Research Article

Hawaii Cohort Study of Serum Micronutrient Concentrations and Clearance of Incident Oncogenic Human Papillomavirus Infection of the Cervix

Marc T. Goodman,[1] Yurii B. Shvetsov,[1] Katharine McDuffie,[1] Lynne R. Wilkens,[1] Xuemei Zhu,[1] Adrian A. Franke,[1] Cathy Cramer Bertram,[5] Bruce Kessel,[5] Marge Bernice,[5] Christian Sunoo,[6] Lily Ning,[2] David Easa,[3] Jeffrey Killeen,[7] Lori Kamemoto,[4] and Brenda Y. Hernandez[1]

[1]Cancer Research Center of Hawaii, [2]University Health Services, [3]Clinical Research Center, and [4]John A. Burns School of Medicine, University of Hawaii; [5]Queen's Medical Center; [6]Kaiser Hawaii Permanente Medical Systems; and [7]Kapiolani Medical Center for Women and Children, Honolulu, Hawaii

Abstract

The degree to which the resolution of human papillomavirus (HPV) infection parallels exposure to other factors, particularly those related to nutritional status, is a relatively unexplored area of research. We established a cohort of women for long-term follow-up to examine the association of serum retinol, carotenoid, and tocopherol concentrations with the clearance of incident cervical HPV infection. Interviews and biological specimens were obtained at baseline and at 4-month intervals. At each visit, a cervical cell specimen for HPV DNA analysis and cytology and a fasting blood sample to measure micronutrient levels were collected. A Cox proportional hazards model was used to study the relationship between clearance of 189 incident (type-specific) oncogenic HPV infections and the levels of 20 serum micronutrients among 122 women. Higher circulating levels of *trans*-zeaxanthin, total *trans*-lutein/zeaxanthin, cryptoxanthin (total and β), total *trans*-lycopene and *cis*-lycopene, carotene (α, β, and total), and total carotenoids were associated with a significant decrease in the clearance time of type-specific HPV infection, particularly during the early stages of infection (≤120 days). HPV clearance time was also significantly shorter among women with the highest compared with the lowest serum levels of α-tocopherol and total-tocopherol, but significant trends in these associations were limited to infections lasting ≤120 days. Clearance of persistent HPV infection (lasting >120 days) was not significantly associated with circulating levels of carotenoids or tocopherols. Results from this investigation support an association of micronutrients with the rapid clearance of incident oncogenic HPV infection of the uterine cervix. [Cancer Res 2007;67(12):5987–96]

Introduction

Human papillomavirus (HPV) has been established as the primary causal agent in squamous intraepithelial lesions (SIL) and carcinoma of the uterine cervix for more than a decade (1). The natural history of cervical carcinogenesis is thought to begin from an initial infection of the metaplastic epithelium of the cervical transformation zone by an oncogenic HPV type (2). The target cell for HPV infection in the cervix is the primitive basal keratinocyte (3). Viral replication is dependent on cellular replication and, importantly, squamous cell differentiation specific to epithelial maturation. Most HPV infections seem to clear quickly through an innate immune response or some other mechanism (2–4). Understanding the determinants of persistent infections, in which the same viral type is detected on consecutive examinations, has been the subject of intensive study. Likely co-factors in the infectious process and cervical carcinogenesis include characteristics of the infection (HPV genotype, concurrent HPV infections, and viral load), concomitant infection (*Chlamydia trachomatis* and herpes simplex virus), host factors (human leukocyte antigen and parity), and behavioral factors (tobacco smoking and oral contraceptive pill use; refs. 5–7).

The role of diet and nutrition on the risk of HPV persistence and cervical carcinogenesis was recently reviewed by Garcia-Closas et al. (8). Only a few longitudinal investigations have examined the association of diet and circulating micronutrient concentrations with the persistence or clearance of cervical HPV infection (9–14). Although the results of these studies have been mixed, a protective effect for fruit and vegetable, vitamin E, and carotenoid intakes against HPV persistence is suggested. Immune cells are particularly vulnerable to oxidative stress; thus, the antioxidant levels in their membranes play an important role in maintaining a reduced environment and preserving cellular function. Antioxidants maintain the function of membrane lipids that, if peroxidized, can lead to loss of membrane integrity and altered membrane fluidity resulting in altered intracellular signaling (15). Dietary components that possess antioxidant properties may protect the immune system from oxidative damage and enhance immune responses (16).

In 1998, we initiated a multiethnic cohort study of women for long-term follow-up to identify factors that influence the persistence or resolution of type-specific HPV infection of the cervix. In addition to measuring HPV infection at each 4-month study visit, repeated measures of circulating levels of carotenoids, tocopherols, and retinol were obtained for each visit. A unique aspect of this analysis was our ability to account for the relative duration of infection through examination of the association of circulating antioxidant concentrations with the clearance of incident, rather than prevalent, oncogenic HPV infection.

Materials and Methods

Subject Recruitment

We established a longitudinal study of cervical HPV infection between 1998 and 2003 among women attending five clinics on Oahu, Hawaii, who

Requests for reprints: Marc T. Goodman, Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Honolulu, HI 96813. Phone: 808-586-2987; Fax: 808-586-2982; E-mail: marc@crch.hawaii.edu.
©2007 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-07-0313

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

met the following initial eligibility requirements: (*a*) able to speak and understand English; (*b*) at least 18 years of age; (*c*) resident of Oahu; (*d*) not pregnant or postpartum within the past 6 months; (*e*) intact cervix (no hysterectomy); (*f*) not immune compromised, taking immunosuppressive drugs, transplant recipient, HIV positive, or within 1 year of cancer chemotherapy; (*g*) sexually active within the past 6 months; (*h*) no abnormal cytologic smear within the past 18 months; (*i*) no history of blood-clotting disorders or on anticoagulant therapy; (*j*) no plans to relocate in the next year; and (*k*) able and willing to read, understand, and sign an informed consent form and medical release form approved by the University of Hawaii Institutional Review Board.

At each of the clinics, women who were scheduled for gynecology appointments were approached for participation in the cohort. The study coordinators explained that we were conducting research under the auspices of the clinic and the University of Hawaii. Willing participants were screened, and written informed consent was obtained before the health care examination. At the first and subsequent visits, a gynecologic examination was done; an exfoliated cervical specimen for a Papanicolaou smear and HPV DNA analysis was collected; and a blood sample was drawn to measure micronutrient levels. Upon completion of the examination, the study coordinator administered a study questionnaire to each participant. The baseline interview included demographics and detailed histories of tobacco and alcohol use as well as sexual activity.

Selection of the Cohort and Follow-up

The results of the baseline cervical smear and HPV DNA testing were necessary to establish final eligibility. Women with inadequate (i.e., negative for the human *β-globin* gene) specimens at baseline were not eligible. Women with abnormal cytology were referred to their physician for appropriate follow-up. All women who were treated for SIL or a more severe lesion (e.g., through conization) were censored at the time of treatment and no longer contributed to follow-up after this time. Women with a biopsy only were retained in the study. Women with low-grade SIL or changes consistent with HPV who were not treated were retained in the study and followed. Women entering the cohort were asked to return to the clinic every 4 months for repeat examination and testing. Participants were called a day or two before their clinic visit to remind them about their scheduled appointment and fasting for blood sample collection, if they were able.

A more detailed interview was conducted during the second visit that took 30 to 40 min to complete. The reference period was the date at entry on the study. The questionnaire included a gynecologic, menstrual, reproductive, and sexual history; hormone use; medical history; history of sexually transmitted infections; and income. An update since the first visit was collected for information covered in more detail in the baseline questionnaire (sexual activity and tobacco and alcohol use). The questionnaire used at subsequent interviews was modified slightly for use during the follow-up period. Questions focused on changes in sexual and reproductive information during the intervening period between clinic visits and checks on the reliability of certain data items.

At baseline, 2,398 women were recruited and tested for HPV DNA by PCR, and 972 of these women completed at least one follow-up visit. Most women who were HPV DNA negative at baseline and the subsequent follow-up visit were terminated because of the cost constraints of continued follow-up. Among the remaining women with two or more study visits, 484 were positive with an oncogenic HPV type on at least one visit, and 182 of these women experienced at least one incident HPV infection (oncogenic and non-oncogenic) defined as an HPV genotype not identified on the previous visit. We limited this analysis to the 122 women with incident oncogenic HPV infections and serum samples. Among these women, a total of 1,057 follow-up visits were completed in which we collected cervical and blood specimens (median, 6 visits per subject). The follow-up experience accumulated for this cohort was 3,199 woman-months (median, 15.3 months per participant; range, 6.5–51.6 months). The follow-up rate on the cohort was 91% overall and 93% for those women remaining in the cohort with two or more visits.

Laboratory Analyses

Detection and genotyping of HPV. HPV DNA was extracted from exfoliated cervical cell specimens using commercial reagents (Qiagen, Inc.). Specimens were analyzed for the presence or absence of HPV DNA by PCR using a modified version of the PGMY09/PGMY11 primer system (17). HPV DNA–positive specimens were genotyped using a reverse line blot detection method for 37 different HPV types, including 6, 11,16,18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, CP6108, and IS39 (refs. 18, 19; Roche Molecular Systems). PCR products were denatured and hybridized to a nylon membrane containing the immobilized HPV probes. This genotyping assay included probes for high and low levels of human *β-globin* gene. Amplicons hybridized to probes were detected using streptavidin/horseradish peroxidase–mediated color precipitation.

Serum micronutrient analysis. All of the blood specimens were drawn into heparin tubes after a 10- to 12-h fast and were used to determine serum micronutrient levels of both *cis* and *trans* forms of lutein/zeaxanthin; α-cryptoxanthin, β-cryptoxanthin, and total cryptoxanthin; *trans* and *cis* forms of lycopene; α-carotene; *trans* and *cis* forms of β-carotene; total carotene; total carotenoids; retinol; α-tocopherol, γ-tocopherol, δ-tocopherol, and total tocopherol (20 micronutrients). The serum was separated from the cells by centrifugation (4°C for 15 min, 1,800 × *g*) and frozen (−80°C) until analyzed. Sample extracts were analyzed by isocratic reverse-phase high-performance liquid chromatography methodology with photodiode array detection, and absorption spectra and retention times for each peak were compared with those of known standards (20). 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 (Gaithersburg, MD).

Statistical Analysis

The statistical analysis was limited to the 122 women with incident oncogenic HPV infections based on genotypes defined by Muñoz et al. (21) and at least one subsequent visit after observation of the infection so that clearance could be observed. Adjustment variables were identified by a prior analysis of the baseline demographic and behavioral risk factors associated with acquisition of HPV infection at subsequent visits by subjects who were HPV negative at the first visit. These risk factors included age and the number of lifetime sexual partners (log-transformed). Age at first sexual intercourse and current tobacco smoking were also included in all models because of a significant interaction of these factors with several micronutrients. A Cox proportional hazards model was used to examine the association of cervical HPV infection clearance with serum concentrations of the micronutrients, after adjustment for risk factors identified at baseline and for the amount of time a blood sample was kept frozen between blood draw and laboratory analysis. We combined measurements for *cis*-anhydrolutein, *trans*-anhydrolutein, *cis*-lutein/zeaxanthin, and *trans*-lutein/zeaxanthin into a single value (total *cis*-lutein/zeaxanthin and *trans*-lutein/zeaxanthin) because these dietary constituents are generally found in trace amounts. Hazard ratios and 95% confidence intervals were calculated for micronutrient levels divided into quartiles and represented by three indicator variables using the lowest quartile as the reference category. A trend variable was created based on the median micronutrient level within each of the quartiles. The test for trend was based on the Wald χ^2 statistic.

The time scale for the Cox-based models was chosen to be the time in days since an incident HPV infection was detected; that is, each infection is set to start at time 0. Other time scales were tested, such as study month, but the results were similar. A woman could experience a subsequent incident infection with the same HPV type during follow-up and could be infected with more than one HPV type at one time. Among the 122 women included in this analysis, a total of 189 incident infections were identified. We assigned a separate infection path to every HPV genotype detected. An HPV type–specific infection was considered resolved after one negative visit for this genotype, and the timing of infection clearance was defined as the time of the first negative visit. Other clearance times were investigated, such as the midpoint between the last visit where the infection was observed and the first negative visit, but the results were not altered. The presence of

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

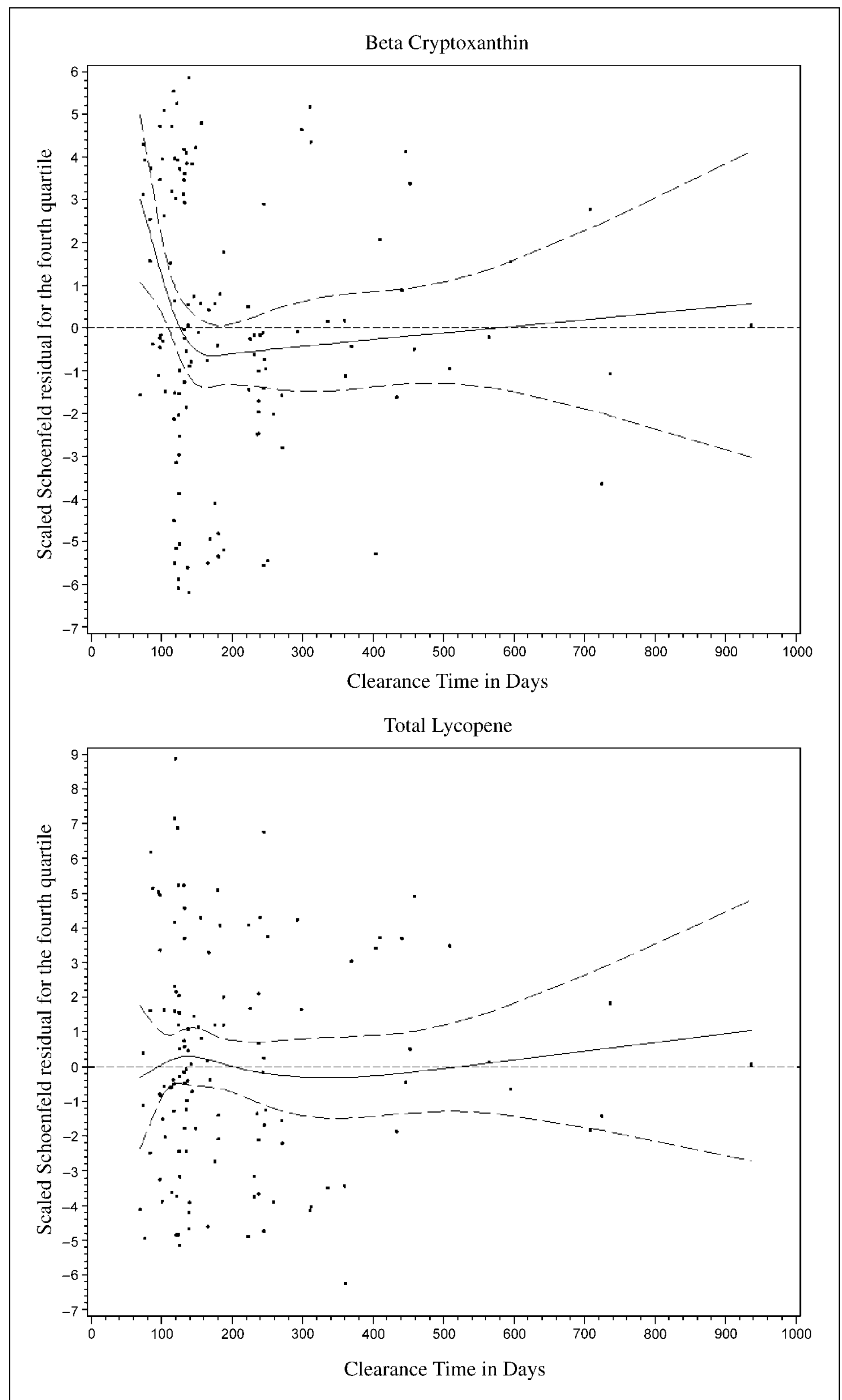

Figure 1. Scaled Schoenfeld residuals for β-cryptoxanthin (*top*) and total lycopene (*bottom*) as a function of time. *Solid line,* hazard function estimate; *dotted lines,* 95% confidence limits.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

multiple HPV infections was accounted for by adjusting for a time-dependent coinfection status indicator variable, which was measured at every visit for each subject. Serum micronutrient levels and the adjustment variables of smoking and drinking were also time-dependent variables, where the values for previous visit ($t - 1$) were used as the exposure variables for clearance at visit t. Because there is no evidence to suggest that micronutrient levels would have different effects on the clearance of HPV by genotype or event number (e.g., first versus subsequent infections),

Table 1. Baseline risk factors for HPV acquisition

Risk factor*	Incident high-risk, HPV (N = 122)	Odds ratio (95% confidence limits)
Age (y)		
<25	50	1.00
25-34	37	0.50 (0.30–0.85)
35-44	22	0.25 (0.14–0.45)
≥45	13	0.09 (0.04–0.17)
Ethnicity		
Japanese	13	1.00
Caucasian	45	1.11 (0.54–2.29)
Hawaiian	14	0.83 (0.35–1.98)
Filipino	7	0.95 (0.32–2.80)
Other	43	1.12 (0.54–2.35)
Education		
≤ High School	26	1.00
Some college	49	0.97 (0.55–1.72)
College grad	33	1.03 (0.57–1.89)
Graduate degree	14	1.04 (0.49–2.20)
No. pregnancies		
None	55	1.00
1	29	1.48 (0.84–2.61)
≥2	38	1.04 (0.61–1.79)
Ever been pregnant		
No	55	1.00
Yes	67	1.22 (0.76–1.95)
Age at first sexual intercourse		
<16	25	1.00
16-17	40	1.40 (0.77–2.54)
18-19	35	1.44 (0.78–2.67)
≥20	22	1.47 (0.74–2.94)
No. lifetime sexual partners		
<2	6	1.00
2-5	47	3.46 (1.38–8.72)
6-10	35	5.15 (1.99–13.36)
≥11	32	3.61 (1.40–9.34)
Ever use oral contraceptives before visit 2		
No	23	1.00
Yes	99	0.75 (0.43–1.31)
Current tobacco smoker		
No	107	1.00
Yes	18	1.18 (0.65–2.16)
Current alcohol drinker		
No	71	1.00
Yes	51	1.16 (0.70–1.93)

*Adjusted for age as a continuous variable.

no stratification was done by these variables and our modeling approach followed that of Andersen and Gill (22). Because each subject was allowed to experience more than one clearance event throughout the course of the study, we used a robust sandwich variance estimate (23), aggregated over subjects, to prevent artificially deflated standard errors and confidence interval estimates.

The proportionality assumption was checked using Schoenfeld residual test statistic and Schoenfeld residual plots, which may serve as hazard function estimates (24–26). Figure 1 displays these plots for β-cryptoxanthin (*top*) and total lycopene (*bottom*). Although the assumption was not significantly violated, there was evidence that for some micronutrients, the effect was not constant over time. Therefore, in addition to the overall model, a change-point estimation approach was adopted (27, 28) to account for the possibility of differential micronutrient effects on the risk of HPV clearance in the early and late stages of an infection. The change-point selection was conducted by visual inspection of Schoenfeld residual plots. The plots clearly showed that the change point was located between 120 and 150 days after infection. Because 120 days was the target time interval between consecutive visits in the study, this time interval was chosen as the change point for all models. However, other cutoffs were examined, and similar results were found. For the 20 micronutrients, Cox models were fit, and hazard ratios were obtained separately for the early (transient) stage of an infection, defined as the first 120 days, and late (persistent) stage, defined to be from day 121 until clearance or censoring. Possible effect modification by age of the micronutrient associations with clearance times for transient, persistent, and transient plus persistent infections was tested through stratification by median age (28 years). All statistical analyses were done using SAS version 9.1.3 (SAS Institute, Inc.). All P values were two sided, and $P < 0.05$ was defined as significant.

Results

Table 1 shows the association of the risk factors for HPV infection in the baseline model with HPV acquisition. Both older age and fewer lifetime numbers of sexual partners were the only baseline risk factors that were significantly associated with decreased HPV acquisition. All adjustment variables were entered in the micronutrient Cox models however. The median age of the participants was 28 years (mean, 32 years; first and third quartiles, 22 and 38 years, respectively). The cohort members were generally non-White with mixed ethnicity ("other") and some college education. More than one half of the cohort had a positive pregnancy history. The average age at first sexual intercourse was 17 years. The average lifetime number of sexual partners was 7. The majority of women used oral contraceptive pills or had used them in the past. Most women were not current tobacco smokers or alcohol drinkers.

The results from the Cox models showed that higher circulating levels of several carotenoids, including *trans*-zeaxanthin, total *trans*-lutein/zeaxanthin, cryptoxanthin (total and β), total *trans*-lycopene plus *cis*-lycopene, carotene (α, β, and total), and total carotenoids, were associated with a significant decrease in the clearance time of type-specific HPV infection (Table 2). Women in the highest quartile for total *trans*-lycopene plus *cis*-lycopene, *cis*-β-carotene, and total carotene were nearly twice as likely to clear their infections as women in the lowest quartile. Trends were particularly strong for several components of carotene, suggesting a general effect of this micronutrient on the risk of clearance. Little association of type-specific HPV clearance with serum tocopherols or retinol was found.

The micronutrient analyses were repeated separately for the early (transient) stage of an infection, defined as the first 120 days, and late (persistent) stage, defined to be from day 121 until clearance or censoring (Table 3). Overall, 188 infections with 908 person-months of follow-up were included in the analysis of transient stage, and 147 infections with 910 person-months of follow-up were included in the analysis of persistent stage. The magnitude of the association of circulating micronutrients with HPV clearance in the early stages of infection was greater than the association with transient and persistent infection combined. Clearance of cervical HPV infection was strongly associated with high circulating levels of the luteins/zeaxanthins, β-cryptoxanthin, total cryptoxanthin, total *trans*-lycopene plus *cis*-lycopene, and the carotenes. Here, the likelihood of clearance was three to seven times greater for the highest compared with the lowest quartile. In addition to the carotenoids, high serum levels of α-tocopherol and total tocopherol were associated with clearance of transient infections.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

Table 2. Hazard ratios and 95% confidence intervals for clearance of incident high-risk HPV infection by levels of circulating plasma micronutrients

Micronutrient/quartile	Quartile range (ng/mL)	No. cleared/infections (mo)*	Hazard ratio[†] (95% confidence limits)	P_{trend}[‡]
Trans-lutein				
Low	41.87-106.63	33/345	1.00	
2	106.64-156.70	35/362	1.23 (0.82–1.85)	
3	156.71-225.21	31/326	1.26 (0.79–2.01)	
High	225.22-624.18	35/342	1.49 (0.91–2.43)	0.16
Trans-zeaxanthin				
Low	20.28-46.40	36/356	1.00	
2	46.41-58.22	30/349	0.88 (0.58–1.32)	
3	58.23-82.69	29/332	0.94 (0.57–1.53)	
High	82.70-281.10	39/338	1.50 (0.94–2.39)	0.04
Total *trans*-lutein/zeaxanthin				
Low	73.53-158.68	35/356	1.00	
2	158.69-212.03	30/344	1.05 (0.67–1.65)	
3	212.04-303.92	31/339	1.14 (0.72–1.83)	
High	303.93-905.29	38/337	1.59 (1.01–2.51)	0.04
Total *cis*-lutein/zeaxanthin and *trans*-lutein/zeaxanthin				
Low	112.67-259.20	37/354	1.00	
2	259.21-332.78	26/345	0.97 (0.62–1.52)	
3	332.79-452.23	38/341	1.50 (0.95–2.38)	
High	452.24-1,287.6	33/335	1.42 (0.88–2.31)	0.12
α-Cryptoxanthin				
Low	0.33-33.52	37/348	1.00	
2	33.53-43.53	33/355	0.84 (0.53–1.33)	
3	43.54-54.44	29/333	1.21 (0.68–2.15)	
High	54.45-106.45	35/339	1.12 (0.70–1.80)	0.33
β-Cryptoxanthin				
Low	17.54-113.85	34/353	1.00	
2	113.86-158.65	36/362	0.99 (0.63–1.57)	
3	158.66-242.59	29/339	1.05 (0.65–1.71)	
High	242.60-1,114.6	35/321	1.64 (0.99–2.73)	0.04
Total cryptoxanthin				
Low	17.87-148.75	33/350	1.00	
2	148.76-207.65	36/359	1.02 (0.64–1.61)	
3	207.66-296.12	30/346	1.14 (0.70–1.84)	
High	296.13-1,164.6	35/320	1.72 (1.02–2.88)	0.03
Total *trans*-lycopene and *cis*-lycopene				
Low	0.00-29.49	31/355	1.00	
2	29.50-42.89	37/348	1.23 (0.76–1.98)	
3	42.90-59.30	31/359	1.24 (0.78–1.95)	
High	59.31-245.11	35/314	2.06 (1.23–3.44)	0.01
Total lycopene				
Low	55.96-251.67	34/334	1.00	
2	251.68-337.62	34/334	1.04 (0.66–1.65)	
3	337.63-449.99	36/353	0.96 (0.65–1.43)	
High	450.00-941.04	30/355	0.71 (0.44–1.16)	0.13
α-Carotene				
Low	0.07-26.81	32/354	1.00	
2	26.82-46.00	33/363	0.89 (0.56–1.40)	
3	46.01-68.81	31/334	1.14 (0.66–1.97)	
High	68.82-388.36	38/323	1.78 (1.03–3.06)	0.009
Trans-β-carotene				
Low	1.87-86.14	35/356	1.00	
2	86.15-160.90	27/343	0.85 (0.51–1.41)	
3	160.91-252.15	35/354	1.25 (0.80–1.96)	
High	252.16-1,405.5	37/322	1.84 (1.13–2.99)	0.004
Cis-β-carotene				
Low	0.00-7.78	38/361	1.00	
2	7.79-14.76	25/344	0.84 (0.51–1.40)	

(*Continued on the following page*)

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

Table 2. Hazard ratios and 95% confidence intervals for clearance of incident high-risk HPV infection by levels of circulating plasma micronutrients (Cont'd)

Micronutrient/quartile	Quartile range (ng/mL)	No. cleared/infections (mo)*	Hazard ratio † (95% confidence limits)	P_{trend} ‡
3	14.77-23.95	34/346	1.22 (0.76–1.96)	
High	23.96-97.36	37/324	1.94 (1.20–3.13)	0.002
Total β-carotene				
Low	2.53-96.54	36/362	1.00	
2	96.55-172.86	27/345	0.86 (0.52–1.41)	
3	172.87-281.15	35/344	1.31 (0.84–2.06)	
High	281.16-1,496.4	36/324	1.74 (1.07–2.85)	0.007
Total carotene				
Low	2.60-127.16	35/357	1.00	
2	127.17-225.29	27/347	0.89 (0.54–1.46)	
3	225.30-379.95	33/349	1.22 (0.77–1.94)	
High	379.96-1,724.4	39/324	1.98 (1.21–3.23)	0.001
Total carotenoids				
Low	198.91-983.25	32/348	1.00	
2	983.26-1,329.3	34/354	1.13 (0.71–1.81)	
3	1,329.4-1,755.8	34/340	1.31 (0.84–2.04)	
High	1,755.9-3,979.0	34/333	1.75 (1.03–2.97)	0.03
Retinol				
Low	0.00-569.45	35/356	1.00	
2	569.46-719.20	32/317	1.15 (0.72–1.83)	
3	719.21-865.26	37/353	1.02 (0.67–1.55)	
High	865.27-1,624.2	30/350	0.77 (0.48–1.24)	0.21
α-Tocopherol				
Low	3,490.4-7,536.0	39/358	1.00	
2	7,536.1-9,391.3	33/325	0.94 (0.57–1.56)	
3	9,391.4-12,375	26/367	0.75 (0.48–1.17)	
High	12,376-36,621	36/325	1.38 (0.80–2.38)	0.22
β-Tocopherol and γ-tocopherol				
Low	29.80-643.85	36/352	1.00	
2	643.86-1,073.0	33/342	0.83 (0.52–1.33)	
3	1,073.1-1,573.6	32/341	0.74 (0.45–1.20)	
High	1,573.7-3,564.0	33/341	0.91 (0.58–1.42)	0.66
δ-Tocopherol				
Low	24.28-165.59	33/339	1.00	
2	165.60-243.57	32/348	0.88 (0.56–1.38)	
3	243.58-417.73	36/352	0.90 (0.54–1.49)	
High	417.74-1,090.3	33/337	0.91 (0.56–1.49)	0.85
Total tocopherol				
Low	4,528.2-9,336.0	37/354	1.00	
2	9,336.1-10,885	34/334	1.03 (0.65–1.63)	
3	10,886-13,604	26/356	0.84 (0.53–1.34)	
High	13,605-37,092	37/332	1.47 (0.89–2.41)	0.13

*Cumulative infection length in months, from first positive to first negative visit.

† Adjusted for age at first visit, current tobacco smoking status, number of lifetime sexual partners, age at first sex, blood frozen over 1 y indicator variable, and coinfection status.

‡ P_{trend} is based on a Wald statistic for a trend variable assigned the median for the appropriate quartile.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

In contrast to the enhanced clearance of transient cervical HPV infections associated with serum micronutrients, clearance of persistent HPV infection was not significantly associated with circulating concentrations of carotenoids, retinols, or tocopherols (Table 3).

We examined the possibility of effect modification by age of the micronutrient associations with clearance times for transient, persistent, and transient plus persistent infections through stratification by median age (28 years; results not shown). Stratified models exhibited some variation in hazard ratio estimates for several micronutrients, but the direction of the effects (protective or otherwise) remained the same. The interaction term between micronutrient trend and age group was found not statistically significant for any of the 20 micronutrients.

Discussion

Results of this study suggest that circulating concentrations of several antioxidant micronutrients may decrease the length of incident HPV type–specific infection. The increased risk of HPV

Table 3. Hazard ratios and 95% confidence intervals for clearance of incident transient and persistent high-risk HPV infection by levels of circulating plasma micronutrients

Micronutrient/ quartile	Quartile range (ng/mL)	Transient infections (first 120 d since detection)			Persistent infections (after 120 d of detection)		
		No. cleared/ infections (mo)*	Hazard ratio† (95% confidence limits)	P_{trend}‡	No. cleared/ infections (mo)*	Hazard ratio† (95% confidence limits)	P_{trend}‡
Trans-lutein							
Low	41.87-106.63	6/249	1.00		27/290	1.00	
2	106.64-156.70	6/248	0.93 (0.28–3.12)		28/308	1.40 (0.84–2.36)	
3	156.71-225.21	13/213	3.03 (1.20–7.70)		18/226	0.93 (0.52–1.68)	
High	225.22-624.18	7/249	1.74 (0.50–5.99)	0.33	28/282	1.40 (0.82–2.36)	0.38
Trans-zeaxanthin							
Low	20.28-46.40	6/245	1.00		29/290	1.00	
2	46.41-58.22	8/254	1.99 (0.67–5.87)		22/279	0.71 (0.43–1.18)	
3	58.23-82.69	10/232	3.24 (1.02–10.28)		19/253	0.58 (0.32–1.08)	
High	82.70-281.10	8/229	2.18 (0.52–9.17)	0.36	31/283	1.27 (0.73–2.22)	0.17
Total *trans*-lutein/zeaxanthin							
Low	73.53-158.68	4/242	1.00		31/311	1.00	
2	158.69-212.03	6/252	1.08 (0.27–4.34)		23/266	1.09 (0.63–1.87)	
3	212.04-303.92	13/229	4.75 (1.62–13.89)		18/254	0.73 (0.39–1.35)	
High	303.93-905.29	9/237	3.15 (1.04–9.53)	0.03	23/275	1.26 (0.75–2.11)	0.42
Total *cis*-lutein/zeaxanthin and *trans*-lutein/zeaxanthin							
Low	112.67-259.20	4/252	1.00		33/310	1.00	
2	259.21-332.78	8/238	1.80 (0.59–5.51)		17/266	0.88 (0.50–1.56)	
3	332.79-452.23	12/232	4.53 (1.32–15.54)		26/267	1.15 (0.65–2.04)	
High	452.24-1,287.6	8/239	3.05 (0.87–10.74)	0.11	25/262	1.11 (0.63–1.93)	0.60
α-Cryptoxanthin							
Low	0.33-33.52	6/219	1.00		31/296	1.00	
2	33.53-43.53	5/242	0.83 (0.24–2.82)		28/312	0.71 (0.41–1.21)	
3	43.54-54.44	10/251	1.81 (0.65–5.05)		18/239	0.84 (0.43–1.65)	
High	54.45-106.45	11/248	1.97 (0.76–5.10)	0.06	24/258	0.73 (0.39–1.37)	0.54
β-Cryptoxanthin							
Low	17.54-113.85	5/237	1.00		29/306	1.00	
2	113.86-158.65	6/244	1.31 (0.34–5.10)		29/301	0.85 (0.52–1.38)	
3	158.66-242.59	7/234	1.78 (0.51–6.17)		22/267	0.86 (0.50–1.46)	
High	242.60-1,114.6	14/245	4.65 (1.65–13.09)	0.003	21/231	0.94 (0.51–1.73)	0.97
Total cryptoxanthin							
Low	17.87-148.75	6/235	1.00		27/295	1.00	
2	148.76-207.65	5/245	0.87 (0.20–3.75)		30/306	0.91 (0.54–1.53)	
3	207.66-296.12	7/237	1.46 (0.44–4.85)		23/274	0.99 (0.57–1.71)	
High	296.13-1,164.6	14/244	3.47 (1.36–8.87)	0.003	21/230	1.02 (0.54–1.94)	0.84
Total *trans*-lycopene and *cis*-lycopene							
Low	0.00-29.49	3/241	1.00		28/312	1.00	
2	29.50-42.89	10/239	2.44 (0.51–11.75)		26/277	0.99 (0.60–1.64)	
3	42.90-59.30	4/241	1.51 (0.27–8.34)		27/300	1.15 (0.70–1.88)	
High	59.31-245.11	15/238	7.03 (1.61–30.70)	0.004	20/216	1.14 (0.64–2.02)	0.57
Total lycopene							
Low	55.96-251.67	9/236	1.00		25/258	1.00	
2	251.68-337.62	9/213	1.28 (0.42–3.91)		25/267	1.03 (0.60–1.74)	
3	337.63-449.99	7/262	0.50 (0.16–1.54)		29/296	1.16 (0.71–1.89)	
High	450.00-941.04	7/249	0.61 (0.18–2.09)	0.32	22/285	0.60 (0.35–1.04)	0.07
α-Carotene							
Low	0.07-26.81	6/246	1.00		26/305	1.00	
2	26.82-46.00	8/248	1.16 (0.45–2.98)		25/297	0.76 (0.44–1.29)	
3	46.01-68.81	4/225	0.84 (0.20–3.51)		26/270	0.91 (0.49–1.72)	
High	68.82-388.36	14/241	3.02 (1.07–8.53)	0.02	24/233	1.27 (0.67–2.39)	0.25
Trans-β-carotene							
Low	1.87-86.14	7/245	1.00		28/296	1.00	
2	86.15-160.90	5/229	0.79 (0.25–2.46)		22/280	0.85 (0.47–1.52)	

(*Continued on the following page*)

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

Table 3. Hazard ratios and 95% confidence intervals for clearance of incident transient and persistent high-risk HPV infection by levels of circulating plasma micronutrients (Cont'd)

Micronutrient/ quartile	Quartile range (ng/mL)	Transient infections (first 120 d since detection)			Persistent infections (after 120 d of detection)		
		No. cleared/ infections (mo)*	Hazard ratio† (95% confidence limits)	P_{trend}‡	No. cleared/ infections (mo)*	Hazard ratio† (95% confidence limits)	P_{trend}‡
3	160.91-252.15	6/268	0.49 (0.14–1.76)		28/292	1.34 (0.79–2.28)	
High	252.16-1,405.5	14/218	3.52 (1.44–8.62)	0.008	23/238	1.43 (0.76–2.69)	0.11
Cis-β-carotene							
Low	0.00-7.78	5/249	1.00		33/304	1.00	
2	7.79-14.76	8/224	1.31 (0.37–4.65)		17/271	0.74 (0.40–1.39)	
3	14.77-23.95	8/258	1.22 (0.31–4.88)		25/279	1.03 (0.60–1.77)	
High	23.96-97.36	11/228	3.43 (1.18–9.93)	0.03	26/252	1.63 (0.90–2.95)	0.04
Total β-carotene							
Low	2.53-96.54	6/242	1.00		30/300	1.00	
2	96.55-172.86	5/239	0.90 (0.29–2.77)		22/286	0.82 (0.46–1.46)	
3	172.87-281.15	8/259	0.88 (0.27–2.92)		26/278	1.23 (0.72–2.08)	
High	281.16-1,496.4	13/220	3.79 (1.59–9.00)	0.005	23/242	1.31 (0.70–2.47)	0.19
Total carotene							
Low	2.60-127.16	6/247	1.00		29/295	1.00	
2	127.17-225.29	5/213	1.08 (0.35–3.32)		22/294	0.86 (0.49–1.50)	
3	225.30-379.95	7/272	0.82 (0.25–2.66)		25/281	1.15 (0.67–1.99)	
High	379.96-1,724.4	14/228	3.72 (1.50–9.23)	0.007	25/236	1.56 (0.82–2.96)	0.08
Total carotenoids							
Low	198.91-983.25	5/229	1.00		27/283	1.00	
2	983.26-1,329.3	10/256	1.70 (0.44–6.51)		24/302	1.01 (0.58–1.75)	
3	1,329.4-1,755.8	6/242	1.42 (0.36–5.55)		27/271	1.12 (0.62–2.02)	
High	1,755.9-3,979.0	11/233	2.73 (0.79–9.38)	0.11	23/248	1.29 (0.69–2.43)	0.36
Retinol							
Low	0.00-569.45	9/284	1.00		26/279	1.00	
2	569.46-719.20	10/244	0.91 (0.32–2.60)		21/231	1.04 (0.56–1.92)	
3	719.21-865.26	8/224	1.03 (0.36–2.92)		29/282	0.87 (0.52–1.44)	
High	865.27-1,624.2	5/208	0.86 (0.22–3.41)	0.87	25/314	0.65 (0.40–1.07)	0.051
α-Tocopherol							
Low	3,490.4-7,536.0	9/269	1.00		30/296	1.00	
2	7,536.1-9,391.3	6/231	1.05 (0.31–3.56)		27/253	1.01 (0.55–1.86)	
3	9,391.4-12,375	5/288	0.74 (0.24–2.29)		20/295	0.80 (0.46–1.39)	
High	12,376-36,621	12/171	3.48 (1.33–9.11)	0.02	24/262	1.18 (0.60–2.33)	0.68
β-Tocopherol and γ-tocopherol							
Low	29.80-643.85	8/244	1.00		28/292	1.00	
2	643.86-1,073.0	11/228	1.43 (0.46–4.43)		22/277	0.66 (0.37–1.19)	
3	1,073.1-1,573.6	4/246	0.34 (0.08–1.51)		28/275	0.83 (0.47–1.47)	
High	1,573.7-3,564.0	9/241	0.98 (0.33–2.95)	0.56	23/261	0.88 (0.51–1.52)	0.89
δ-Tocopherol							
Low	24.28-165.59	9/251	1.00		23/258	1.00	
2	165.60-243.57	9/225	1.41 (0.38–5.31)		23/297	0.78 (0.44–1.38)	
3	243.58-417.73	7/246	1.04 (0.29–3.78)		29/271	1.02 (0.56–1.85)	
High	417.74-1,090.3	7/237	1.05 (0.29–3.79)	0.88	26/280	1.01 (0.56–1.82)	0.66
Total tocopherol							
Low	4,528.2-9,336.0	8/258	1.00		29/292	1.00	
2	9,336.1-10,885	7/236	1.19 (0.39–3.68)		27/264	1.03 (0.54–1.96)	
3	10,886-13,604	6/289	0.96 (0.35–2.68)		19/274	0.88 (0.45–1.71)	
High	13,605-37,092	11/178	3.25 (1.21–8.71)	0.03	26/276	1.32 (0.69–2.50)	0.37

*Cumulative infection length in months, from first positive to first negative visit.
† Adjusted for age at first visit, current tobacco smoking status, number of lifetime sexual partners, age at first sex, blood frozen over 1 y indicator variable, coinfection status.
‡ The P for trend is based on a Wald statistic for a trend variable assigned the median for the appropriate quartile.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

type–specific clearance was strongest among women with high circulating levels of several carotenes with vitamin A potential, although no association was found for retinol. Carotenoids with less vitamin A potential, such as β-cryptoxanthin and total *trans*-lycopene and *cis*-lycopene, were also associated with reduced clearance times. The association of high circulating levels of micronutrients with the likelihood of HPV clearance was most evident among women who resolved their infections rapidly (within 120 days). Among women with transient infections, most carotenoids measured seemed to enhance time to clearance. In contrast, serum micronutrients were generally unrelated to clearance of longer-term HPV infections, suggesting a time-related association with immune response to the virus. In addition, a modest association was found for the tocopherols among the subgroup of women with rapid clearance and high circulating levels of this micronutrient.

The failure of nutrient-based phase III chemoprevention trials to enhance the regression of cervical SIL supports the observation that micronutrients may only be active early in the natural history of cervical carcinogenesis (29). Only a few investigators have examined the association of circulating micronutrient concentrations with the clearance of cervical HPV infection. In the first of these studies, Giuliano et al. (9) collected two cervical specimens 3 months apart for HPV DNA testing by Hybrid Capture among 123 women. Blood was collected at the second visit for antioxidant nutrient assessment. Mean serum concentrations of β-carotene, β-cryptoxanthin, lutein, α-tocopherol, and γ-tocopherol were lower among women who were HPV positive on both visits compared with women who were always HPV negative or HPV positive on a single visit. In the Young Women's Health Study, Sedjo et al. (11) compared serum antioxidant levels taken at the first follow-up visit among 101 women who were oncogenic HPV-positive by Hybrid Capture II on two or three consecutive visits (persistent) with 58 women who were positive for an oncogenic HPV infection on a single visit. Although none of the differences in antioxidant levels were statistically significant, the odds ratio associated with the highest compared with the lowest tertile of *cis*-lycopene was 0.44 (95% confidence interval, 0.19–1.01), suggesting that higher levels were associated with more transient infections. In an analysis of a subsample of 84 participants in the Young Women's Health Study who were positive for at least one oncogenic HPV type by PCR at baseline, Sedjo et al. (12) reported that the likelihood of clearing an oncogenic HPV infection was significantly greater among women with higher levels of *cis*-lycopene and *trans*-lycopene measured at their first follow-up visit. Although these investigations are consistent with an association of circulating and dietary micronutrient concentrations with cervical HPV clearance, in a 9-month clinical trial of β-carotene against cervical dysplasia among 69 women, Palan et al. (24) found no association of persistent HPV infection detected by Southern blot and PCR with mean levels of circulating retinol, α-carotene, β-carotene, or lycopene. This was in agreement with Siegel et al. (14) who found no association of type-specific oncogenic HPV persistence with circulating levels of retinol, tocopherols, or carotenoids measured over four clinical visits among 229 women in the Ludwig-McGill Cohort study.

A unique aspect of our study design was the use of PCR to examine type-specific clearance of cervical HPV infection. Most longitudinal studies of circulating micronutrients relied on Hybrid Capture (9, 11) to detect a panel of HPV types and were therefore susceptible to misclassification of HPV persistence. This method cannot distinguish single from multiple infections or whether persistent HPV-positive tests represent infection with the same or newly acquired HPV types. Patterns of multiple and newly acquired infections in our cohort suggest that the potential for misclassification is great in studies that do not have HPV type–specific information. Only the recent study by Siegel et al. (14) modeled type-specific HPV persistence, but, in contrast to our study, these investigators did not distinguish prevalent from incident HPV infections and were hence unable to examine the effect of duration of infection on the risk of viral persistence. This distinction would seem important based on the divergent results for incident compared with persistent infections found in our analysis.

A further advantage of our study design was the measurement of serum micronutrient concentrations at each visit. This allowed us to examine the relation of change in micronutrient level over time with the persistence or clearance of HPV infection. With the exception of the Ludwig-McGill Cohort study analysis (14), previous investigations were limited to a single serum/plasma micronutrient measurement; thus, it was unclear how HPV persistence and nutrient levels were related over time. Furthermore, Cooney et al. (30) reported substantial inter-individual and intra-individual variability in serum antioxidant concentrations taken from the same individual at 2- to 4-week intervals, underscoring the benefit of multiple blood samples on cohort members.

In this study, we defined clearance based on a single HPV-negative test. Under this definition, if there is a period of viral latency in which HPV DNA levels are undetectable, a woman who seems to have cleared her infection may still be persistent. If viral latency occurs, it is possible that a fraction of the "incident" cases of cervical HPV might reflect a reactivation of a prior infection rather than a new acquisition. Among these women, the rapid clearance of HPV may represent a robust response to reactivation rather than a rapid clearance of a new infection. Moscicki et al. (31) used a more stringent definition of clearance requiring two or more HPV-negative tests to reduce this source of potential bias. Although this more conservative definition limits the possibility of false-negative results, it would have reduced the sample of women available for analysis. We examined the potential influence of our clearance definition on the results by counting the number of re-infections with the same genotype after that genotype was cleared (i.e., after at least one negative was observed). Overall, 7 of 189 infections reappeared after at least one negative visit. One of the seven infections reappeared after six negatives; thus, it is unlikely that it was a reactivation of a latent infection. The remaining six infections reappeared after one negative. This finding is similar to that of Molano et al. (32) who reported that only 5 of 223 HPV infections that had cleared on one visit were positive again for the same HPV type on a subsequent visit. Based on these results, it would seem unlikely that the more stringent definition would have materially altered our findings.

The immune response is the key determinant in the persistence of cervical HPV infection and involves both innate and adaptive immunity (3, 4, 33). Although numerous immune pathways are likely involved in the host defense against cervical HPV infection, women with transient infections are less likely to develop humoral or cell-mediated immune responses than women with persistent infections, suggesting a role for innate immunity in the rapid clearance of the virus (2). Dietary components that possess antioxidant properties may protect the immune system from oxidative damage and enhance immune responsiveness (34). Santos et al. (35) found that β-carotene supplementation resulted

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

in significantly greater natural killer cell activity compared with placebo, suggesting that β-carotene may induce natural killer cell-enhancing cytokines. Carotenoids may regulate antigen presentation by immature dendritic cells in the presence of inflammatory stimuli (e.g., tumor necrosis factor-α), but the precise mechanism for this immune enhancement is unknown (36). Immune cells are particularly vulnerable to oxidative stress; thus, the antioxidant levels in these cells play an important role in maintaining a reduced environment and preserving cellular function. Antioxidants maintain the function of membrane lipids that, if peroxidized, can lead to loss of membrane integrity and altered membrane fluidity resulting in altered intracellular signaling (16). The few investigations of the influence of other carotenoids, such as lycopene and lutein, on immune function show little association with monocyte surface molecules involved with antigen presentation (37). It is possible that the plasma levels of these compounds achieved with physiologic doses are not sufficient to induce measurable changes in immunity. Furthermore, the immune response in healthy, well-nourished individuals may not be easily influenced by vitamin supplementation.

In conclusion, results from this investigation support a role for several carotenoids or dietary factors associated with carotenoids in the rapid clearance of incident oncogenic HPV infection of the cervix. It is possible that this association with HPV clearance might explain the protective effect against cervical neoplasia found for α-carotene, β-carotene, lycopene, lutein/zeaxanthin, and cryptoxanthin (8). In future studies, we will be able to investigate the relation of circulating micronutrient levels with the clearance of specific HPV types. We will also be able examine the joint association of local cervical cytokine and micronutrient levels with the risk of HPV clearance. Increased understanding of the joint role of innate and cellular immunity with nutritional status in the clearance of HPV infections will contribute to the understanding of effective anti-HPV cellular responses.

Acknowledgments

Received 1/24/2007; revised 3/16/2007; accepted 4/11/2007.

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.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/12/5987/2569695/5987.pdf by guest on 05 February 2023

References

1. Bosch FX, Manos MM, Munoz N, et al.; International biological study on cervical cancer (IBSCC) Study Group. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 1995; 87:796–802.
2. Moscicki AB, Schiffman M, Kjaer S, Villa LL. Chapter 5: updating the natural history of HPV and anogenital cancer. Vaccine 2006;24:S42–51.
3. Stanley M. Immune responses to human papillomavirus. Vaccine 2006;24:S16–22.
4. Konya J, Dillner J. Immunity to oncogenic human papillomaviruses. Adv Cancer Res 2001;82:205–38.
5. Castellsague X, Bosch FX, Munoz N. Environmental co-factors in HPV carcinogenesis. Virus Res 2002;89: 191–9.
6. Castellsague X, Munoz N. Chapter 3: cofactors in human papillomavirus carcinogenesis-role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 2003;31:20–8.
7. Wang SS, Hildesheim A. Chapter 5: viral and host factors in human papillomavirus persistence and progression. J Natl Cancer Inst Monogr 2003;31:35–40.
8. Garcia-Closas R, Castellsague X, Bosch X, Gonzalez CA. The role of diet and nutrition in cervical carcinogenesis: a review of recent evidence. Int J Cancer 2005;117:629–37.
9. Giuliano AR, Papenfuss M, Nour M, Canfield LM, Schneider A, Hatch K. Antioxidant nutrients: associations with persistent human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 1997;6: 917–23.
10. Palan PR, Chang CJ, Mikhail MS, Ho GY, Basu J, Romney SL. Plasma concentrations of micronutrients during a nine month clinical trial of β-carotene in women with precursor cervical cancer lesions. Nutr Cancer 1998;30:46–52.
11. Sedjo RL, Roe DJ, Abrahamsen M, et al. Vitamin A, carotenoids, and risk of persistent oncogenic human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 2002;11:876–84.
12. Sedjo RL, Papenfuss MR, Craft NE, Giuliano AR. Effect of plasma micronutrients on clearance of oncogenic human papillomavirus (HPV) infection (United States). Cancer Causes Control 2003;14:319–26.
13. Giuliano AR, Siegel EM, Roe DJ, et al. Dietary intake and risk of persistent human papillomavirus (HPV) infection: the Ludwig-McGill HPV Natural History Study. J Infect Dis 2003;188:1508–16.
14. Siegel EM, Craft NE, Duarte-Franco E, Villa LL, Franco EL, Giuliano AR. Associations between serum carotenoids and tocopherols and type-specific HPV persistence: the Ludwig-McGill cohort study. Int J Cancer 2007;120:672–80.
15. Calder PC, Grimble RF. Polyunsaturated fatty acids, inflammation and immunity. Eur J Clin Nutr 2002;563: S14–9.
16. Hughes DA. Dietary carotenoids and human immune function. Nutrition 2001;17:823–7.
17. Gravitt P, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38:357–61.
18. Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by single-hybridization, reverse line blot detection method. J Clin Microbiol 1998;36:3020–7.
19. Peyton CL, Gravitt PE, Hunt WC, et al. Determinants of genital human papillomavirus detection in a US population. J Infect Dis 2001;183:1554–64.
20. Franke AA, Custer LJ, Cooney RV. Synthetic carotenoids as internal standards for plasma micronutrient analysis by high-performance liquid chromatography. J Chromatogr B 1993;614:43–57.
21. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348:518–27.
22. Andersen PK, Gill RD. Cox's regression model for counting processes: a large sample study. Ann Stat 1982; 10:1100–20.
23. Lin DY, Wei LJ. The robust inference for the Cox proportional hazards model. J Am Stat Assoc 1989;84: 1074–8.
24. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 1994;81:515–26.
25. Therneau TM, Grambsch PM, Fleming TR. Martingale-based residuals for survival models. Biometrika 1990;77:147–60.
26. Schoenfeld D. Partial residuals for the proportional hazards regression model. Biometrika 1982;69: 239–41.
27. Quandt RE. The estimation of the parameter of a linear regression system obeying two separate regimes. J Am Stat Assoc 1958;53:873–80.
28. Hinkley DV. Inference in two-phase regression. J Am Stat Assoc 1971;66:736–43.
29. Castle PE, Giuliano AR. Chapter 4: genital tract infections, cervical inflammation, and antioxidant nutrients-assessing their roles as human papillomavirus cofactors. J Natl Cancer Inst Monogr 2003;31: 29–34.
30. Cooney RV, Franke AA, Hankin JH, et al. Seasonal effects on intra-individual variations in plasma micronutrients and antioxidants. Cancer Epidemiol Biomarkers Prev 1995;4:207–15.
31. Moscicki AB, Shiboski S, Broering J, et al. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr 1998;132:277–84.
32. Molano M, Van den Brule A, Plummer M, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am J Epidemiol 2003;158:486–94.
33. Frazer IH. Immunology of papillomavirus infection. Curr Opin Immunol 1996;8:484–91.
34. Geissmann F, Revy P, Brousse N, et al. Retinoids regulate survival and antigen presentation by immature dendritic cells. J Exp Med 2003;198:623–34.
35. Santos MS, Meydani SN, Leka L, et al. Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. Am J Clin Nutr 1996;64:772–5.
36. Stephensen CB. Vitamin A, infection, and immune function. Annu Rev Nutr 2001;21:167–92.
37. Hughes DA, Wright AJA, Finglas PM, et al. Effects of lycopene and lutein supplementation on the expression of functionally associated surface molecules on blood monocytes from healthy male nonsmokers. J Infect Dis 2000;182:S11–5.