RESEARCH COMMUNICATION

The Association of Plasma Micronutrients with the Risk of Cervical Atypical Squamous Cells of Undetermined Significance (ASCUS)

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Abstract

Little is known about factors that favor the development of cervical atypical squamous cells of undetermined significance (ASCUS), nor how these factors might affect the pathogenesis of cervical neoplasia. The primary focus of this case-control study among the multiethnic population of Hawaii was to identify biomarkers of diet in the recent past that may influence the risk of ASCUS, after carefully accounting for the presence of HPV DNA. Cases included 185 women with ASCUS and 191 cytologically-normal controls diagnosed between 1992 and 1996 from three clinics in Honolulu, Hawaii. In-person interviews were conducted in the subjects' homes, a fasting blood sample was drawn to measure plasma levels of various micronutrients, and the presence and type of HPV was determined in exfoliated cell samples using Polymerase Chain Reaction (PCR) dot blot hybridization.

As results, we found an inverse dose-response gradient with increasing plasma concentrations of α-cryptoxanthin and δ-tocopherol for the development of ASCUS. The odds ratio for ASCUS among women in the highest quartile compared with women in the lowest quartile of total cryptoxanthin was 0.5 (95% confidence interval (CI): 0.3-1.0), α-cryptoxanthin was 0.4 (0.2-0.8), total tocopherol was 0.5 (0.2-1.0), α-tocopherol was 0.5 (0.2-1.0), and δ-tocopherol was 0.4 (0.2-0.8). Little association of plasma levels of lutein/zeaxanthin, lycopene, α- or β-carotene, retinol, vitamin C, or cholesterol, with disease risk was evident. Our findings suggest that women with high circulating concentrations of cryptoxanthin and tocopherol may be at a reduced risk of ASCUS.

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Introduction

Although HPV has been identified as a primary causal agent in cervical dysplasia and carcinoma, the virus is also detected with some frequency among women who are cytologically normal (Schiffman et al, 1993). Among cytologically normal women with latent HPV infection, as well as women with clinically detectable cervical dysplasia, infection with HPV is often short-lived and transient, with high-grade squamous intraepithelial lesions (SIL) and cervical cancer occurring among a fraction of women with infection (Hildescheim et al, 1994; Ho et al, 1995; Remmink et al, 1995). The search for cofactors with HPV in cervical carcinogenesis has included a variety of host (immunological response, hormones) and environmental (tobacco smoking, infection with other sexually transmitted diseases) agents,

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but little attention has focused on the association of diet with early cellular changes in the cervix that may be pathognomonic of neoplasia.

With the increased availability and prevalence of cervical cancer screening, microscopic cervical cell abnormalities are being detected with greater frequency. The poor classification of these abnormalities has contributed to considerable controversy regarding their clinical management (Kurman and Solomon, 1994; National Cancer Institute Workshop, 1989; Williams et al, 1997). A high percentage of women with ASCUS on initial Papanicolaou (Pap) smear demonstrate distinct cellular abnormalities on subsequent colposcopy-directed biopsy (Williams et al, 1997). Few studies have been conducted that have examined risk factors for ASCUS. In particular, there has been only one epidemiological investigation of the association of ASCUS with dietary or plasma micronutrient levels (Wideroff et al, 1998). The objective of this analysis was to determine whether various plasma micronutrients correlate with early histopathological changes in cervical cells, and whether these associations influence the effect of HPV on disease risk.

Materials and Methods

Population

We conducted a case-control study on the island of Oahu, Hawaii, among women attending three hospital-based clinics for cervical cytological screening between June, 1992 and December, 1996 (Goodman et al, 1998). Eligible subjects included non-hysterectomized women from 18 to 84 years of age who were residents of Oahu. Women who had been pregnant or lactating within 6 months of enrollment, who had a diagnosis of cervical abnormalities within the past three years, or who were clinic referrals were considered ineligible for study.

Eligible cases were identified through the cytology logs of the participating clinics and included all women with a cytological classification of ASCUS according to the Bethesda system (Kurman and Solomon, 1994). These women were contacted by letter, followed by a telephone call, for consent to participate in the study before their return to the clinic for a follow-up examination and cervical smear. As part of the study, a colposcopy was performed and cervical cells were collected for HPV testing. All smears were read by one of three cytopathologists and categorized histopathologically. Only women diagnosed with ASCUS conforming to the Bethesda classification were included as cases in this analysis. Interview information was obtained from 254 cases, which was 78% of the estimated 325 eligible women. We were able to draw blood and perform plasma micronutrient analyses on 185 (73%) of the 254 ASCUS cases completing interviews.

Controls were women with negative cytological smears attending the same clinics as the cases. Eligible women were selected from the admission logs of the participating clinics on a randomly selected day of the month. Potential controls were met at the clinics by one of the interviewers who explained the purpose of the study. An exfoliated cervical cell specimen was obtained at the time of the cervical cytological smear among consenting women. Of the 318 qualified women with negative cytological results, 271 (85%) were interviewed and 191 (70%) further consented to have their blood drawn and were used as controls in the present analysis. Most (>95%) blood was drawn within six weeks of the cytological smear.

Participants were scheduled for a personal interview at their homes or other convenient location. A standardized questionnaire was used to elicit a detailed sexual and reproductive history, including age at first intercourse, number of sexual partners and methods of contraception, diet, a lifetime history of tobacco and alcohol use, and other demographic and lifestyle information. In this article, we have focused on the association of plasma micronutrients and HPV with the risk of ASCUS. Other exposures, such as diet, and tobacco smoking and alcohol drinking, were analyzed as potential confounders, but details of these analyses will be reported elsewhere.

Micronutrient analysis

Blood was drawn after a 10-12 hour fast and was used to determine plasma levels of both *cis* and *trans* forms of lutein/zeaxanthin, lycopene, total cryptoxanthin, α-cryptoxanthin, β-cryptoxanthin, total carotene, α-carotene, β-carotene, retinol, total tocopherol, α-tocopherol, γ-tocopherol, δ-tocopherol, vitamin C, and cholesterol. The plasma was separated from the cells by centrifugation (4° C for 15 min, 1800 x g) and frozen (-70° C) until analyzed. Sample extracts were analyzed isocratically by reverse-phase HPLC methodology (Franke et al, 1993), and absorption spectra and retention times for each peak were compared to those of known standards. Each sample was analyzed by HPLC in duplicate and the results were reported as means.

Plasma samples for vitamin C analysis were prepared by the addition of 0.4 ml plasma to each of two 1.0 ml cryogenic storage vials that contained 0.4 ml 10% aqueous meta-phosphoric acid. Vitamin C levels were determined with a spectrophotometric assay using dichlorophenol-indophenol (Omaye et al, 1979). Plasma cholesterol levels were determined spectrophometrically using commercial enzymatic kits #339-50, 352-50 and 352-3 (Sigma Chemical, 1996). These measurements were based on a sequence of enzymatic reactions hydrolyzing the esterified analytes followed by specific enzymatic oxidation. The quality of all laboratory analyses was evaluated by performance in round-robin trials organized by the U.S. National Institute for Standards and Technologies (Gaitherburg, Maryland).

HPV DNA detection

Frozen cervical cell specimens (1 ml) were shipped to Harborview Medical Center where they were prepared for HPV-DNA detection by polymerase chain reaction (PCR) amplification and dot blot hybridization of the amplicons. The PCR amplifies the highly conserved L1 region of the viral genome using degenerate primers (MY09 and MY11)

(Ting and Manos, 1990) which amplify over 45 types of HPV, including the most common oncogenic types found in the cervix (Bauer et al, 1991). PCR products were assessed through dot blot hybridization using biotin-labeled oligonucleotide DNA probes. We used five biotin-labeled type-specific probe mixes for HPV types 6/11, 16, 18, 31/33/35/39, and 45, as well as a biotin-labeled generic probe which detects a broad spectrum of HPV types.

Statistical analysis

A preliminary examination of the data included comparisons of cases and controls with respect to several demographic characteristics and risk factors of interest. We used analysis of covariance to compare the mean, log-transformed plasma micronutrient concentrations between cases and controls while adjusting for age, ethnicity and for tocopherol, cholesterol (Snedecor and Cochran, 1967). Partial Pearson correlations of continuous log-transformed plasma micronutrients and other variables, adjusting for age, ethnicity, and other potential confounders were calculated to evaluate colinearity. These means and correlations guided subsequent analyses.

The odds ratios associated with different levels of the exposure variables were evaluated by unconditional multiple logistic regression modeling case-control status (Breslow and Day, 1980). Odds ratios and 95% confidence intervals (CI) were computed by exponentiating the coefficients (and the 95% CIs) for the binary indicator variables representing the quartile levels of the plasma micronutrients. The quartile cutpoints were based on the distributions for the controls. Adjustment variables included age, as a continuous variable, ethnicity by indicator variables, and the following additional variables: tobacco use (ever vs never smoked cigarettes), alcohol use (ever drank vs never drank), number of sex partners before age 20 (continuous), HPV detection (yes versus no) by PCR dot blot and, for tocopherol only, cholesterol (continuous). The addition of indicator variables for education (>14 years vs _14 years) and family income (>$37,500 vs _$37,500) or continuous variables for body mass index and calories to the logistic models did not change the findings. We performed a test for linear trend in the logit of risk by comparing twice the difference in log-likelihoods for models with and without a trend variable, based on a $\_^2$ distribution with one degree of freedom. The trend variable was assigned the median for the appropriate quantile or category.

Logistic regression models were used to explore the interaction between all plasma micronutrients and HPV detection by dichotomizing the micronutrient level at the median into low and high groups. Three dummy variables were created to model each level of interaction between the pairs of variables using subjects whose plasma micronutrient levels were low and who were HPV negative as the reference category. Similar models were evaluated consisting of the joint effect of various plasma micronutrients, tobacco smoking history, and alcohol drinking history. The likelihood ratio test was used to evaluate the interaction between plasma micronutrients and the other variables on the risk of ASCUS. This test compared a no-interaction model containing main effect terms with a fully-parameterized model containing all possible interaction terms for the variables of interest.

Results

Table 1 shows odds ratios for factors potentially associated with ASCUS. Controls were older (mean age = 39.2 years) than cases (mean age = 37.6 years), more likely to be Japanese, somewhat better educated, and married. None of these differences between cases and controls was significant. A positive relation of tobacco smoking to ASCUS was found, particularly among current smokers compared to never smokers (OR: 2.2; 95% CI: 1.2-4.2) (data not shown). A higher estimated risk for ASCUS was also found in association with alcohol drinking, although the odds ratios were attenuated after adjustment for tobacco smoking and HPV prevalence (OR: 1.4; 95% CI: 0.9-2.1) (data not shown). Thirty-four percent of women with ASCUS and 10% of controls were positive for HPV by PCR.

With the exception of lutein/zeaxanthin, covariate-adjusted mean concentrations of the carotenoids were lower among cases than among controls, with the largest differences (ng/ml) found for total cryptoxanthin (166 vs. 185) and α-cryptoxanthin (27 vs. 33) (Table 2). We also found substantial variation between cases and controls in mean plasma concentrations of the tocopherols, with the largest differences in the level of δ-tocopherol: mean concentrations (ng/ml) were 56% lower among cases than controls. Smaller percentage differences between cases and controls were found for mean concentrations of retinol (2%), vitamin C (-8%), and cholesterol (1%).

Table 3 shows the association of plasma micronutrients with the odds ratios for ASCUS after adjustment for age and ethnicity (multivariate model 1), and after additional adjustment for tobacco smoking, alcohol drinking, number of sex partners before age 20, and HPV detection by PCR dot blot hybridization (multivariate model 2). Women with higher plasma concentrations of cryptoxanthin, especially α-cryptoxanthin, had substantially lower odds ratios for ASCUS even after adjustment for HPV and potential confounders. Odds ratios were 60% lower among women in the highest compared with the lowest quartile of plasma α-cryptoxanthin in both multivariate models. No gradient in risk was apparent by plasma level of other carotenoids or retinol. We found a weak, inverse relation between increased plasma concentration of total tocopherol and the odds ratios for ASCUS that was strengthened slightly in multivariate model 2: the odds ratio for the highest compared with the lowest quartile of plasma tocopherol was 0.5 (95% CI: 0.2-1.0) after additional adjustment for potential confounders. Specific tocopherol components were generally inversely related to the estimated risk of ASCUS, although these relations were weak for α- and γ-tocopherol. Women with plasma levels of δ-tocopherol in the highest quartile had odds ratios that were 60% lower than women in the lowest quartile,

Table 1. Background Characteristics of Women with Cervical Atypia (ASCUS) and Controls, Hawaii, 1992-1996

Variable	Cases(n=185)	Controls(n=191)	Odds ratio[a]	95% confidence interval	p for trend
Age					
<25	35	34	1[b]		
26-35	50	50	1.0	0.5-1.8	
36-45	56	56	1.2	0.6-2.2	
>45	44	44	0.7	0.4-1.3	0.69
Ethnicity					
Caucasian	86	83	1[b]		
Japanese	15	31	0.5	0.2-1.0	
Hawaiian	31	28	1.0	0.6-1.9	
Other	53	49	1.0	0.6-1.7	
Education (years)					
<12	36	41	1[b]		
13-14	56	43	1.5	0.8-2.7	
15-16	41	49	0.9	0.5-1.8	
>16	52	58	1.1	0.6-2.0	0.14
Family income ($)[c]					
<22,500	46	40	1[b]		
22,501-37,500	45	48	0.9	0.5-1.6	
37,501-62,500	47	57	0.8	0.4-1.4	
>62,500	46	44	1.2	0.6-2.0	0.79
Marital status					
Single	35	28	1[b]		
Married	100	120	0.7	0.4-1.2	
Other	50	43	1.0	0.5-1.9	
Tobacco use					
Never smoker	106	119	1[b]		
Ever smoker	79	72	1.4	0.7-1.8	
Alcohol use					
Never drinker	79	105	1[b]		
Ever drinker	106	86	1.6	1.0-2.4	
Number of sexual partners before age 20[c]					
None	51	67	1[b]		
<1	44	53	1.0	0.6-1.8	
2-3	49	41	1.5	0.8-2.6	
>4	40	26	1.9	1.0-3.7	0.05
HPV DNA[d]					
None	122	172	1[b]		
Any HPV-DNA	63	19	4.6	2.5-8.2	
16	7	3	4.5	1.2-17	
18	4	1	6.8	0.8-61	
31, 33, 35, 39	5	2	5.2	1.0-27	
6, 11	2	0	∞		
Other HPV	40	13	4.2	2.1-8.4	
Multiple HPV	5	0	∞		

[a] After adjustment by unconditional multiple logistic regression for age and ethnicity, when appropriate. [b] Reference category. [c] One case and two controls were missing information on family income; one case and four controls were missing information on number of sexual partners before age 20. [d] Determined by polymerase chain reaction (PCR) amplification and dot blot hybridization of the amplicons; subtypes given in numbers.

Table 2. Means[a] and 95% Confidence Intervals for Plasma Nutrient Concentrations among Cervical Atypia (ASCUS) Cases and Controls, Hawaii, 1992-1996

	Cases (n=185)		Controls (n=191)		
Nutrient	Mean	95% Confidence Interval	Mean	95% Confidence Interval	p[b]
Total carotenoids (ng/ml)	1230	1160-132	1260	1180-1340	0.68
Lutein	422	397-448	410	388-434	0.47
Lycopene	290	269-312	313	292-336	0.12
Total cryptoxanthin	166	151-181	185	170-201	0.06
α-cryptoxanthin	27	24-30	33	29-37	0.01
β-cryptoxanthin	101	91-112	109	99-120	0.29
Total carotene	259	240-313	265	234-299	0.80
α-carotene	54	48-60	55	49-62	0.73
β-carotene	199	175-226	203	179-229	0.85
Retinol (ng/ml)	620	590-650	610	580-630	0.46
Total tocopherol (ng/ml)[c]	118	107-129	126	116-138	0.01
α-tocopherol	9800	9400-10300	10500	10000-11000	0.0024
γ-tocopherol	1360	1230-1490	1460	1340-1600	0.23
δ-tocopherol	27	18-41	62	42-91	0.002
Vitamin C x 100 (mg/dl)	122	111-134	133	122-145	0.15
Cholesterol (mg/dl)	155	145-166	154	145-164	0.87

[a] Adjusted by analysis of covariance for age and ethnicity. Nutrients were log-transformed before entry into the model. Means and 95% confidence intervals were transformed back into their original units (ng/ml for vitamin C and mg/dl for cholestrerol). [b] p-values comparing log plasma nutrients between cases and controls. [c] After additional adjustment for cholesterol.

although the inverse trend in risk was not monotonic. No association with ASCUS was observed for plasma retinol, vitamin C, or cholesterol concentrations.

We modeled the joint association of plasma micronutrients and HPV with the odds ratios for ASCUS by creating three dummy variables, one representing the main effects of micronutrients with dichotomization at the median (e.g., >32.8 ng/ml α-cryptoxanthin), one representing HPV detection by PCR (any type versus none), and one representing the interaction term. Results shown in Table 4 are limited to α-cryptoxanthin, selected because of its association with risk and its high correlation with the other carotenoids. Human papillomavirus-negative women with α-cryptoxanthin levels above the 50th percentile were at 60% the risk of ASCUS compared to other HPV-negative women. Among HPV-positive women, the odds ratio was higher among women with low plasma α-cryptoxanthin levels than among women with high plasma cryptoxanthin levels. Similar patterns were found for the joint association of HPV and the tocopherols (data not shown). We found no statistical interactions (i.e., departure from additivity in the coefficients on a log scale) between HPV detection and any of the micronutrients on the risk of ASCUS.

In addition to HPV detection, we modeled the joint effects of cryptoxanthin and tobacco smoking, alcohol drinking, and other plasma micronutrients on the odds ratios for ASCUS (data not shown). Risks were consistently higher among women with low plasma α-cryptoxanthin measurements regardless of the category of the second exposure (e.g., alcohol drinker or abstainer).

Discussion

Few studies have examined risk factors for ASCUS, in part because of the lack of a standardized definition and the subjectivity of the diagnosis (Kurman and Solomon, 1994; Williams et al, 1997). Results of our analysis suggest that women with high plasma concentrations of α-cryptoxanthin, total tocopherol, and δ-tocopherol, may be at reduced risk of early malignant changes in the cervical epithelium largely associated with HPV infection (Sherman et al, 1994). These findings are generally in accord with an earlier analysis of plasma micronutrient concentrations among women with biopsy-confirmed SIL and normal controls in which we found an inverse dose-response of α-cryptoxanthin, total tocopherol, and α-tocopherol to the odds ratios for disease (Goodman et al, 1998). Although the same control group was used in both analyses, the possibility that our findings were a chance occurrence was lessened by the consistency of the results among cases with ASCUS (equivocal SIL) and cases with biopsy-confirmed SIL.

The hypothesis that hematologic or dietary markers of nutrition in the recent past may be correlated with the presence of cervical dysplasia has been tested in numerous epidemiological investigations (Potischman and Brinton, 1996) but few investigators have considered HPV or other potential confounders. Adjustment for HPV infection may be critical to studies of cervical carcinogenesis because the

Table 3. Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association of Quartiles (Q)[a] of Plasma Nutrients with the Risk of Cervical Atypia (ASCUS), Hawaii, 1992-1996

	Multivariate model 1[b]							Multivariate model 2[c]						
	Q_2 vs Q_1[d]		Q_3 vs Q_1		Q_4 vs Q_1		p for	Q_2 vs Q_1		Q_3 vs Q_2		Q_4 vs Q_1		p for
Nutrient	OR	95%CI	OR	95% CI	OR	95% CI	trend	OR	95% CI	OR	95% CI	OR	95% CI	trend
Carotenoids	0.8	0.4-1.4	0.5	0.2-0.9	0.9	0.5-1.6	0.58	0.6	0.3-1.2	0.5	0.2-0.9	1.0	0.6-1.9	0.94
Lutein/zeaxanthin	0.4	0.2-0.7	0.9	0.5-1.6	1.1	0.6-2.0	0.29	0.4	0.2-0.7	0.9	0.5-1.6	1.1	0.6-2.0	0.35
Lycopene	1.0	0.6-1.7	1.0	0.6-1.9	0.6	0.3-1.1	0.17	0.9	0.5-1.6	1.0	0.5-1.9	0.6	0.4-1.2	0.27
Total cryptoxanthin	0.6	0.3-1.1	0.5	0.3-0.8	0.5	0.3-1.0	0.04	0.5	0.3-1.0	0.5	0.3-0.9	0.5	0.3-1.0	0.08
α-cryptoxanthin	0.5	0.3-1.0	0.5	0.2-0.9	0.4	0.4-0.7	0.0009	0.5	0.3-0.9	0.5	0.3-1.0	0.4	0.2-0.8	0.008
β-cryptoxanthin	1.0	0.5-1.8	0.7	0.4-1.2	0.7	0.4-1.4	0.21	1.0	0.5-1.8	0.7	0.4-1.3	0.8	0.4-1.5	0.36
Total carotene	0.9	0.5-1.6	0.8	0.4-1.4	0.9	0.5-1.7	0.95	0.9	0.5-1.7	0.9	0.5-1.7	0.9	0.5-1.8	0.97
α-carotene	0.8	0.5-1.5	0.6	0.3-1.1	0.8	0.4-1.5	0.32	0.9	0.5-1.7	0.7	0.4-1.2	1.0	0.5-1.8	0.70
β-carotene	0.8	0.4-1.4	1.0	0.5-1.8	0.9	0.5-1.7	0.95	0.8	0.4-1.6	1.2	0.6-2.2	1.0	0.5-2.0	0.69
Retinol	1.4	0.8-2.5	1.2	0.6-1.1	1.4	0.8-2.5	0.39	1.4	0.8-2.6	1.0	0.5-1.8	1.1	0.6-2.2	0.97
Total tocopherol[e]	0.6	0.3-1.1	0.6	0.3-1.2	0.6	0.3-1.1	0.37	0.5	0.3-1.0	0.6	0.3-1.2	0.5	0.2-1.0	0.15
α-tocopherol	0.4	0.2-0.8	0.5	0.3-1.0	0.6	0.3-1.2	0.61	0.4	0.2-0.8	0.5	0.3-1.0	0.5	0.2-1.0	0.22
γ-tocopherol	0.6	0.4-1.1	0.6	0.3-1.1	0.8	0.4-1.4	0.38	0.6	0.4-1.2	0.6	0.4-1.2	0.7	0.4-1.3	0.31
δ-tocopherol	0.5	0.3-0.9	0.8	0.5-1.4	0.3	0.2-0.6	0.004	0.5	0.3-0.9	0.8	0.4-1.4	0.4	0.2-0.8	0.05
Vitamin C	1.2	0.7-2.2	0.6	0.3-1.1	0.8	0.4-1.4	0.22	1.2	0.6-2.2	0.8	0.4-1.4	0.8	0.4-1.5	0.39
Cholesterol	0.5	0.3-1.0	1.1	0.6-1.9	1.0	0.6-1.8	0.18	0.5	0.3-0.9	0.9	0.5-1.7	0.9	0.5-1.7	0.88

[a]The quartile cutpoints for nutrients were as follows: total carotenoid 932, 1190, 1530 ng/ml; lutein/zeaxanthin, 325, 398, 502 ng/ml; lycopene, 231, 319, 406 ng/ml; total cryptoxanthin 115, 158, 233 ng/ml; α-cryptoxanthin, 23.9, 32.8, 41.6 ng/ml; β-cryptoxanthin, 63.0, 93.6, 149 ng/ml; total carotene, 136, 238, 415 ng/ml; α-carotene, 32.8, 52.6, 86.5 ng/ml; β-carotene, 103, 172, 330 ng/ml; retinol, 507, 635, 737 ng/ml; total tocopherol, 9400, 11,200, 37,400 ng/ml; α-tocopherol, 7540, 9140, 11,600 ng/ml; γ-tocopherol, 1100, 1540, 2070 ng/ml; δ-tocopherol, 62, 102, 444 ng/ml; vitamin C, 1.02, 1.44, 1.79 mg/dl; cholesterol, 137, 156, 182 mg/dl. [b]After adjustment by unconditional multiple logistic regression for age and ethnicity. [c]After adjustment by unconditional multiple logistic regression for age, ethnicity, tobacco smoking, alcohol drinking, number of sex partners before age 20, and human papillomavirus (HPV) detection by polymerase chain reaction (PCR) amplification and dot blot hybridization of the amplicons, [d]Lowest micronutrient quartile (Q_1) is the reference category. [e]After additional adjustment for cholesterol.

Table 4. Odds Ratios[a] (OR) and 95% Confidence Intervals (CI) for the Joint Association of Human papillomavirus (HPV) and Plasma α-cryptoxanthin on the Risk of Cervical Atypia (ASCUS),Hawaii, 1992-1996

	< 32.8 ng/ml α-cryptoxanthin				> 32.8 ng/ml α-cryptoxanthin				
>Any Type= HPV[b]	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI	p-value[c]
No	68	77	1[d]		54	95	0.6	0.4-1.0	
Yes	41	11	4.0	1.9-8.5	22	8	2.9	1.2-7.0	1.00

[a] Adjusted by unconditional multiple logistic regression for age, ethnicity, tobacco smoking, alcohol drinking, and number of sexual partners before age 20. [b] Determined by polymerase chain reaction (PCR) amplification and dot blot hybridization of the amplicons. [c] Based on the likelihood ratio test comparing models with and without an interaction term. [d] Reference category.

virus may be a necessary, but perhaps insufficient cause of disease; and may interact with other agents, such as micronutrients or tobacco smoke constituents, in cervical tissue to produce malignant change (Schiffman et al, 1996). The majority of dietary and hematologic investigations of micronutrient concentrations have been small (<100 cases), with a focus on retinol, carotene, and folate (Potischman and Brinton, 1996).

The most consistent data from epidemiological investigations of micronutrient concentrations and the risk of cervical dysplasia and cancer have been found for deficiencies in serum or plasma concentrations of carotenoids (Van Eenwyk et al, 1991; Brock et al, 1988; Harris et al, 1986; Potischman et al, 1991). Case-control studies have shown a reduced risk of cervical lesions associated with serum lycopene (Van Eenwyk et al, 1991; Kanetsky et al, 1998) and β-carotene (Brock et al, 1988; Harris et al, 1986; Potischman et al, 1991); and a cohort study suggests that the risk of cervical cancer is higher among women with lower prediagnostic serum concentrations of α-carotene, β-carotene, total cryptoxanthin, lycopene, and total carotenoids (Batieha et al, 1993). In our study, only cryptoxanthin levels, especially plasma concentrations of α-cryptoxanthin, were associated with differences in risk between cases and controls.

Little relation of other carotenoids to the odds ratio for ASCUS was evident. Blood concentrations of cryptoxanthin have also been reported to be lower among cases than among controls in three studies (Van Eenwyk et al, 1991; Potischman et al, 1991; Batieha et al, 1998), but confidence intervals included unity.

We found a weak, inverse relation of total plasma tocopherol to the risk for ASCUS. A greater than 50% reduction in estimated risk was found for subjects in the highest compared with the lowest quartile of total tocopherol concentration. We also report a negative (non-monotonic) trend in the risk for ASCUS among women with increasing plasma levels of α-tocopherol, a micronutrient that has not been previously examined. α-tocopherol is the predominant vitamin E component in plasma with approximately 200 times the concentration of α-tocopherol in our study population. Although the effect of δ-tocopherol on risk was not dose-responsive, it is possible that a minimum threshold level of this micronutrient is needed to reduce risk. Given the relatively small quantity of δ-tocopherol in the blood, it is not clear whether this micronutient is directly associated with ASCUS or simply a correlate of total tocopherol or some other underlying factor.

Aside from our own study, two other investigations of the relation of serum tocopherol to the risk of cervical dysplasia have been conducted. In a small case-control study, Cuzick et al (1990) showed an inverse dose-response association of α-tocopherol with the odds ratios for cervical intraepithelial neoplasia (CIN) 1 and CIN 3, although no adjustment was made for HPV. Kwasniewska et al (1997) found four-fold higher serum levels of α-tocopherol among HPV-negative controls with normal pap smears than among HPV-positive cervical dysplasia cases. Findings regarding an association of vitamin E with the risk of cervical cancer are equivocal (Slattery et al, 1990; Verreault et al, 1989; Knekt, 1988), but one dietary case-control study (Verreault et al, 1989) and one serologic cohort study (Knekt, 1988) report significant inverse relations to risk.

Although ASCUS cases had lower mean concentrations of vitamin C than controls, no trend in the odds ratios for disease was found by plasma level of this micronutrient. Case-control studies of the association of dietary vitamin C with the risk for dysplasia (De Vet et al, 1991, Van Eenwyk et al, 1992) and cancer (Slattery et al, 1990; Verreault et al, 1989; De Vet et al, 1991; Van Eenwyk et al, 1992; Brock et al, 1989; Herrero et al, 1991) have generally reported inverse relations, although only three of these were significant (Verreault et al, 1989; Van Eenwyk et al, 1992; Herrero et al, 1991). Of the four case-control studies of vitamin C and the risk of SIL (Goodman et al, 1998; Basu et al, 1991; Romney et al, 1985; Butterworth et al, 1992), three have found a modest inverse dose-response association (Goodman et al, 1998; Basu et al, 1991; Romney et al, 1985).

Results from a case-control study (Butterworth et al, 1992) and two prospective studies (Hiatt and Fireman, 1986; Schatzkin et al, 1988) suggest an increased risk for cervical cancer associated with low serum cholesterol levels. We found no effect of cholesterol levels on the odds ratios for ASCUS.

Several mechanisms have been hypothesized through which micronutrients, such as carotenoids and tocopherols can inhibit the neoplastic process. Experimental evidence suggests that these micronutrients may protect cells against oxidative damage by chemical carcinogens (Gebremichael et al, 1984; Burton, 1989, Bendich, 1989) However, an antioxidant mechanism is unlikely for the association of α-cryptoxanthin and δ-tocopherol with ASCUS in this analysis because other plasma micronutrients with substantial antioxidant potential, such as vitamin C, were unrelated to risk. The inverse association of cryptoxanthin with the odds ratio for ASCUS in our analysis suggests a biological mechanism that is independent of retinol; other carotenoids that have provitamin A activity, such as α- and β-carotene, were not found to be risk factors for disease. Nutrients, such as retinoic acid, decrease immortalization of HPV-infected cells and decrease the production of the early transforming proteins of HPV, E6 and E7 (Khan et al, 1993) Giuliano et al (1997) have hypothesized that carotenoids and tocopherols may reduce the risk of persistent HPV infections and progression of disease by up-regulating natural killer cell activity and T cell proliferation effects on immune cell function. Long-term follow-up studies with serial measurements of HPV DNA, plasma micronutrients, and immunity are needed to test these hypotheses.

The use of sensitive and specific HPV testing and quantitative micronutrient assessment was an advantage of our study design. Adjustment for potential confounders on the odds ratio for ASCUS strengthens the probability that our findings did not result from differences between cases and controls in exposures to other risk factors. Most previous studies of the etiology of cervical dysplasia have used proxies for HPV exposure, such as age at first intercourse or number of sexual partners, that serve as imprecise measures of viral infection (Potischman and Brinton, 1996). Adjustment for these behaviors in our analyses did little to alter the association of plasma micronutrients with the odds ratio for ASCUS. Varying dietary history methods, problems with diet recall, differences in food composition databases, and other problems may contribute to the heterogeneity in the results of previous investigations of nutrition and cervical neoplasia. The use of easily quantifiable biochemical assays for plasma micronutrients, although limited to recent dietary exposure, avoids the pitfalls of dietary assessment methods that rely on incomplete memory.

A weakness of this study was the relatively small sample size making it difficult to examine potential interactions between micronutrients, HPV, and other risk factors for ASCUS. Confidence intervals were wide, especially for those variables with skewed distributions. We attempted to investigate the independent effects of plasma micronutrients on risk, but correlations among micronutrients were high, and it is likely that a combination of dietary factors may better explain differences in plasma micronutrient concentrations between cases and controls. The response rate for subjects

(57% for cases, 60% for controls), although low, compares favorably with other hematologic investigations of this type (Potischman and Brinton,1991). Human papillomavirus infections are transient in nature and the study design did not allow us to measure incident infections. Our cross-sectional design could bias our findings if those women with long-term infections have different risk profiles than women with infections that rapidly resolve.

Misclassification of disease status is a potential problem in most studies, but this issue is particularly troublesome in investigations of early pre-invasive lesions in which interpretation is difficult and biopsies are unavailable. We attempted to standardize slide preparation and slide reading among the participating pathologists, and a single pathologist was used to resolve equivocal cases. Nonetheless, there was no mechanism to ensure precise classification of study subjects. Future analyses of these data will evaluate the association of plasma micronutrients with HPV prevalence as an outcome variable, irrespective of cytologic classification. We are now conducting a follow-up study with serial blood and cervical cell collection to examine the importance of nutrient status to the subsequent diagnosis of high-grade SIL in HPV-infected women with equivocal and mild cervical cytological diagnoses.

In summary, this study has shown an inverse association of several plasma micronutrients, particularly cryptoxanthin and tocopherol, with the estimated risk for ASCUS. The data further suggest that these micronutrients may influence the association of HPV infection with microscopic cervical disease. It is likely that one or more of these micronutrients will prove to be useful biomarkers of HPV persistence or disease progression among women with cervical dysplasia.

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Symbols used: ASCUS, atypical squamous cells of undetermined significance; HPLC, high pressure liquid chromatography; HPV, human papillomavirus; CI; confidence interval, PCR, polymerase chain reaction; OR; odds ratio, SIL, squamous intraepithelial lesions.

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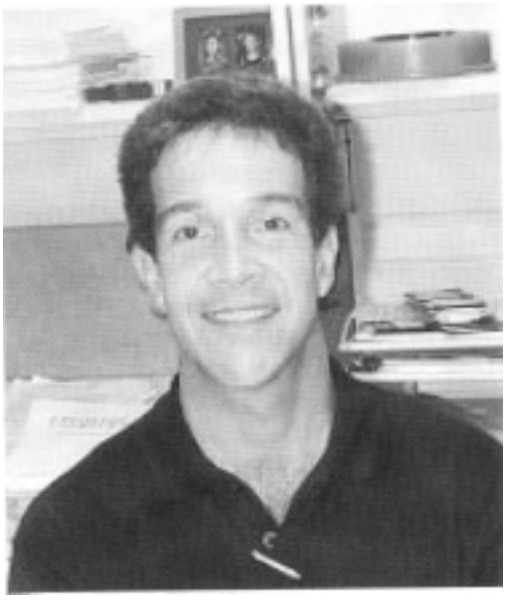

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