthin in the upper 2 quartiles and intake values of vitamin C in the upper quartile, compared with those reporting intake in the lowest quartile. Consumption of papaya ≥1 time/week was inversely associated with persistent HPV infection.

Epidemiologic research conducted during the past 2 decades has shown that infection with the human papillomavirus (HPV) is a cause of most cases of cervical cancer [1]. Prospective studies have shown that women infected with HPV are more likely to develop cervical intraepithelial neoplasia (CIN) and that those with persistent oncogenic-type HPV infections are at a significantly increased risk of developing CIN, compared with women who are transiently infected [2, 3]. In addition, women who persistently test positive for HPV appear to be 4 times more likely to have persistent cervical lesions [4]. As a result, persistent HPV infection is considered to be a strong intermediate biomarker of risk of cervical cancer.

Although HPV infection is a cause of cervical cancer, it may be an insufficient cause, requiring the presence of other factors for the infection to progress to a significant cervical lesion. Nutritional status may be an important cofactor, affecting both persistence of HPV infection and progression of persistent HPV infection to CIN [5, 6]. However, the association between nutritional status and cervical carcinogenesis has not been adequately tested, since most nutritional epidemiological studies have had methodological limitations [7]. First, most studies were conducted before a reliable test for HPV status was available. Second, few studies controlled for potential confounders, such as smoking and oral contraceptive use. This is especially important when evaluating risk of cervical cancer associated with carotenoids, vitamin C, and folate, nutrients that appear to be influenced by other risk factors for cervical cancer. Finally, most studies used a retrospective design and, therefore, were unable to examine where in the carcinogenesis continuum nutrients were active.

Of the published case-control and cohort studies examining the association between nutritional factors and

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cervical cancer, most focused on assessing risk of higher grades of CIN and invasive cervical cancer, relatively later events in cervical carcinogenesis. Among the studies that included a dietary evaluation, several found inverse associations between consumption of dark green and yellow vegetables, β-carotene, and vitamins C and E and the risk of CIN and cancer [7].

Our recently published studies [8, 9] are the only at present that have examined the association between dietary intake and persistence of HPV infection. In a prospective study of US women attending a family planning clinic for routine care, we observed significant inverse associations between dietary intake of lutein, vitamin E, and vegetables and persistence of HPV infection [8]. However, this was a relatively small cohort, and an assessment of persistence of type-specific HPV infection was not possible, because of small numbers of participants. In the present study, we evaluated the association between dietary intake and persistence of type-specific HPV infection (≥2 consecutive tests positive for the same HPV type), during a 12-month period, among Brazilian women at high risk of cervical neoplasia participating in the Ludwig-McGill HPV Natural History Study.

PATIENTS, MATERIALS, AND METHODS

The on-going Ludwig-McGill HPV Natural History Study is an epidemiologic cohort investigation of women attending a comprehensive maternal and child health maintenance program catering to low-income families, in São Paulo, Brazil. The clinical setting where participants were recruited is part of a network of primary, secondary, and tertiary health care institutions maintained by the municipal health department. Cohort participants are examined every 4 months during the first year and twice yearly thereafter, for a total of 5 years [10]. As described elsewhere, the majority of women had only 1–2 sex partners during their lifetime.

The mean age at enrollment was 31 years (median age, 33 years) [3]. Each study participant signed an informed-consent document. All study procedures and the informed-consent document were approved by the institutional review boards and ethical committees of all institutions with which the authors are affiliated. Human-experimentation guidelines of the US Department of Health and Human Services and of the authors' institutions were followed.

Study sample. For the present study, a nested case-control design was used. A subcohort of 1392 women was selected, representing mostly those who entered the study during the first 2 years (1993–1995) and who received long-term follow-up. In the present study, HPV status is based on the 4 HPV evaluations conducted at enrollment and during the first year of follow-up. To study the association between dietary nutrients and persistence of HPV infection, only women who tested positive for HPV in any 1 of the 4 evaluations were included in this analysis. Two groups of women were identified: (1) those who tested positive for HPV in only 1 of the 4 evaluations or nonconsecutively positive for the same type (transient group) and (2) those who tested positive for the same HPV type at ≥2 consecutive visits during the first year of the study (persistent group). Of the 1392 women selected, 248 had transient HPV infection, and 185 had persistent HPV infection, during the first year of study. Complete follow-up compliance for this subcohort, at 12, 24, 36, and 48 months, has been 83%, 78%, 74%, and 71%, respectively. HPV results from this subcohort are complete for the first year of follow-up.

To test the hypothesis that diet is associated with persistent HPV infection, in this study, women with transient infections formed the control group, and women with persistent infections formed the case group. At each study visit, participants were interviewed on the basis of a structured questionnaire specific for the current visit and had cervical specimens taken for Pap smear cytologic testing and HPV testing. A 10-mL blood sample was drawn by venipuncture, in vacutainer tubes without coagulant.

Study questionnaires. Data from the baseline questionnaire were used in the present study. This questionnaire consisted of 107 questions, which included questions on sociodemographic characteristics, tobacco and alcohol consumption, menstrual status, sexually transmitted disease history, and gynecologic, sexual, contraceptive, and anal and oral intercourse history.

Diet questionnaire. Information on the frequency of consumption of selected food items and the consumption of vitamin and mineral supplements was obtained at the second visit (month 4). Participants were asked to recall the usual frequency of consumption, during the past 5 years, of the following 15 food items: oranges, lemons, carrots, pumpkin, papaya, cauliflower, spinach, broccoli, lettuce, other vegetables, eggs, milk and yogurt, cheese, butter, and liver. These foods contribute substantially to variation in intake of carotenoids and tocopherols, among Brazilian women living in São Paulo. Food consumption–frequency categories were as follows: never, <1 time/month, 2–3 times/month, 1–3 times/week, 4–6 times/week, and ≥1 time/day. A similar food-frequency questionnaire (FFQ) was used in an epidemiologic study conducted in the same region of Brazil, a study that produced strong evidence for the association between specific dietary intake and risk of oral cancer [11].

Nutrient values were calculated from the participants' reported dietary intake by use of the United States Drug Administration's Continuing Survey of Food Intake of Individuals (CSFII-86) and Nationwide Food Consumption Survey (NFCS 87–88), unless Brazil-specific nutrient values were available. When available, food carotenoid values (e.g., β-carotene, lutein/zeaxanthin, and

β-cryptoxanthin) were derived from published values for foods consumed in São Paulo, Brazil [12]. Dietary-intake calculations used age-specific portion sizes for women, as described elsewhere [13]. From these databases, nutrient values were obtained for vitamin A, carotenoids (α and β-carotene, β-cryptoxanthin, and lutein/zeaxanthin), and vitamin C.

Since the questionnaire used in this study was short, we examined the association between consumption of certain fruits and vegetables and corresponding serum concentrations of carotenoids, for 100 women in the subcohort. Using serum concentrations of carotenoid from the first visit, we observed strong associations ($r \geq 0.40$) between the consumption of citrus fruits and serum concentrations of lutein, zeaxanthin, *cis*-zeaxanthin, α-cryptoxanthin, lycopene, *cis*-lycopene, and α-carotene, and between the consumption of carrots and serum concentrations of lutein, α-cryptoxanthin, β-cryptoxanthin, lycopene, *cis*-lycopene, α-carotene, and β-carotene. These correlation coefficients are similar to those observed in studies comparing the intake of specific carotenoids estimated from FFQs and the corresponding serum concentrations [14–16]. In addition, we evaluated the crude and energy-adjusted [17] associations between intake of carotenoids and serum concentrations of carotenoids and found stronger associations when the energy-adjusted intake values for carotenoids were used (data not shown). The primary purpose of energy-adjustment of nutrient values was to adjust for the tendency of individuals to consistently under- or overreport intake values when a frequency checklist is used [17]. Therefore, all diet–HPV persistence analyses used energy-adjusted nutrient values.

Cervical cell specimens. At each of the visits, an accelon biosampler was used to collect a sample of ectocervical and endocervical cells. After the smear was prepared on a glass slide and fixed in 95% ethanol, the sampler containing exfoliated cells was immersed in a tube containing Tris-EDTA buffer (pH 7.4), was swirled to release the adhered cells, and was maintained at the clinic for, at most, 5 days at 4°C. Once they were brought to the laboratory at the Ludwig Institute, the tubes containing cell suspensions were frozen until testing.

HPV DNA detection methods—polymerase chain reaction (PCR) methods. All HPV analyses were performed at the Ludwig Institute for Cancer Research, São Paulo, Brazil. Cervical-specimen DNA was extracted and purified in accordance with standard techniques. In brief, cells were digested with 100 μg/mL proteinase K for 3 hours at 55°C, followed by organic extraction and ethanol precipitation. Specimens were tested for the presence of HPV DNA by a previously described PCR protocol amplifying a highly conserved 450-bp segment in the L1 viral gene (flanked by primers MY09/11) [18, 19]. Typing of the amplified products was performed by hybridization with individual oligonucleotide probes specific for all 27 HPV genital types whose nucleotide sequences for probes within the MY09/11 fragment have been published elsewhere [19], namely types 6/11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–59, 66, 68, 73, 82, 83, and 84 . The PCR amplification products were further tested by restriction fragment–length polymorphism (RFLP) analysis of the L1 fragment [20], to resolve dubious results from the dot-blot hybridization and to distinguish among HPVs that could not be typed by dot-blot hybridization with the specific probes. This allowed us to extend the detection range to >40 genital HPV types, including HPV types 32, 34, 44, 62, 64, 67, 69–72, CP6108, and IS39. Amplified products that hybridized with the generic probe but not with any of the type-specific probes and that also did not produce a recognizable band pattern in the RFLP analysis were considered to be positive for HPV of unknown types.

In this subcohort of women, 99 had persistent oncogenic infections, and 86 had persistent nononcogenic infections, during a 12-month period (data not shown). Among all women with persistent infections, 93 had 2 consecutive type-specific infections, 51 had 3 consecutive type-specific infections, and 41 tested positive for the same HPV type at all 4 clinical visits.

High-pressure liquid chromatography analysis of serum carotenoids and tocopherols. For the determination of serum concentrations of carotenoids and tocopherols, a modification of the procedures described by Craft et al. [21] was used. This system has the following lower limits of detection: 0.15 μg/mL for the tocopherols, 0.01 μg/mL for the carotenoids, and 0.02 μg/mL for retinyl palmitate. Samples below the detectable limit of the assay were assigned a value half-way between zero and the lower limits of detection.

Statistical analyses. To test the hypothesis that nutrient intake is associated with persistence of HPV infection, 2 groups were compared: women with persistent type-specific HPV infections ($n = 185$) and women with transient infections ($n = 248$). Quartiles of each nutrient were calculated on the basis of the nutrient distribution of women with transient HPV infection. Energy-adjusted nutrient intake values were computed as the residual values from the regression model with total energy intake (independent variable) and absolute nutrient intake (dependent variable) [17]. Several variables that could potentially confound the diet–HPV persistence association were considered, including those previously associated with persistence of HPV infection and dietary nutrient intake. Only those factors that altered the risk estimate by ⩾10% were retained in the final multivariate model (income, education, number of individuals in the household, smoking, number of sex partners during the past 5 years, age at first intercourse, and number of pregnancies). In addition, we evaluated energy as a covariate and found that it significantly contributed to the models. Logistic regression was performed to estimate the association (odds ratio [OR]) and 95% confidence interval (CI) of each nutrient or food group with both persistent and transient HPV

infection. Tests for trends were preformed by treating all categorical nutrient variables as continuous, in the multivariate logistic regression models. All statistical tests performed were 2-sided. Statistical analyses were performed by use of Intercooled STATA (version 7.0; Stata).

RESULTS

Dietary-intake values for women with transient and persistent HPV infection are reported in table 1. For both groups, the most commonly consumed carotenoid was β-carotene, followed by lutein/zeaxanthin, β-cryptoxanthin, and α-carotene. Compared with women who persistently tested positive for HPV, women with transient HPV infection had higher mean daily intakes of β-cryptoxanthin (177 vs. 149 μg; $P = .025$) and lutein/zeaxanthin (296 vs. 249 μg; $P = .03$). No differences in the mean daily intake of the methyl-donor nutrients (folate, vitamins B_{12} and B_6, methionine, and cystine) were observed.

The association between intake of carotenoids and vitamin C (unadjusted mean intake values) and risk factors for cervical cancer is presented in table 2. Intake of carotenoids was marginally ($P < .10$) associated with educational attainment and number of individuals living in the household. On average, women who were illiterate consumed ~30% less dietary carotenoids than those who completed a minimum of a high-school education. Lutein consumption was highest among women with fewer household members. Income and smoking were marginally ($P < .10$) associated with intake of vitamin C.

To develop a final statistical model with which to examine the independent associations between nutrient intake and persistence of HPV infection, we tested nonnutrient factors to find which were associated statistically with both exposure and outcome. The variables identified by this process were energy intake (Kcal), smoking, education, number of persons in a household, income, total number of sex partners during the past five years, and total number of pregnancies. The association between specific dietary nutrient intake and persistence of HPV infection, resulting from this final model, is reported in table 3. Increased intake of β-cryptoxanthin and lutein/zeaxanthin was significantly inversely associated with persistent HPV infection. Risk of persistent HPV infection was lower among women reporting intake values of β-cryptoxanthin in the upper 2 quartiles (quartile 3: adjusted OR [AOR], 0.48; 95% CI 0.27–0.87; quartile 4: AOR, 0.47; 95% CI 0.26–0.85), compared with reference intake values. Similarly, the upper 2 quartiles of dietary intake values of lutein/zeaxanthin were inversely associated with persistence of HPV infection (quartile 3: AOR, 0.44; 95% CI, 0.24–0.78; quartile 4: AOR, 0.49; 95% CI, 0.27–0.87). In addition, risk of persistent HPV infection was associated with intake of vitamin C (AOR, 0.50; 95% CI, 0.27–0.92 [highest vs. lowest quartile]).

Table 1. Unadjusted mean (SD) values of nutrient intake, by human papillomavirus (HPV) infection status.

Nutrient	Transient HPV infection (n = 248)	Persistent HPV infection (n = 185)	P[a]
Vitamin A, μg RE	1168.14 (843.21)	1158.74 (817.39)	.7749
Vitamin E, α-TE, mg	1.56 (0.72)	1.59 (0.71)	.6077
Vitamin C, ascorbic acid, mg	57.75 (34.71)	53.69 (33.19)	.1841
β-Carotene, μg[b]	979.64 (580.15)	995.85 (613.09)	.4303
α-Carotene, μg[b]	143.64 (143.15)	141.80 (138.90)	.8819
β-Cryptoxanthin, μg[b]	176.96 (205.25)	149.33 (195.32)	.0245
Lutein plus zeaxanthin, μg[b]	296.38 (348.68)	248.88 (331.09)	.0303
Folate, μg	107.33 (56.24)	105.26 (53.25)	.8727
Vitamin B-12, μg	5.07 (5.11)	4.80 (4.87)	.7145
Vitamin B-6, mg	0.28 (0.13)	0.28 (0.12)	.9583
Methionine, g	0.38 (0.17)	0.39 (0.16)	.5589
Cystine, g	0.18 (0.08)	0.18 (0.08)	.4197

NOTE. Means are presented as untransformed values. RE, retinol equivalent; TE, tocopherol equivalent.

[a] One-way analysis of variance of log-transformed energy-adjusted nutrient intake values.

[b] Nutrient intake based on Brazilian values [12].

The association between consumption of selected fruits and vegetables and persistent type-specific HPV infection is shown in table 4. Consumption of papaya reported as >1 time/week was inversely associated with persistence of HPV infection (AOR, 0.30; 95% CI, 0.14–0.64). Risk of persistence of HPV infection was also marginally reduced among women consuming oranges $\geq$1 time/week (AOR, 0.51; 95% CI, 0.24–1.06).

DISCUSSION

The present study is the first study to evaluate the association between dietary consumption of selected nutrients and foods and persistence of type-specific HPV infection, a strong biomarker of risk of cervical cancer. In this population of Brazilian women at high risk of cervical neoplasia, dietary intake of lutein/zeaxanthin, β-cryptoxanthin, and vitamin C were significantly independently associated with reduced risk of type-specific, persistent HPV infection. Correspondingly, consumption of papaya, a major source of dietary carotenoids, was associated with decreased risk of persistent infection.

It is now well accepted that HPV is a cause of cervical cancer and that other factors may be related to cervical cancer, but these need to be understood within the context of the natural history of HPV infection. At present, most studies examining dietary factors have not been designed to evaluate nutrients within this context, since most were retrospective studies with either CIN II/III or cancer as the case groups. In our earlier studies, we evaluated the role of dietary antioxidants and risk of persistent HPV infections among US women [8]. Because

Table 2. Distribution of unadjusted mean intake of carotenoids, according to risk factors for cervical cancer among women with transient and persistent human papillomavirus infections.

Variable	No.	β-Carotene, μg	α-Carotene, μg	β-Cryptoxanthin, μg	Lutein plus zeaxanthin, μg	Vitamin C, mg
Ethnicity						
White	267	1002.7	144.1	164.5	275.1	55.9
Nonwhite	165	962.8	140.7	166.8	278.7	56.3
Education						
Illiterate	24	858.8	121.2[a]	145.9	242.6	52.6
<Elementary school	65	877.3	110.3	140.8	234.1	45.7
Elementary school	244	993.1	147.3	167.3	279.5	56.6
<High school	52	1010.1	124.2	154.3	258.9	54.0
⩾High school	47	1150.8	196.5	211.8	356.2	71.7
No. of persons living in household						
1–2	31	1121.5	126.4	200.7	335.4[a]	64.1
3	69	848.9	113.2	173.6	291.0	52.7
4	102	1005.2	149.3	192.1	322.2	63.2
5	83	1026.5	147.3	159.7	267.6	59.1
6	61	970.1	144.3	117.2	193.1	49.6
⩾7	83	996.5	157.1	149.4	249.0	48.4
Monthly income, US$						
<240	211	907.3	139.0	156.4	261.1	55.9[a]
240–379	211	995.0	152.7	160.7	268.4	54.6
380–659	194	988.7	143.2	143.2	239.5	51.4
660–2985	204	1163.7	226.9	226.9	380.9	64.1
Cigarette smoking						
Never	198	972.3	134.8	176.3	295.2	58.7[a]
Current	162	974.8	146.2	144.5	241.2	50.1
Former	72	1057.7	157.5	182.2	304.5	61.0
Age at first sexual intercourse, years						
⩽15	130	927.1	129.0	162.3	271.1	62.1
16–17	116	971.4	142.7	158.6	267.2	56.4
18–19	93	1043.4	156.7	145.1	241.9	55.2
20–50	93	1035.8	148.4	198.5	332.6	52.3
No. of sex partners during lifetime						
0–1	155	1004.1	138.9	160.5	268.2	59.2
2–3	167	1000.4	149.6	162.8	271.9	54.3
⩾4	110	944.2	138.1	176.2	295.0	54.3
No. of sex partners during past 5 years						
0–1	282	1023.2	153.7	164.8	275.3	57.5
⩾2	150	920.2	122.4	166.4	278.6	53.3
No. of sex partners during past year						
0–1	385	1006.7	147.1	165.9	277.2	56.9
⩾2	42	866.0	114.0	160.9	269.4	48.6
Oral contraceptive use						
Never	70	1042.9	142.1	183.0	305.6	58.5
<6 years	247	965.0	139.4	149.5	249.8	53.5
⩾6 years	115	1002.0	150.6	188.8	316.0	60.1
No. of pregnancies						
0–1	84	981.9	137.1	135.9	226.7	54.9
2–3	177	948.1	138.9	175.5	294.4	58.5
4–6	123	1075.2	156.9	176.1	293.5	56.8
⩾7	44	904.4	132.8	154.4	257.4	47.9

[a] $P < .10$; 1-way analysis of variance of log-transformed, energy-adjusted nutrient-intake values.

Table 3. Association between energy-adjusted intake of carotenoids and antioxidant vitamins and persistence of type-specific human papillomavirus infection.

Nutrient	Infection, no.		Crude OR (95% CI)	Adjusted OR (95% CI)[a]
	Transient	Persistent		
Carotenoids				
β-Carotene, μg[b]				
Quartile 1	64	53	1.00	1.00
Quartile 2	60	50	1.00 (0.60–1.70)	0.94 (0.54–1.66)
Quartile 3	62	34	0.66 (0.38–1.15)	0.62 (0.34–1.13)
Quartile 4	62	48	0.93 (0.55–1.58)	0.83 (0.47–1.46)
α-Carotene, μg[c]				
Quartile 1	62	42	1.00	1.00
Quartile 2	63	52	1.22 (0.71–2.08)	1.13 (0.63–2.03)
Quartile 3	62	43	1.02 (0.59–1.78)	1.02 (0.56–1.86)
Quartile 4	61	48	1.16 (0.67–2.00)	1.10 (0.61–1.99)
β-Cryptoxanthin, μg[d]				
Quartile 1	61	61	1.00	1.00
Quartile 2	62	44	0.71 (0.42–1.20)	0.60 (0.33–1.09)
Quartile 3	62	39	0.63 (0.37–1.07)	0.48 (0.27–0.87)
Quartile 4	63	41	0.65 (0.38–1.11)	0.47 (0.26–0.85)
Lutein plus zeaxanthin, μg[e]				
Quartile 1	61	63	1.00	1.00
Quartile 2	62	43	0.67 (0.40–1.13)	0.58 (0.32–1.05)
Quartile 3	62	36	0.56 (0.33–0.97)	0.44 (0.24–0.78)
Quartile 4	63	43	0.66 (0.39–1.11)	0.49 (0.27–0.87)
Antioxidant vitamin				
Vitamin C, ascorbic acid, mg[f]				
Quartile 1	61	54	1.00	1.00
Quartile 2	60	40	0.75 (0.44–1.30)	0.63 (0.35–1.15)
Quartile 3	65	53	0.92 (0.55–1.54)	0.84 (0.47–1.48)
Quartile 4	62	38	0.69 (0.40–1.19)	0.50 (0.27–0.92)

NOTE. CI, confidence interval; OR, odds ratio.

[a] Logistic-regression model adjusted simultaneously for energy intake (Kcal), income, education, no. of persons in household, smoking, no. of sex partners during past 5 years, and total no. of pregnancies.

[b] $P = .307$, test for trend.

[c] $P = .843$, test for trend.

[d] $P = .007$, test for trend.

[e] $P = .007$, test for trend.

[f] $P = .066$, test for trend.

of the small size of the cohort in the latter study, we were unable to assess associations with type-specific infections and were limited to a relatively short period of follow-up. Results of the present study of Brazilian women are consistent with our previous findings, which demonstrate an inverse association between lutein consumption and persistence of HPV infection. In addition, among Brazilian women, higher consumption of β-cryptoxanthin and vitamin C appeared to be associated with decreased risk of persistent infection.

The mechanism by which carotenoids might prevent cervical cancer remains unclear. However, carotenoids and vitamin C have a multitude of effects that may be chemopreventive. It is possible that the observed effects with β-cryptoxanthin and lutein are due to their pro–vitamin A activity; however, few studies have found an association between consumption of vitamin A and risk of cervical neoplasia [6, 7]. Alternatively, these compounds may be chemopreventive because of their activity as antioxidants. As antioxidants, carotenoids and vitamin C have been shown to quench reactive oxygen species (ROS). These highly reactive compounds can lead to cellular damage, disregulate cell signaling, and increase viral replication and viral expression [22]. Carotenoids and vitamin C may not only quench ROS but may also potentiate host cellular and humoral immunity [23]. The examples from human immu-

Table 4. Association between consumption of select fruits and vegetables and persistent type-specific human papillomavirus (HPV) infection.

Fruit/vegetable, frequency	Infection, no.		Crude OR (95% CI)	Adjusted OR (95% CI)[a]
	Transient	Persistent		
Carrots[b]				
Never or <1 time/year	14	14	1.00	1.00
⩾1 time/year, <1 time/month	32	13	0.41 (0.15–1.08)	0.30 (0.10–0.87)
1–3 times/month	53	41	0.77 (0.33–1.80)	0.63 (0.26–1.55)
⩾1 time/week	149	117	0.78 (0.36–1.71)	0.66 (0.28–1.54)
Pumpkin[c]				
Never or <1 time/year	57	35	1.00	1.00
⩾1 time/year, <1 time/month	79	61	1.26 (0.73–2.15)	1.14 (0.64–2.05)
1–3 times/month	59	44	1.21 (0.68–2.16)	1.12 (0.60–2.09)
⩾1 time/week	53	45	1.38 (0.78–2.47)	1.13 (0.60–2.14)
Papaya[d]				
Never or <1 time/year	20	26	1.00	1.00
⩾1 time/year, <1 time/month	45	31	0.53 (0.25–1.11)	0.43 (0.19–0.97)
1–3 times/month	62	53	0.66 (0.33–1.31)	0.52 (0.24–1.14)
⩾1 time/week	121	75	0.48 (0.25–0.91)	0.30 (0.14–0.64)
Spinach[e]				
Never or <1 time/year	146	107	1.00	1.00
⩾1 time/year, <1 time/month	50	34	0.93 (0.56–1.53)	0.89 (0.51–1.54)
⩾1 time/month	52	44	1.15 (0.72–1.85)	0.99 (0.60–1.66)
Broccoli[f]				
Never or <1 time/year	105	76	1.00	1.00
⩾1 time/year, <1 time/month	52	37	0.98 (0.59–1.64)	0.93 (0.52–1.65)
1–3 times/month	59	55	1.29 (0.80–2.06)	1.10 (0.66–1.86)
⩾1 time/week	32	17	0.73 (0.38–1.42)	0.53 (0.24–1.12)
Oranges[g]				
Never or <1 time/month	18	24	1.00	1.00
1–3 times/month	37	32	0.65 (0.30–1.40)	0.79 (0.34–1.81)
⩾ 1 time/week	193	129	0.50 (0.26–0.96)	0.51 (0.24–1.06)

NOTE. CI, confidence interval; OR, odds ratio.

[a] Logistic regression model adjusted simultaneously for energy intake (Kcal), income, education, no. of persons in household, smoking, no. of sex partners during past 5 years, and total no. of pregnancies.

[b] $P = .683$, test for trend.

[c] $P = .755$, test for trend.

[d] $P = .004$, test for trend.

[e] $P = .918$, test for trend.

[f] $P = .387$, test for trend.

[g] $P = .036$, test for trend.

nodeficiency virus (HIV) and influenza virus indicate a role for antioxidants, in particular nutrient antioxidants, in the down-regulation of viral replication and expression. ROS appear to play a central role in cell signaling, by activating transcription factors activator protein–1 (AP-1) and NF-κB, cell proliferation, and apoptosis [24]. In animal and in vitro models, ROS increased viral titer [25] and the infectivity of the influenza virus [26]. Administration of antioxidants to animals infected with the influenza virus protected them from the lethal effects of influenza [27]. In vitro, increases in the cellular oxidant load have been shown to increase the replication of HIV [25, 28]. This effect is thought to be due to the fact that ROS activate NF-κB, a nuclear transcriptional factor that is obligatory for HIV replication [28]. In vitro studies have consistently demonstrated inhibition of NK-κB activation by the antioxidants N-acetylcysteine and pyrrolidine-dithiocarbamate (PDTC) and nutrient antioxidants [29].

Evidence is accumulating to suggest that ROS, and their down-regulation by antioxidants, may work in a similar manner in HPV infection. Activation of the transcriptional factor AP-1, a central transcription factor for the expression of the oncoproteins E6 and E7 of the oncogenic-type HPVs [30, 31], has been

shown to be inhibited by antioxidants in in vitro assays. Using an HPV-16–immortalized human keratinocyte culture, Rösl et al. [32] have demonstrated that PDTC selectively suppressed AP-1–induced HPV-16 gene expression. These authors suggested that manipulation of the redox potential may be a novel therapeutic approach to interfere with the expression of oncogenic HPVs.

In addition to the effects of the oxidant-antioxidant balance, on target cells and cell signaling, this balance is an important determinant of immune cell function, affecting maintenance of immune cell membrane lipids, controlling signal transduction, as well as gene expression of immune cells [23, 33, 34], events important to the loss of HPV infection and CIN regression.

As with any epidemiological study, there are limitations and strengths to the present study, which must be considered in interpreting results. The questionnaire used to assess usual diet contained only 15 items and, as a result, has not captured the full range of nutrient intake in this population. For example, the observed intake of vitamin E was very low in this population, compared with observed values obtained by use of an extensive FFQ among US women (data not shown). Nonetheless, there is evidence [35] that even an incomplete index can account for a substantial proportion of variability in nutrient intake. In addition, the use of an unweighted fruit and vegetable index can be a substantial predictor of serum concentrations of at least some carotenoids and tocopherols, as we have demonstrated. Potential misclassification of dietary intake, due to measurement error and inaccurate recall, was also possible. Recall of diet may have been biased, although women were unaware of their HPV status at the time that they completed the FFQ, and knowledge of any possible association between diet and HPV status is unlikely to have been substantial. Interviewer bias was minimized, since interviewers were unaware of a woman's HPV status at the time of a clinical visit. Another potential limitation of this study is that the present study included women with prevalent infections at enrollment, which limits our ability to calculate duration of infection. Since 12 months is the average duration of an oncogenic infection, it is difficult to distinguish between long-term (i.e., >12 months) and short-term persistence, in this study. Despite these limitations, the Ludwig-McGill HPV Natural History Study offered a unique opportunity to assess the association between dietary consumption and persistence of HPV infection in a large, well-characterized cohort, with multiple measures of type-specific HPV infection during a 12-month period. To increase accuracy in estimating intake of carotenoids in this population, Brazil-specific carotenoid food-content values were used.

In conclusion, increasing dietary intake of lutein/zeaxanthin, β-cryptoxanthin, and vitamin C appear to be associated with reduced risk of persistence of type-specific HPV infection, a strong determinant of risk of cervical cancer. Correspondingly, consumption of papaya, a major source of dietary carotenoids, was associated with reduced risk of persistent infection. These results suggest that, among populations with low levels of intake of antioxidant nutrients, increasing dietary consumption of certain fruits may confer protection against cervical neoplasia.

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