Vitamin A, Carotenoids, and Risk of Persistent Oncogenic Human Papillomavirus Infection[1]

Rebecca L. Sedjo, Denise J. Roe, Martha Abrahamsen, Robin B. Harris, Neal Craft, Susie Baldwin, and Anna R. Giuliano[2]

Arizona Cancer Center [R. L. S., D. J. R., M. A., R. B. H., S. B., A. R. G.], Arizona College of Public Health [D. J. R., R. B. H., A. R. G.], and Obstetrics and Gynecology [S. B.], University of Arizona, Tucson, Arizona 85724, and Craft Technologies, Inc., Wilson, North Carolina 27893 [N. C.]

Abstract

Oncogenic human papillomavirus (HPV) infection is the main etiologic factor for cervical neoplasia, although infection alone is insufficient to produce disease. Cofactors such as nutritional factors may be necessary for viral progression to neoplasia. Results from previous studies have suggested that higher dietary consumption and circulating levels of certain micronutrients may be protective against cervical neoplasia. This study evaluated the role of vitamin A and carotenoids on HPV persistence comparing women with intermittent and persistent infections. As determined by the Hybrid Capture II system, oncogenic HPV infections were assessed at baseline and at approximately 3 and 9 months postbaseline. Multivariate logistic regression analysis was used to determine the risk of persistent HPV infection associated with each tertile of dietary and circulating micronutrients. Higher levels of vegetable consumption were associated with a 54% decrease risk of HPV persistence (adjusted odds ratio, 0.46; 95% confidence interval, 0.21–0.97). Also, a 56% reduction in HPV persistence risk was observed in women with the highest plasma *cis*-lycopene concentrations compared with women with the lowest plasma *cis*-lycopene concentrations (adjusted odds ratio, 0.44; 95% confidence interval, 0.19–1.01). These data suggest that vegetable consumption and circulating *cis*-lycopene may be protective against HPV persistence.

Introduction

HPV[3] infection causes most, if not all, invasive cervical cancers (1, 2). Whereas numerous types of HPV infect the anogenital tract, the development of cervical cancer has been associated with only a few specific genital oncogenic types (1). HPV infection is common among young women; however, most HPV infections appear to be transient (3, 4), with persistent oncogenic infections conferring the highest risk for cervical dysplasia and cancer (5–7). This high prevalence of HPV infection relative to the low incidence of cervical intraepithelial neoplasia and cervical cancer suggests that HPV infection alone may be insufficient to produce disease. Factors that may contribute to the progression of HPV infection to neoplasia include OC use, cigarette smoking, infection with other sexually transmitted diseases, immunosuppression, and nutrient status (8).

Previous case-control studies have suggested that specific dietary or circulating micronutrients including vitamin A and the carotenoids may be protective against cervical neoplasia (9–42). However, due to inconsistent results from these investigations, definitive roles for these nutrients in cervical neoplasia have been elusive. Only two studies, both using circulating nutrient measures, have addressed whether these nutrients were associated with HPV persistence, an early carcinogenic event (30, 31). In the study of Giuliano *et al.* (30), lower serum levels of β-carotene, β-cryptoxanthin, and lutein were associated with HPV persistence. In contrast, Palan *et al.* (31) found no association in mean levels of circulating retinol, α-carotene, β-carotene, and lycopene when women with persistent HPV infection were compared with women with intermittent or no HPV infection. Both studies (30, 31) had relatively small sample sizes ($<$125 women), and their analyses included women who were HPV negative, women not at risk for developing a persistent infection. No previous study has reported on the association between dietary intake of these nutrients and oncogenic HPV persistence risk.

To expand on our previous findings (30), we examined whether dietary and circulating concentrations of vitamin A and specific carotenoids were associated with HPV persistence in the Young Women's Health Study, a study with a larger sample of women followed over a longer time period. This study tested the hypothesis that higher dietary levels of vitamin A (retinol) and the carotenoids (α-carotene, β-carotene, lutein, and lycopene) or circulating levels of retinol and the carotenoids (α-carotene, *trans*-β-carotene, *cis*-β-carotene, lutein, zeaxanthin, α- and β-cryptoxanthin, *trans*-lycopene, and *cis*-lycopene) reduce the risk of HPV persistence.

Materials and Methods

The Young Women's Health Study was a prospective cohort study whereby participants were examined at baseline and approximately 3 and 9 months postbaseline. Details of this

Received 7/27/01; revised 4/30/02; accepted 5/14/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported in part by grants from the Arizona Disease Control Research Commission (Contracts 9710 and 10004). R. L. S. was supported by a National Cancer Institute Pre-Doctoral Cancer Prevention Fellowship (R25-CA78447). A. R. G. was supported in part by a National Cancer Institute Career Development Award (K07-CA60885).
[2] To whom requests for reprints should be addressed, at Arizona Cancer Center, 1515 North Campbell Avenue, Room 4977 C, Tucson, AZ 85724-5024. Phone: (520) 626-3341; Fax: (520) 626-33343; E-mail: agiuliano@azcc.arizona.edu.
[3] The abbreviations used are: HPV, human papillomavirus; AOR, adjusted odds ratio; CI, confidence interval; RLU, relative light unit(s); AFFQ, Arizona Food Frequency Questionnaire; USDA, United States Department of Agriculture; BMI, body mass index; AP-1, activator protein 1; OC, oral contraceptive.

cohort have been published previously (43). The University of Arizona's Human Subjects Committee approved all study procedures. Before entry, all participants signed an informed consent form.

Subjects. From September 1996 to August 1999, all healthy women receiving routine gynecological care at a reproductive health care clinic were approached by a study interviewer for participation in the Young Women's Health Study. One thousand and forty-two women who met the following eligibility criteria were enrolled on study: (*a*) age, 18–35 years; (*b*) currently sexually active or seeking birth control; (*c*) resident of the Tucson metropolitan area; (*d*) no treatment for cervical intraepithelial neoplasia within the last 18 months; (*e*) no abnormal Pap smear in the last 18 months; (*f*) no history of chronic illness; (*g*) not currently pregnant and more than 2 months postpartum; (*h*) still having menstrual periods (*i.e.*, no hysterectomy); and (*i*) no relocation plans over the next 12 months. At the baseline visit, each participant completed an extensive self-administered questionnaire regarding demographics, tobacco history, sexual behaviors, and reproductive history. During each participant's routine gynecological examination, exfoliated cervical cells for HPV analysis were collected after Pap smear sample collection.

Three hundred and forty-six women with a baseline Pap smear of normal or abnormal squamous cells of underdetermined significance were enrolled into the first follow-up visit of this study conducted at approximately 3 months postbaseline. Of the 346 women who completed at least one follow-up visit, 187 (54%) were positive for an oncogenic HPV infection at baseline, and the remainder were HPV negative. Furthermore, 206 women completed the third study visit conducted at approximately 9 months postbaseline. Whereas the study visits were scheduled for 3 and 9 months postbaseline, the actual median follow-up period was 10 months (range, 6–18 months). Participants were monetarily compensated ($50) for their participation in each of the follow-up visits.

HPV Analysis. HPV status at the baseline and follow-up visits was determined using Hybrid Capture II (Digene Corp., Beltsville, MD) high-risk probe (Probe B) to detect oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Positive samples were defined as those with a ratio of RLU of sample:RLU of a positive control of $\geq$1. Repeat testing was conducted on samples with equivocal readings (range, 1–2 RLU), with results of $\geq$1 RLU classified as positive. Samples with repeated values of $<$1 RLU were analyzed a third time and classified based on the results of the two concordant results. Approximately 1 pg viral DNA/ml sample can be detected by this method (44).

The objective of this investigation was to test the association between either dietary ($n = 201$) or circulating ($n = 159$) micronutrients and HPV persistence. Therefore, only participants who tested positive for a HPV infection and had a least one follow-up visit were included in these analyses. Women who were positive for any oncogenic HPV infection at two or more consecutive time points (dietary $n = 131$, plasma $n = 101$) were categorized as having a persistent infection, whereas women who were positive for any oncogenic type infection at one or more nonconsecutive time points were classified as having intermittent infections (dietary $n = 70$, plasma $n = 58$). Women who tested negative for HPV infection until the last clinic visit and then tested positive (dietary $n = 24$, plasma $n = 22$) and women who were consistently HPV negative (dietary $n = 121$, plasma $n = 100$) were excluded from the analyses.

Dietary Intake Analysis. Trained interviewers administered the AFFQ at the 3 month visit. The AFFQ is a modification of the dietary portion of the Block National Cancer Institute Health Habits and History Questionnaire (45) and includes foods common to the Southwestern United States (*i.e.*, salsa and burritos) as well as low-fat food items. Vitamin supplement duration, dosage, and brand are included. This optically scannable, semiquantitative questionnaire measured participants' usual intake by asking questions regarding the frequency and portion size of 153 food items over the previous 3 months. The validity and reliability of this questionnaire have been investigated through comparisons with four 24-h food recalls, as well as repeated administration after 1 year of both AFFQ and four 24-h recalls (46) or four dietary records (47) in two different prospective studies.

Nutrient values were calculated from the participants' reported dietary and supplement intake using the USDA's Continuing Survey of Food Intake of Individuals (CSFII-86) and National Food Consumption Survey (NFCA 87–88). Carotenoids were updated from the USDA-Nutrition Coordinating Center (University of Minnesota) Carotenoid Database (1998). The dietary calculations used age-specific portion sizes for women. From these databases, nutrient values were obtained for vitamins A and the carotenoids (α- and β-carotene, lutein, and lycopene).

Circulating Nutrient Measures. At the 3 month clinic visit, fasting blood samples were collected for quantitative assessment of circulating nutrient values by venipuncture using Monoject evacuated tubes containing EDTA (Sherwood Medical, St. Louis, MO). All samples were processed within 1 h of collection and stored at -80°C until analyses were conducted.

Plasma samples were obtained from 281 participants (81%) at the 3 month study visit. Plasma samples were not obtained from 65 participants because the phlebotomist was unable to find a vein, only a small amount of blood was obtained, or the participant refused. Participants with and without a sample were comparable on age, race, education, marital status, and cigarette smoking but were significantly different based on BMI ($P = 0.02$).

Nutrient analyses were conducted using a modification of the procedure described by Craft (48) for reverse phase high-pressure liquid chromatography (Craft Technologies, Inc., Wilson, NC). This assay provided a quantitative measure of circulating concentrations of retinol and nine carotenoids (α-carotene, *trans*-β-carotene, *cis*-β-carotene, lutein, zeaxanthin, α- and β-cryptoxanthin, *trans*-lycopene, and *cis*-lycopene). Briefly, 150-μl aliquots of plasma were diluted with 150 μl of water. Samples were then deproteinated with 300 μl of ethanol containing tocol as an internal standard. Samples were extracted twice with 1 ml of hexane, and the combined supernatant was evaporated under nitrogen. The residue was dissolved in 35 μl of ethyl acetate and then diluted with 100 μl of mobile phase and ultrasonically agitated for 15 s before placement in the autosampler. A 15-μl volume was injected into the chromatographic system for analysis. The relative SD for pooled controls ranged from 3–10%.

Statistical Analysis. Nutrient intake levels were categorized into tertiles based on the distribution observed in the intermittent group. Bivariate analyses were conducted for baseline demographic variables and cervical neoplasia risk factors determined from the literature, as well as variables that would account for under- and over-reporting of dietary intake such as energy and BMI. χ^2 analyses for categorical variables and independent *t* tests or analysis of variances for continuous

variables were used. Non-nutrient risk factors associated with both HPV persistence and any dietary or circulating nutrient at $P \leq 0.2$ were assessed in multivariate logistic models. To maximize statistical power, only those variables associated with both nutrient status and HPV persistence were retained as covariates using multivariate analysis. Two independent multivariate logistic regression models were developed for the risk of HPV persistence with either dietary ($n = 201$) or circulating ($n = 159$) nutrients using backwards stepwise elimination. The final dietary nutrient model included the significant variables at $P < 0.05$ (age at first intercourse, marital status, and BMI), as well as age to be consistent with the literature. The final plasma nutrient model included only one significant variable $P < 0.05$ (race), as well as age, BMI, and cigarette smoking ($P = 0.052$) to be consistent with the literature. Due to the high intercorrelation of the nutrients, the risk of HPV persistence was estimated individually for each nutrient. Multivariate logistic regression modeling of HPV persistence was performed to obtain an estimate of the association (AOR) and 95% CIs. Treating the categorical nutrient variables as continuous variables in multivariate logistic regression models assessed linear trends. Pearson correlation coefficients were estimated between the dietary or plasma nutrient levels using the log-transformed values because these data were highly skewed. All statistical tests performed were two-sided with an α level of 0.05 and were performed using Intercooled STATA (Stata Statistical Software, Release 6.0; Stata Corp., College Station, TX).

Results

The demographic and cervical neoplasia risk factor characteristics of participants with either intermittent or persistent HPV infection are presented in Table 1. At the baseline interview, the majority of this cohort was unmarried, 22 years of age or older, white, and had completed some college. Approximately half of the participants reported smoking more than 100 cigarettes during their life-times, 69% of participants had 5 or more lifetime number of sexual partners, and 55% had initiated sexual activity before 17 years of age. The majority of this cohort were current users of OCs or had used them in the past. Seventy-seven percent of participants reported never giving birth.

Table 1 Demographic and risk factor characteristics of study participants

	n	Percentage
Age (yrs)		
≤22	95	47.26
23–25	41	20.40
≥26	65	32.34
Race		
White	160	79.60
Hispanic	31	15.42
Other	10	4.98
Education		
≤High school/vocational	57	28.36
≥Some college	144	71.64
Marital status		
Single	141	70.15
Married	17	8.46
Cohabiting	23	11.44
Divorced/separated	20	9.95
BMI		
<21.46	80	39.40
21.46–23.40	47	23.38
>23.40	74	36.82
Cigarette smoking		
Never	99	49.25
Ever	102	50.75
Age at 1st intercourse (yrs)		
≤16	63	31.3
17–18	80	39.8
≥19	58	28.9
Lifetime no. of sexual partners		
1	10	5.03
2–4	51	25.63
5–9	66	33.17
≥10	72	36.18
Parity		
None	158	78.6
1–6	43	21.4
OC use		
Never	29	14.43
Current	83	41.29
Past	89	44.28

The unadjusted mean values of dietary and circulating nutrients by case-control status are reported in Table 2. No significant differences were observed in dietary intake for total energy, total fat, protein, carbohydrates, vitamin A, α-carotene, β-carotene, lutein, and lycopene when participants with persistent and intermittent HPV infections were compared. Dietary α-carotene, β-carotene, lutein, and lycopene were intercorrelated with Pearson correlation coefficients ranging between $r = 0.41$ ($P < 0.01$) and $r = 0.84$ ($P < 0.01$; data not shown). Similarly, no significant differences were observed in circulating concentrations for retinol, α-carotene, *trans*-β-carotene, *cis*-β-carotene, lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, *trans*-lycopene, and *cis*-lycopene when participants with persistent and intermittent HPV infections were compared. Plasma carotenoid concentrations were intercorrelated with Pearson correlation coefficients ranging from 0.10 ($P = 0.22$) for *cis*-β-carotene and *cis*-lycopene to 0.90 ($P < 0.01$) for *trans*-lycopene and *cis*-lycopene (data not shown). Correlations were low between dietary and circulating measures (range, $r = 0.18$ to $r = 0.43$; data not shown).

Associations between fruit, vegetable, and juice intake and HPV persistence are reported in Table 3. Vegetable consumption was significantly inversely associated with HPV persistence (AOR, 0.46; 95% CI, 0.21–0.97) when participants in the lowest and highest tertiles were compared. Juice consumption was marginally inversely associated with HPV persistence (AOR, 0.46; 95% CI, 0.19–1.06).

The association between dietary vitamin A, dietary carotenoids, and HPV persistence is reported in Table 4. Lutein intake above reference levels was associated with a decrease in HPV persistence risk of 50–63% (second tertile AOR, 0.37; 95% CI, 0.13–0.82; third tertile AOR, 0.50; 95% CI, 0.24–1.07).

Unadjusted and multivariate adjusted associations between circulating retinol, carotenoids, and HPV persistence are reported in Table 5. Higher circulating levels of *cis*-lycopene were associated with linear decreased risk of HPV persistence in both unadjusted and adjusted models (P for trend = 0.046). The highest tertile of circulating *cis*-lycopene was associated with a 56% reduction in HPV persistence risk compared with women in the lowest tertile (AOR, 0.44; 95% CI, 0.19–1.01). A nonsignificant linear increase in HPV persistence risk was observed with increased plasma *cis*-β-carotene levels (AOR, 2.00; 95% CI, 0.87–4.61).

Table 2 Unadjusted nutrient mean[a] values of nutrient intake by case-control status

	Intermittent HPV infection		Persistent HPV infection		*P*
	Mean	SD	Mean	SD	
Dietary nutrients					
Macronutrients[b]					
Total energy (kcal)	2370	1310	2219	1013	0.665
Total fat (g)	73.5	47.2	69.9	39.6	0.771
Protein (g)	92.7	54.5	90.0	45.2	0.925
Carbohydrates (g)	337.8	186.7	307.8	149.5	0.413
Dietary intake of micronutrients (foods only[b])					
Preformed vitamin A (RE)	498.7	369.7	573.1	459.1	0.457
α-Carotene (μg)	520.6	672.0	689.3	1605.9	0.994
β-Carotene (μg)	3329.5	2551.3	3766.0	4589.6	0.640
Lutein (μg)	2180.1	2154.9	2324.1	3399.9	0.289
Lycopene (μg)	4278.0	4445.1	4415.6	3946.9	0.691
Plasma nutrients[c]					
Vitamin A (μM)					
Retinol	1.890	0.047	1.977	0.051	0.310
Carotenoids (μM)					
α-Carotene	0.075	0.069	0.069	0.048	0.779
trans-β-Carotene	0.307	0.220	0.278	0.216	0.318
cis-β-Carotene	0.043	0.024	0.046	0.022	0.184
α-Cryptoxanthin	0.045	0.014	0.047	0.018	0.841
β-Cryptoxanthin	0.139	0.071	0.132	0.056	0.588
Lutein	0.185	0.056	0.188	0.070	0.876
Zeaxanthin	0.058	0.016	0.060	0.025	0.713
trans-Lycopene	0.423	0.142	0.425	0.188	0.666
cis-Lycopene	0.410	0.136	0.393	0.181	0.293

[a] Means are presented as untransformed values, but *P*s were calculated from log-transformed values.
[b] Intermittent = 70, persistent = 131.
[c] Intermittent = 58, persistent = 101.

Table 3 Associations between fruit, juice, vegetable intake, and HPV persistence

	Intermittent *n*	Persistent *n*	Crude OR[a]	AOR[a,b] (95% CI)
Fruits (g)				
Low (<64.5)	23	54	1.00	1.00
Medium (64.5–204.6)	23	47	0.87	0.89 (0.42–1.87)
High (>204.6)	23	29	0.54	0.59 (0.27–1.30)
P for trend				0.206
Juices (g)				
Low (<110.4)	23	50	1.00	1.00
Medium (110.4–406.7)	23	44	0.88	0.82 (0.38–1.75)
High (>406.7)	22	29	0.67	0.46 (0.19–1.06)
P for trend				0.077
Vegetables (g)				
Low (<117.0)	23	64	1.00	1.00
Medium (117.0–207.4)	23	30	0.47	0.38 (0.17–0.84)
High (>207.4)	23	37	0.58	0.46 (0.21–0.97)
P for trend				0.033

[a] OR, odds ratio; *n* = 191–200.
[b] Adjusted for age, age at first intercourse, marital status, and BMI.

Discussion

HPV infection alone may be insufficient to produce disease, necessitating the presence of other cofactors for HPV infection to progress to neoplasia. The present study was conducted to examine the association between dietary and circulating vitamin A, the carotenoids, and the risk of oncogenic HPV persistence. To our knowledge, this is the first study to measure dietary vitamin A and the carotenoids as well as the circulating carotenoid isomers (*trans*- or *cis*-β-carotene and *trans*- or *cis*-lycopene) and their association to HPV persistence. Our findings suggest that increased vegetable consumption, a significant source of dietary carotenoids, may be associated with decreased risk of HPV persistence (AOR, 0.46; 95% CI, 0.21–0.97). We also observed a 56% reduction in HPV persistence risk for women in the highest tertile compared with women in the lowest tertile of plasma *cis*-lycopene (AOR, 0.44; 95% CI, 0.19–1.01).

In the current study, higher consumption of vegetables was associated with decreased risk of HPV persistence. Numerous studies have assessed the role of vegetable consumption in cervical neoplasia (12–15, 18, 21, 23–25, 27), with half of these studies showing a protective effect (13, 18, 21, 24, 25). Data such as these from food group analyses have led to a search for specific nutrient components that account for this protective effect.

Table 4 Associations between specific dietary nutrient intake and HPV persistence, $n = 201$

	Intermittent *n*	Persistent *n*	Crude OR	AOR[a] (95% CI)
Preformed vitamin A (RE)				
Low (<259.8)	24	27	1.00	1.00
Medium (259.8–549.4)	23	50	1.93	1.51 (0.68–3.35)
High (>549.4)	23	54	2.09	1.57 (0.71–3.46)
P for trend				0.297
α-Carotene (μg)				
Low (<195.5)	24	45	1.00	1.00
Medium (195.9–449.4)	23	41	0.95	0.96 (0.45–2.06)
High (>449.4)	23	45	1.04	0.90 (0.42–1.90)
P for trend				0.774
β-Carotene (μg)				
Low (<1899.1)	24	52	1.00	1.00
Medium (1899.1–3334.8)	23	34	0.68	0.56 (0.25–1.23)
High (>3334.8)	23	45	0.90	0.81 (0.39–1.71)
P for trend				0.567
Lutein (μg)				
Low (<1042.4)	24	67	1.00	1.00
Medium (1042.4–2377.2)	23	27	0.42	0.37 (0.13–0.82)
High (>2377.2)	23	37	0.58	0.50 (0.24–1.07)
P for trend				0.054
Lycopene (μg)				
Low (<1971.0)	24	35	1.00	1.00
Medium (1971.0–4771.7)	23	54	1.61	1.37 (0.63–2.97)
High (>4771.7)	23	42	1.25	0.99 (0.44–2.20)
P for trend				0.958

[a] Adjusted for age, age at first intercourse, marital status, and BMI.

When individual nutrients were examined in our study, we observed a marginally significant association between dietary lutein and HPV persistence. This association is suggestive of a possible threshold effect, with decreased HPV persistence risk observed for dietary lutein intake levels above reference values ($\geq$1042.4 μg/day). Only two previous studies have examined dietary lutein levels and the risk of cervical neoplasia (10, 11). Both investigations reported no significant association between dietary lutein levels and risk of cervical dysplasia (10, 11). Whereas the dietary lutein association observed in this study may be spurious, it may also reflect an effect that is limited to very early cervical carcinogenic events previously unexamined or differences in food sources by study populations. In addition, lutein may be a marker of other unmeasured compounds. For example, indole-3-carbinol, which is found in many of the same foods as lutein, such as cruciferous vegetables, and is not currently included in nutrient databases, may prevent HPV oncogene expression (49).

In this investigation we also observed a significant inverse association between HPV persistence and plasma *cis*-lycopene concentrations. Similar to our findings, a protective association between circulating lycopene levels and cervical dysplasia has been reported previously (10, 29). After adjustment for HPV infection and smoking, Nagata *et al.* (29) reported a statistically significant inverse association among 152 dysplasia cases and 152 aged-matched controls. A protective association between both dietary and serum levels of lycopene and cervical dysplasia was also reported by Van Eenwyk *et al.* (10). In contrast to these studies, no association was observed between circulating lycopene levels and cervical neoplasia in six studies (11, 30–34). Two prospective studies of HPV persistence also reported no association (30, 31). In our current study, the protective association observed was specific to *cis*-lycopene. Because none of the previous publications, which measured circulating levels of lycopene, distinguished between the *trans*- and *cis*-isomer forms of lycopene (10, 11, 29–34), these studies were unable to assess the relationship of HPV persistence or cervical neoplasia to *cis*-lycopene.

Caution must be taken when comparing dietary and circulating concentrations of carotenoids. Dietary measures simply assess what is consumed, whereas circulating measures assess the sum of consumption, absorption, utilization, and excretion. In validation studies, correlations between circulating and dietary carotenoids have ranged from 0.24–0.51 for α-carotene, 0.21–0.58 for β-carotene, 0–0.37 for lycopene, and 0.09–0.45 for lutein + zeaxanthin (50–56), similar to correlations observed in this study for lycopene ($r = 0.16$–0.18) and lutein ($r = 0.37$). In addition, there is evidence to suggest that plasma carotenoids are differentially metabolized in response to an oxidant load; plasma lycopene appears to be more rapidly oxidized than lutein and, as such, may be a more predictive measure of risk (57, 58). Finally, carotenoid isomers are not captured in nutrient databases, therefore, only total dietary lycopene can be reported. Whereas lycopene is found primarily as *trans*-lycopene in food sources, the *cis* form of lycopene comprises approximately 50% of blood lycopene levels due to the metabolism of this compound (59, 60). For example, consumption of tomato juice, containing lycopene in the *trans*-isomer, has been shown to greatly increase plasma concentrations of *cis*-lycopene (59). Therefore, dietary intake of lycopene food sources increases the plasma lycopene levels of both isomers. The role of the *cis*-isomer of lycopene has not been elucidated (61), although the elevated percentage of the *cis*-isomer of lycopene in blood compared with the diet suggests a physiological role for this isomer. However, the lack of internal consistency between dietary and circulating measures leads to uncertainty regarding the association between nutritional status and the risk of oncogenic HPV persistence. Clearly, there is a need for improved nutritional assessment methods.

Table 5 Association between circulating retinol, carotenoids, and HPV persistence, $n = 159$

Nutrients (μM)	Intermittent *n*	Persistent *n*	Crude OR	AOR[a] (95% CI)
Retinol				
Low (<1.675)	20	31	1.00	1.00
Medium (1.675–1.992)	19	20	0.68	0.71 (0.29–1.72)
High (>1.992)	19	50	1.70	1.78 (0.78–4.03)
P for trend				0.144
α-Carotene				
Low (<0.045)	20	40	1.00	1.00
Medium (0.045–0.066)	19	28	0.74	0.76 (0.33–1.74)
High (>0.066)	19	33	0.87	0.96 (0.42–2.19)
P for trend				0.905
trans-β-Carotene				
Low (<0.181)	20	37	1.00	1.00
Medium (0.181–0.325)	19	39	1.11	1.10 (0.48–2.58)
High (>0.325)	19	25	0.71	0.79 (0.32–1.94)
P for trend				0.603
cis-β-Carotene				
Low (<0.030)	20	27	1.00	1.00
Medium (0.030–0.048)	19	31	1.21	1.20 (0.51–2.82)
High (>0.048)	19	43	1.68	2.00 (0.87–4.61)
P for trend				0.101
α-Cryptoxanthin				
Low (<0.038)	20	32	1.00	1.00
Medium (0.038–0.050)	19	29	0.95	0.90 (0.39–2.10)
High (>0.050)	19	40	1.32	1.68 (0.73–3.89)
P for trend				0.218
β-Cryptoxanthin				
Low (<0.114)	20	42	1.00	1.00
Medium (0.114–1.443)	19	23	0.58	0.86 (0.36–2.07)
High (>1.443)	19	36	0.90	2.00 (0.78–5.15)
P for trend				0.154
Lutein				
Low (<0.152)	20	35	1.00	1.00
Medium (0.152–0.194)	19	25	0.75	0.82 (0.35–1.91)
High (>0.194)	19	41	1.23	1.41 (0.62–3.22)
P for trend				0.413
Zeaxanthin				
Low (<0.050)	20	39	1.00	1.00
Medium (0.050–0.0596)	19	19	0.51	0.63 (0.26–1.51)
High (>0.0596)	19	43	1.16	1.56 (0.67–3.65)
P for trend				0.303
trans-Lycopene				
Low (<0.375)	20	46	1.00	1.00
Medium (0.375–0.490)	19	24	0.55	0.49 (0.21–1.14)
High (>0.490)	19	31	0.71	0.78 (0.35–1.78)
P for trend				0.496
cis-Lycopene				
Low (<0.357)	20	51	1.00	1.00
Medium (0.357–0.468)	19	26	0.54	0.57 (0.25–1.29)
High (>0.468)	19	24	0.50	0.44 (0.19–1.01)
P for trend				0.046

[a] Adjusted for age, race (white *versus* non-white), smoking status at baseline (never *versus* ever), and BMI.

A hypothesized mechanism by which both lutein and lycopene appear to reduce cancer incidence is through their role as lipid-soluble antioxidants (62). Antioxidants prevent damage due to oxidative stress caused by free radical molecules (57, 63) that produce a decrease in immune function (64) and an increase in viral replication (65). Free radical reactive oxygen species initiate a biological cascade resulting in the phosphorylation of transcriptional factors including AP-1, a transcriptional factor reported to be responsible for the expression of numerous genes that can modify cell growth and apoptosis (66) such as the HPV oncogenic proteins E6 and E7 (67). Antioxidants have been shown to modify the expression of the genes associated with the transcriptional AP-1 complex through their ability to quench free radicals and alter the redox status of cells. An *in vitro* study demonstrated the impedance of HPV viral transcription due to alteration of the AP-1 transcriptional complex after treatment with the antioxidant pyrrolidine-dithiocarbamate (68). Through their role as antioxidants, lutein and lycopene may decrease viral load, thereby decreasing persistence and progression to disease. Whereas the protective associations of lutein and lycopene observed in this study may be due to their antioxidant properties, other biological functions may also mediate this effect. For example, non-provitamin A carotenoids have been shown to decrease cell proliferation (69).

Several strengths should be noted when interpreting the results of this study. HPV status was determined with multiple

measures of HPV infection over a scheduled 9-month period. Whereas the follow-up period was scheduled for 9 months, the median duration of follow-up was 10 months. Previous studies reported median times to clearance of 7 months (70) and 8.1 months (71). Therefore, we believe the length of follow-up allowed for adequate assessment of HPV persistence status. Additionally, nutritional measures of both dietary and plasma levels were assessed in the same women.

The limitations of this study should also be considered when interpreting these findings. Only 346 women participated in the follow-up visits. Thus, the small number of participants limited the statistical power. Whereas this study had appropriate power to detect large associations, more modest effects could not be detected. Misclassification of HPV persistence was possible. This study assessed HPV infection using the Hybrid Capture II system, which detects the presence of a panel of oncogenic HPV types rather than individual types. Repeat positives can indicate retention of a specific HPV type or acquisition of new oncogenic types. Because the risk of cervical dysplasia has been shown to increase with either the same or different type oncogenic HPV persistence (6, 72), we believe that HPV persistence as determined by Hybrid Capture II provides a useful measure of subsequent cervical neoplasia risk.

There are several limitations that are common in the use of nutritional assessment methods. As with any dietary study, assessing dietary intake is dependent on the quality of the nutrient database. Whereas carotenoid values were calculated using the USDA-National Coordinating Center Carotenoid Database (1998), these data are limited due to the available analytical data for certain foods (73). Errors from the use of this database as well as participants' misreporting of their dietary intake may have resulted in misclassification of dietary intake status, leading to bias toward the null. In contrast, blood measures are considered unbiased estimates but only reflect recent dietary consumption of carotenoids, providing a measure of short-term rather than long-term nutritional status. Concentrations of β-carotene as well as lycopene reach their maximum approximately 24 h after dosing (74). It is unclear at what stage of the natural history of HPV infection exposure to these nutrients may play a role. Whereas circulating concentrations of retinol and carotenoids were used as proxy measures for concentrations found in cervical tissue, not all plasma micronutrient concentrations reflect cervical tissue concentrations (75). Furthermore, substantial variability in circulating retinol and carotenoid concentrations has been observed, suggesting that more than one sample may be necessary to ensure reliable estimates of nutritional status (76). Because we used a definition of HPV status that included women with prevalent infections at baseline, and plasma samples were collected at the 3 month study visit, no conclusions can be drawn regarding the temporal relationship between nutrient status and oncogenic HPV persistence.

In conclusion, results from this study support a role for vegetable consumption and circulating *cis*-lycopene in decreasing the risk of HPV oncogenic infection persistence. These associations need to be assessed in larger prospective studies that include longer follow-up periods that include multiple measures of nutrient status and HPV status.

References

1. Bosch, F. X., Manos, M. M., Munoz, N., Sherman, M., Jansen, A. M., Peto, J., Schiffman, M. H., Moreno, V., Kurman, R., and Shah, K. V. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. J. Natl. Cancer Inst. (Bethesda), *87:* 796–802, 1995.

2. Walboomers, J. M., Jacobs, M. V., Manos, M. M., Bosch, F. X., Kummer, J. A., Shah, K. V., Snijders, P. J., Peto, J., Meijer, C. J., and Munoz, N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J. Pathol., *189:* 12–19, 1999.

3. Wheeler, C. M., Greer, C. E., Becker, T. M., Hunt, W. C., Anderson, S. M., and Manos, M. M. Short-term fluctuations in the detection of cervical human papillomavirus DNA. Obstet. Gynecol., *88:* 261–268, 1996.

4. Moscicki, A. B., Palefsky, J., Smith, G., Siboshski, S., and Schoolnik G. Variability of human papillomavirus DNA testing in a longitudinal cohort of young women. Obstet. Gynecol., *82:* 578–585, 1993.

5. Moscicki, A. B., Palefsky, J. M., Gonzales, J., Smith, G., and Schoolnik, G. K. Colposcopic and histologic findings and human papillomavirus (HPV) DNA test variability in young women positive for HPV DNA. J. Infect. Dis., *166:* 951–957, 1992.

6. Ho, G. Y., Bierman, R., Beardsley, L., Chang, C. J., and Burk, R. D. Natural history of cervicovaginal papillomavirus infection in young women. N. Engl. J. Med., *338:* 423–428, 1998.

7. Londesborough, P., Ho, L., Terry, G., Cuzick, J., Wheeler, C., and Singer, A. Human papillomavirus genotype as a predictor of persistence and development of high-grade lesions in women with minor cervical abnormalities. Int. J. Cancer, *69:* 364–368, 1996.

8. Schiffman, M. H., and Brinton, L. A. The epidemiology of cervical carcinogenesis. Cancer (Phila.), *76:* 1888–1901, 1995.

9. Potischman, N., and Brinton, L. A. Nutrition and cervical neoplasia. Cancer Causes Control, *7:* 113–126, 1996.

10. Van Eenwyk, J., Davis, F. G., and Bowen, P. E. Dietary and serum carotenoids and cervical intraepithelial neoplasia. Int. J. Cancer, *48:* 34–38, 1991.

11. Kanetsky, P. A., Gammon, M. D., Mandelblatt, J., Zhang, Z. F., Ramsey, E., Dnistrian, A., Norkus, E. P., and Wright, T. C., Jr. Dietary intake and blood levels of lycopene: association with cervical dysplasia among non-Hispanic, black women. Nutr. Cancer, *31:* 31–40, 1998.

12. Verreault, R., Chu, J., Mandelson, M., and Shy, K. A case-control study of diet and invasive cervical cancer. Int. J. Cancer, *43:* 1050–1054, 1989.

13. de Vet, H. C., Knipschild, P. G., Grol, M. E., Schouten, H. J., and Sturmans, F. The role of β-carotene and other dietary factors in the aetiology of cervical dysplasia: results of a case-control study. Int. J. Epidemiol., *20:* 603–610, 1991.

14. Brock, K. E., Berry, G., Mock, P. A., MacLennan, R., Truswell, A. S., and Brinton, L. A. Nutrients in diet and plasma and risk of *in situ* cervical cancer. J. Natl. Cancer Inst. (Bethesda), *80:* 580–585, 1988.

15. Herrero, R., Potischman, N., Brinton, L. A., Reeves, W. C., Brenes, M. M., Tenorio, F., de Britton, R. C., and Gaitan, E. A case-control study of nutrient status and invasive cervical cancer. I. Dietary indicators. Am. J. Epidemiol., *134:* 1335–1346, 1991.

16. Wideroff, L., Potischman, N., Glass, A. G., Greer, C. E., Manos, M. M., Scott, D. R., Burk, R. D., Sherman, M. E., Wacholder, S., and Schiffman, M. A nested case-control study of dietary factors and the risk of incident cytological abnormalities of the cervix. Nutr. Cancer, *30:* 130–136, 1998.

17. Nagata, C., Shimizu, H., Higashiiwai, H., Sugahara, N., Morita, N., Komatsu, S., and Hisamichi, S. Serum retinol level and risk of subsequent cervical cancer in cases with cervical dysplasia. Cancer Invest., *17:* 253–258, 1999.

18. Kwasniewska, A., Charzewska, J., Tukendorf, A., and Semczuk, M. Dietary factors in women with dysplasia colli uteri associated with human papillomavirus infection. Nutr. Cancer, *30:* 39–45, 1998.

19. Liu, T., Soong, S. J., Wilson, N. P., Craig, C. B., Cole, P., Macaluso, M., and Butterworth, C. E., Jr. A case control study of nutritional factors and cervical dysplasia. Cancer Epidemiol. Biomark. Prev., *2:* 525–530, 1993.

20. Buckley, D. I., McPherson, R. S., North, C. Q., and Becker, T. M. Dietary micronutrients and cervical dysplasia in Southwestern American Indian women. Nutr. Cancer, *17:* 179–185, 1992.

21. Ziegler, R. G., Jones, C. J., Brinton, L. A., Norman, S. A., Mallin, K., Levine, R. S., Lehman, H. F., Hamman, R. F., Trumble, A. C., Rosenthal, J. F., *et al.* Diet and the risk of *in situ* cervical cancer among white women in the United States. Cancer Causes Control, *2:* 17–29, 1991.

22. Slattery, M. L., Abbott, T. M., Overall, J. C., Robison, L. M., French, T. K., Jolles, C., Gardner, J. W., and West, D. W. Dietary vitamins A, C, and E and selenium as risk factors for cervical cancer. Epidemiology, *1:* 8–15, 1990.

23. Ziegler, R. G., Brinton, L. A., Hamman, R. F., Lehman, H. F., Levine, R. S., Mallin, K., Norman, S. A., Rosenthal, J. F., Trumble, A. C., and Hoover, R. N. Diet and the risk of invasive cervical cancer among white women in the United States. Am. J. Epidemiol., *132:* 432–445, 1990.

24. La Vecchia, C., Decarli, A., Fasoli, M., Parazzini, F., Franceschi, S., Gentile, A., and Negri, E. Dietary vitamin A and the risk of intraepithelial and invasive cervical neoplasia. Gynecol. Oncol., *30:* 187–195, 1988.

25. La Vecchia, C., Franceschi, S., Decarli, A., Gentile, A., Fasoli, M., Pampallona, S., and Tognoni, G. Dietary vitamin A and the risk of invasive cervical cancer. Int. J. Cancer, *34:* 319–322, 1984.

26. Wylie-Rosett, J. A., Romney, S. L., Slagle, N. S., Wassertheil-Smoller, S., Miller, G. L., Palan, P. R., Lucido, D. J., and Duttagupta, C. Influence of vitamin A on cervical dysplasia and carcinoma *in situ*. Nutr. Cancer, *6:* 49–57, 1984.

27. Marshall, J. R., Graham, S., Byers, T., Swanson, M., and Brasure, J. Diet and smoking in the epidemiology of cancer of the cervix. J. Natl. Cancer Inst. (Bethesda), *70:* 847–851, 1983.

28. Romney, S. L., Palan, P. R., Duttagupta, C., Wassertheil-Smoller, S., Wylie, J., Miller, G., Slagle, N. S., and Lucido, D. Retinoids and the prevention of cervical dysplasias. Am. J. Obstet. Gynecol., *141:* 890–894, 1981.

29. Nagata, C., Shimizu, H., Yoshikawa, H., Noda, K., Nozawa, S., Yajima, A., Sekiya, S., Sugimori, H., Hirai, Y., Kanazawa, K., Sugase, M., and Kawana, T. Serum carotenoids and vitamins and risk of cervical dysplasia from a case-control study in Japan. Br. J. Cancer, *81:* 1234–1237, 1999.

30. Giuliano, A. R., Papenfuss, M., Nour, M., Canfield, L. M., Schneider, A., and Hatch, K. Antioxidant nutrients: associations with persistent human papillomavirus infection. Cancer Epidemiol. Biomark. Prev., *6:* 917–923, 1997.

31. Palan, P. R., Chang, C. J., Mikhail, M. S., Ho, G. Y., Basu, J., and Romney, S. L. Plasma concentrations of micronutrients during a nine-month clinical trial of β-carotene in women with precursor cervical cancer lesions. Nutr. Cancer, *30:* 46–52, 1998.

32. Potischman, N., Herrero, R., Brinton, L. A., Reeves, W. C., Stacewicz-Sapuntzakis, M., Jones, C. J., Brenes, M. M., Tenorio, F., de Britton, R. C., and Gaitan, E. A case-control study of nutrient status and invasive cervical cancer. II. Serologic indicators. Am. J. Epidemiol., *134:* 1347–1355, 1991.

33. Goodman, M. T., Kiviat, N., McDuffie, K., Hankin, J. H., Hernandez, B., Wilkens, L. R., Franke, A., Kuypers, J., Kolonel, L. N., Nakamura, J., Ing, G., Branch, B., Bertram, C. C., Kamemoto, L., Sharma, S., and Killeen, J. The association of plasma micronutrients with the risk of cervical dysplasia in Hawaii. Cancer Epidemiol. Biomark. Prev., *7:* 537–544, 1998.

34. Batieha, A. M., Armenian, H. K., Norkus, E. P., Morris, J. S., Spate, V. E., and Comstock, G. W. Serum micronutrients and the subsequent risk of cervical cancer in a population-based nested case-control study. Cancer Epidemiol. Biomark. Prev., *2:* 335–339, 1993.

35. Ho, G. Y., Palan, P. R., Basu, J., Romney, S. L., Kadish, A. S., Mikhail, M., Wassertheil-Smoller, S., Runowicz, C., and Burk, R. D. Viral characteristics of human papillomavirus infection and antioxidant levels as risk factors for cervical dysplasia. Int. J. Cancer, *78:* 594–599, 1998.

36. Butterworth, C. E., Jr., Hatch, K. D., Macaluso, M., Cole, P., Sauberlich, H. E., Soong, S. J., Borst, M., and Baker, V. V. Folate deficiency and cervical dysplasia. J. Am. Med. Assoc., *267:* 528–533, 1992.

37. Cuzick, J., De Stavola, B. L., Russell, M. J., and Thomas, B. S. Vitamin A, vitamin E and the risk of cervical intraepithelial neoplasia. Br. J. Cancer, *62:* 651–652, 1990.

38. Harris, R. W., Forman, D., Doll, R., Vessey, M. P., and Wald, N. J. Cancer of the cervix uteri and vitamin A. Br. J. Cancer, *53:* 653–659, 1986.

39. Kwasniewska, A., Tukendorf, A., and Semczuk, M. Content of β-carotene in blood serum of human papillomavirus infected women with cervical dysplasias. Arch. Immunol. Ther. Exp., *44:* 309–313, 1996.

40. Palan, P. R., Mikhail, M. S., Basu, J., and Romney, S. L. Plasma levels of antioxidant β-carotene and α-tocopherol in uterine cervix dysplasias and cancer. Nutr. Cancer, *15:* 13–20, 1991.

41. Lehtinen, M., Luostarinen, T., Youngman, L. D., Anttila, T., Dillner, J., Hakulinen, T., Koskela, P., Lenner, P., and Hallmans, G. Low levels of serum vitamins A and E in blood and subsequent risk for cervical cancer: interaction with HPV seropositivity. Nutr. Cancer, *34:* 229–234, 1999.

42. Liu, T., Soong, S. J., Alvarez, R. D., and Butterworth, C. E., Jr. A longitudinal analysis of human papillomavirus 16 infection, nutritional status, and cervical dysplasia progression. Cancer Epidemiol. Biomark. Prev., *4:* 373–380, 1995.

43. Sedjo, R. L., Inserra, P., Abrahamsen, M., Harris, R. B., Roe, D. J., Baldwin, S., and Giuliano, A. R. Human papillomavirus persistence and nutrients involved in the methylation pathway among a cohort of women. Cancer Epidemiol. Biomark. Prev., *11:* 353–359, 2002.

44. Lorincz, A. T. Hybrid capture method for detection of human papillomavirus DNA in clinical specimens. Papillomavirus Rep., *7:* 1–5, 1996.

45. Block, G., Hartman, A. M., Dresser, C. M., Carroll, M. D., Gannon, J., and Gardner, L. A data-based approach to diet questionnaire design and testing. Am. J. Epidemiol., *124:* 453–469, 1986.

46. Thomson, C. A., Giuliano, A. R., Graver, E., Hartz, V., Whitacre, R., Pierce, J., and Marshall, J. R. Reproducibility and validity of the Arizona Cancer Center food frequency questionnaire (AFFQ) among WHEL cohort participants. 25th Annual ASPO Meeting, New York, New York 2001.

47. Martinez, M. E., Marshall, J. R., Graver, E., Whitacre, R. C., Woolf, K., Ritenbaugh, C., and Alberts, D. S. Reliability and validity of a self-administered food frequency questionnaire in a chemoprevention trial of adenoma recurrence. Cancer Epidemiol. Biomark. Prev., *8:* 941–946, 1999.

48. Craft, N. E. High resolution HPLC method for the simultaneous analysis of carotenoids, retinoids, and tocopherols. FASEB J., A527, 1996.

49. Yuan, F., Chen, D. Z., Liu, K., Sepkovic, D. W., Bradlow, H. L., and Auborn, K. Anti-estogenic activities of indole-3-carbinol in cervical cells: implication for prevention of cervical cancer. Anticancer Res., *19:* 1673–1680, 1999.

50. Tucker, K. L., Chen, H., Vogel, S., Wilson, P. W. F., Schaefer, E. J., and Lammi-Keefe, C. J. Carotenoid intakes, assessed by dietary questionnaire, are associated with plasma carotenoid concentrations in an elderly population. J. Nutr., *129:* 438–445, 1999.

51. Brady, W. E., Mares-Perlman, J. A., Bowen, P., and Stacewicz-Sapuntzakis, M. Human serum carotenoid concentrations are related to physiologic and lifestyle factors. J. Nutr., *126:* 129–137, 1996.

52. Ritenbaugh, C., Peng, Y. M., Aickin, M., Graver, E., Branch, M., and Alberts, D. S. New carotenoid values for foods improve relationship of food frequency questionnaire intake estimates to plasma values. Cancer Epidemiol. Biomark. Prev., *5:* 907–912, 1996.

53. Peng, Y. M., Peng, Y. S., Lin, Y., Moon, T., Roe, D. J., and Ritenbaugh, C. Concentrations and plasma-tissue-diet relationships of carotenoids, retinoids, and tocopherols in humans. Nutr. Cancer, *23:* 233–246, 1995.

54. Yong, L. C., Forman, M. R., Beecher, G. R., Graubard, B. I., Campbell, W. S. Reichman. M. E., Taylor, P. R., Lanza, E., Holden, J. M., and Judd, J. T. Relationship between dietary intake and plasma concentrations of carotenoids in premenopausal women: application of the USDA-NCI carotenoid food-composition database. Am. J. Clin. Nutr., *60:* 223–230, 1994.

55. Forman, M. R., Lanza, E., Yong, L. C., Holden, J. M., Graubard, B. I., Beecher, G. R., Meltiz, M., Brown, E. D., and Smith, J. C. The correlation between two dietary assessments of carotenoid intake and plasma carotenoid concentrations: application of a carotenoid food composition database. Am. J. Clin. Nutr., *58:* 519–524, 1993.

56. Coates, R. J., Eley, J. W., Block, G., Gunter, E. W., Sowell, A. L., Grossman, C., and Greenberg, R. S. An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low-income black women. Am. J. Epidemiol., *134:* 658–671, 1991.

57. Di Mascio, P., Murphy, M. E., and Sies, H. Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. Am. J. Clin. Nutr., *53* (Suppl. 1)*:* 194S–200S, 1991.

58. Siems, W. G., Sommerburg, O., and van Kuijk, F. J. Lycopene and β-carotene decompose more rapidly than lutein and zeaxanthin upon exposure to various pro-oxidants *in vitro*. Biofactors, *10:* 105–113, 1999.

59. Muller, H., Bub, A., Watzl, B., and Rechkemmer, G. Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. Eur. J. Nutr., *38:* 35–44, 1999.

60. Clinton, S. K., Emenhiser, C., Schwartz, S. J., Bostwick, D. G., Williams, A. W., Moore, B. J., and Erdman, J. W. *cis-trans* Lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol. Biomark. Prev., *5:* 823–833, 1996.

61. Clinton, S. K. Lycopene: chemistry, biology, and implications for human health and disease. Nutr. Rev., *56:* 35–51, 1998.

62. Khachik, F., Beecher, G. R., and Smith, J. C., Jr. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J. Cell. Biochem., *22* (Suppl.)*:* 236–246, 1995.

63. Conner, E. M., and Grisham, M. B. Inflammation, free radicals, and antioxidants. Nutrition, *12:* 274–277, 1996.

64. Anderson, R., and Theron, A. J. Physiological potential of ascorbate, β-carotene and α-tocopherol individually and in combination in the prevention of tissue damage, carcinogenesis and immune dysfunction mediated by phagocyte-derived reactive oxidants. World Rev. Nutr. Diet., *62:* 27–58, 1990.

65. Peterhans, E. Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation. J. Nutr., *127* (Suppl. 5)*:* 962S–965S, 1997.

66. Palmer, H. J., and Paulson, K. E. Reactive oxygen species and antioxidants in signal transduction and gene expression. Nutr. Rev., *55:* 353–361, 1997.

67. Rosl, F., and Schwarz, E. Regulation of E6 and E7 oncogenic transcription. *In:* M. Tommasino (ed.), Papillomaviruses in Human Cancer: Role of E6 and E7 Oncoproteins, pp. 25–70. Austin, TX: Landes Bioscience and Chapmena & Hall, 1997.

68. Rosl, F., Das, B. C., Lengert, M., Geletneky, K., and zur Hausen, H. Antioxidant-induced changes of the AP-1 transcription complex are paralleled by a selective suppression of human papillomavirus transcription. J. Virol., *71:* 362–370, 1997.

69. Rock, C. L., Kusluski, R. A., Galvez, M. M., and Ethier, S. P. Carotenoids induce morphological changes in human mammary epithelial cell cultures. Nutr. Cancer, *23:* 319–333, 1995.

70. Moscicki, A. B., Shiboski, S., Broering, J., Powell, K., Clayton, L., Jay, N., Darragh, T. M., Brescia, R., Kanowitz, S., Miller, S. B., Stone, J., Hanson, E., and Palefsky, J. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J. Pediatr., *132:* 277–284, 1998.

71. Franco, E. L., Villa, L. L., Sobrinho, J. P., Prado, J. M., Rousseau, M. C., Desy, M., and Rohan, T. E. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J. Infect. Dis., *180:* 1415–1423, 1999.

72. Schlecht, N. F., Kulaga, S., Robitaille, J., Ferreira, S., Santos, M., Miyamura, R. A., Duarte-Franco, E., Rohan, T. E., Ferenczy, A., Villa, L. L., and Franco, E. L. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. J. Am. Med. Assoc., *286:* 3106–3114, 2001.

73. Mangels, A. R., Holden, J. M., Beecher, G. R., Forman, M. R., and Lanza, E. The carotenoid content of fruits and vegetables: an evaluation of analytical data. J. Am. Diet. Assoc., *93:* 284–296, 1993.

74. Arab, L., Steck-Scott, S., and Bowen, P. Participation of lycopene and β-carotene in carcinogenesis: defenders, aggressors, or passive bystanders?. Epidemiol. Rev., *23:* 211–230, 2002.

75. Peng, Y. M., Peng, Y. S., Childers, J. M., Hatch, K. D., Roe, D. J., Lin, Y., and Lin, P. Concentrations of carotenoids, tocopherols, and retinol in paired plasma and cervical tissue of patients with cervical cancer, precancer, and noncancerous diseases. Cancer Epidemiol. Biomark. Prev., *7:* 347–350, 1998.

76. Cooney, R. V., Franke, A. A., Hankin, J. H., Custer, L. J., Wilkens, L. R., Harwood, P. J., and Le Marchand, L. Seasonal variations in plasma micronutrients and antioxidants. Cancer Epidemiol. Biomark. Prev., *4:* 207–215, 1995.