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Plasma Micronutrients and the Acquisition and Clearance of Anal Human Papillomavirus Infection: the Hawaii HPV Cohort Study

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Abstract

Anal human papillomavirus (HPV) infection is common among women and the cause of most anal malignancies. The incidence of anal cancer has been increasing among U.S. women; yet few co-factors for the natural history of anal HPV infection have been identified. We examined the hypothesis that plasma carotenoid, retinol, and tocopherol concentrations are associated with the acquisition and clearance of anal HPV infection in a cohort of 279 Hawaiian residents followed at 4-month intervals for a mean duration of 16 months. At each visit, interviews were conducted and biological specimens were obtained, including anal cell specimens for HPV DNA detection and genotyping, and a fasting blood sample to measure 27 micronutrients. Cohort participants acquired 189 anal HPV infections, 113 of which cleared during the study period. The most frequently acquired HPV genotypes were HPV-52, 53, 84, and 16. Women in the highest quartile of *trans*-zeaxanthin, *trans*-anhydro-lutein, *trans*-, *cis*-, and total β -carotene had significant 43-50% reduction in the risk of acquisition of any HPV infection compared to women in the lowest quartile. Few associations were observed between micronutrient levels and clearance of transient (≤ 150 days) anal HPV infections. However, clearance of persistent (> 150 days) infections was associated with higher levels of $\beta + \gamma$ -tocopherol and lower levels of carotenoids and retinol. Our findings suggest that several carotenoids can reduce the risk and clearance of anal HPV infections that contribute to anal cancer.

Keywords

human papillomavirus; cohort study; micronutrients; carotenoids; tocopherols

INTRODUCTION

Anal cancer is an uncommon malignancy, occurring at a rate of ~ 1.5 per 100,000 in the US, but its incidence has increased substantially during the past several decades in the US and other countries (1-3). The rate of invasive anal squamous cell carcinoma increased by 1.7% per year, and *in situ* tumors increased by 2.9% per year between 1973 and 2005 (1), but the basis for this increase is not well understood. Anal cancer is one of the few malignancies

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without a known hormonal etiology that is more frequent among women than among men. In the US, there were an estimated 5,290 new cases of anal cancer in 2009, with 3,190 cases occurring in women and 2,100 cases occurring in men (4).

During the past decade, epidemiological studies have shown that the majority of anal cancers in both sexes are associated with human papillomavirus (HPV) (5), the most common sexually-transmitted infection (6). It is unknown why women are at greater risk of squamous cell anal cancer and are more likely to have HPV-positive anal cancer than men (1). Risk factors for anal malignancies in females include a history of other HPV-associated diseases (7,8), anal receptive intercourse (9,10), tobacco smoking (10), and multiple sexual partners (10,11). However, little is known regarding the natural history of anal HPV, particularly the correlates of HPV acquisition and clearance among women. In some populations of women, the prevalence of anal HPV infection may be at least as high as the prevalence of cervical HPV infection (12-14), but the viral infection appears to clear far more rapidly in the anus than in the cervix (15). The biological basis for the relatively rapid clearance of anal HPV infection compared to cervical HPV infection has not been determined; nor whether this rapid clearance explains the comparatively low rate for anal malignancy.

One class of potential etiologic factors that has been investigated in relation to HPV infection of the cervix, but not the anus, is circulating micronutrient levels (16-21). Studies found significant associations of various carotenoids and tocopherols with cervical HPV acquisition and clearance (16-18,21). The objective of the present analysis was to examine the association of anal HPV acquisition and clearance with circulating levels of 27 micronutrients in a longitudinal cohort of Hawaii women.

MATERIALS AND METHODS

Subject Recruitment and Follow-Up

Between 1998 and 2003, a cohort of sexually active women was established for a longitudinal study of cervical and anal HPV infection (15,21,22). Cohort participants were recruited among women attending five clinics on Oahu, Hawaii, who were able to read, understand, and sign an informed consent and medical release form approved by the University of Hawaii Institutional Review Board. Potentially eligible patients included those with appointments for new or annual gynecologic examinations or for family planning services, who were not pregnant or postpartum within the past six months, and had no plans to relocate within a year.

At the first and subsequent clinic visits, a gynecologic examination was performed, exfoliated anal and cervical specimens for a Pap smear and HPV DNA analysis were collected, and a blood sample was drawn to measure circulating micronutrient levels. Anal cell specimens were obtained using a Dacron swab moistened with sterile water. The swab was inserted 1.5 to 2.0 cm into the anus, rotated several times, and placed in 1.0 mL buffered medium (Digene Corp., Gaithersburg, MD). Women entering the cohort were asked to return every four months for repeat examination and testing.

A study questionnaire was administered by the study interviewer after each clinical examination. The baseline questionnaire included demographic and sexual activity data, and histories of tobacco and alcohol use. A more detailed interview, conducted during the second visit after a four-month interval, included gynecological, menstrual, reproductive and sexual histories, hormone use, medical history, history of sexually transmitted infections, and income. Information covered in the baseline questionnaire (sexual activity, tobacco and alcohol use) was also updated. The questionnaire used at subsequent interviews was

modified slightly for use during the follow-up period; questions included changes in sexual and reproductive information during the period since the last clinic visit.

Laboratory Analysis

Detection and genotyping of HPV—HPV DNA was extracted from exfoliated anal and cervical cell specimens using commercial reagents (Qiagen Inc, Valencia CA). Specimens were analyzed for presence of HPV DNA by PCR using a modified version of the PGMY09/PGMY11 primer system (23). HPV DNA-positive specimens were genotyped using a reverse line blot detection method for 36 different HPV types including high-risk (HR) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (24); possible high-risk types 26, 34, 53, 66, 69, 70, 73, 82; low-risk (LR) types 6, 11, 40, 42, 44, 54, 61, 72, 81, and 89; and undetermined-risk types 62, 67, 71, 83, and 84 (25,26). We defined the risk (oncogenic potential) associated with various HPV types using the International Agency for Research on Cancer definition (26). HPV-positive specimens subsequently found to be negative in the genotyping assay were considered unclassified HPV-positive specimens. All specimens were also tested for the human β -globin gene as an internal control for sample sufficiency. The 163 of 1257 anal specimens which tested negative for β -globin were considered insufficient and were excluded from analyses.

Micronutrient analysis—Blood was drawn at the clinic into heparin tubes after a 10-12 hour fast and was used to determine plasma micronutrient levels of both *cis* and *trans* forms of lutein/zeaxanthin, α -, β - and total cryptoxanthin, *trans* and *cis* forms of lycopene, α -carotene, *trans* and *cis* forms of β -carotene, total carotene, retinol, α -, γ - and δ -, and total tocopherol. Total nutrient levels were determined by adding up all individual isoforms. The plasma was separated from the cells by centrifugation (4° C for 15 min, 1800 $\times$ g) and frozen (−80° C) until analyzed. The median time from blood draw to freezer was under two hours. Specimens were protected from light during processing. Sample extracts were analyzed by reversed-phase HPLC with photo diode array detection (27,28). Absorption spectra and retention times for each peak were compared to those of known authentic standards. Briefly, under subdued light 0.5 ml of thawed plasma was vortexed by hand for at least 1 minute with 0.5 ml of ethanol containing 0.3 mg/L *n*-butyl-, β -apo-8'-carotenoate (as an internal standard for carotenoids), 1.0 mg/L retinyl laurate (as an internal standard for retinoids), 6.0 mg/L tocopherol (as an internal standard for tocopherols), and 250 mg/L butylated hydroxytoluene (as preservative) followed by extraction with hexane. After drying the hexane extracts with nitrogen at ambient temperature and redissolving it in mobile phase, 0.01 mL were subjected to isocratic C18 HPLC analysis. The high quality of all laboratory analyses was assured by participation in the proficiency test program for lipid soluble vitamins organized by the U.S. National Institute for Standards and Technologies (Gaithersburg, Maryland) and by the inclusion of pooled plasma from 8 healthy volunteers to measure variation during the three month period between study visits, which showed a coefficient of variation for all analytes between 4 and 10%. The intraclass correlation coefficients were above 0.6 for all but three micronutrients (29), and all of the micronutrients were detectable in each plasma sample (28).

Statistical Analysis

Of the 972 women who completed at least 2 clinic visits, we limited the analyses to 279 women who provided valid anal, cervical and blood samples, as well as interview information at two or more clinical visits. The excluded women had younger age but did not differ in any other baseline characteristics. Anal HPV infections were classified by oncogenic risk: all infections, high-risk (HR) genotypes, and possible, low or undetermined-risk (LR) genotypes. Infections with unclassified HPV types and clinic visits with missing or inadequate specimens were excluded from all analyses. To prevent bias in clearance and

duration estimates due to left censoring, we only considered incident infections, i.e. infections first detected at the second or subsequent visit. The time to clearance was defined as the time from the first detection of an HPV genotype until the first negative result for that genotype. If a study participant reacquired the same HPV genotype after clearance, only the first infection with that genotype was considered.

The association of anal HPV acquisition and clearance with plasma concentrations of micronutrients was modeled through Cox regression. Micronutrient concentrations were measured longitudinally at each clinic visit and included in the models as time-dependent variables. All analyses were adjusted, by inclusion as a strata variable, for the presence or absence of a concordant (same genotype) cervical HPV infection detected at any time a woman was at risk for acquisition or clearance of the index anal infection. Such adjustment was conducted to rule out the possibility that the observed findings were primarily driven by an association of micronutrients with HPV infection in the cervix, rather than the anus. All analyses were repeated for anal infections with no concordant cervical infection, yielding similar results (data not shown). Days since study entry (for acquisition) and days since infection acquisition (for clearance) were used as the time metrics. Adjustment variables were selected among baseline demographic and behavioral risk factors that were significantly associated with acquisition or clearance of anal HPV in our cohort (15,22). These risk factors included age, current tobacco smoking, current practice of anal sex, and history of pregnancy during lifetime. We additionally adjusted for the amount of time a blood sample was kept frozen between blood draw and laboratory analysis. The inclusion of other risk factors, such as the number of sexual partners, in the models did not result in over 10% change in the parameter estimates (30), nor in a significantly better fit according to the likelihood ratio test. We additionally checked for correlation among the micronutrient levels. While isoforms of the same micronutrient were strongly correlated (≥ 0.8), correlation between different micronutrients was moderate to low (0-0.6; data not shown).

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 a reference category. The Wald χ^2 statistic was used to evaluate the linear trend based on the median micronutrient level within each quartile. A woman could be infected with more than one HPV type at one time. We assigned a separate infection path to every genotype detected. 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 (31), aggregated over subjects, to prevent artificially deflated standard errors and confidence interval estimates. Time-dependent indicator variables, measured at every visit for each study subject, were used to account for the presence of a concordant cervical HPV infection and the presence of anal infection with multiple HPV types.

The proportional hazard assumption for Cox models was verified by plotting scaled Schoenfeld residuals against time to HPV acquisition or clearance (32). Although the assumptions were not significantly violated, there was evidence that the effect of some micronutrients on HPV clearance varied with time. Therefore, we adopted a change-point estimation approach for clearance models (33,34), to account for a possible differential effect of micronutrients on HPV clearance at the early (transient) and late (persistent) stage of infection. The change-point selection was performed by visual inspection of Schoenfeld residual plots, which showed an inflection located between 120 and 180 days after infection acquisition. We selected 150 days as the change point for all clearance analyses because this was the median time interval between clinic visits and because most intervals between visits were longer than 120 days, preventing us from detecting clearance events in the first 120 days following infection acquisition. Cox models were fit separately for the transient stage of HPV infection (first 150 days after acquisition) and persistent stage (from day 151 until

clearance or censoring). Other change points were examined, but the results were similar. All analyses were conducted using SAS version 9.1.3 (SAS Institute, Inc., Cary NC). All *p*-values were two-sided, and *p* < 0.05 was defined as statistically significant.

RESULTS

Cohort participants completed a total of 1090 clinical visits, with a mean of 4 visits per woman and an average of 1.3 years follow-up (Table 1). The cohort composition was multiethnic and participants were 38 years of age, on average, at cohort entry. Only 14% of women were current tobacco smokers, and 7% reported history of anal sex. About two thirds of the cohort had a history of pregnancy, and 107 women had prevalent anal HPV infections. A total of 189 incident anal HPV infections were observed during the follow-up period. The most frequently acquired HPV genotypes were HPV-52, 53, and 84, followed by HPV-16, 56, and 62 (Figure 1). We have previously reported on the distribution of micronutrient levels in the same group of women (29).

Higher circulating carotenoid levels were generally associated with a reduced risk of anal HPV acquisition (Table 2). Significant inverse associations of incident anal HPV with carotenoid levels were observed for *trans*-zeaxanthin, total β -carotene, and total carotenoids. Women in the highest quartile of *trans*-zeaxanthin and total β -carotene had significant 43-50% reductions in the risk of any-HPV infection compared to women in the lowest quartile. The magnitude and direction of the associations of circulating carotenoids with the risk of high-risk and low-risk HPV infections were generally similar (Supp. Table 1). Levels of *trans*-zeaxanthin in the highest quartile were significantly associated with lower risk of LR HPV, and *cis*- β -carotene was significantly related to reduced risk of both HR- and LR-HPV. No significant trends in the risk of anal HPV acquisition were associated with circulating levels of the tocopherols, retinol, or retinyl palmitate.

We examined the association of circulating micronutrient levels with anal HPV clearance (Table 3; Supp. Table 2). Higher levels of *cis*- β -cryptoxanthin were significantly associated with decreased clearance of any-genotype and LR anal HPV, and higher levels of β + γ -tocopherol were associated with faster clearance. The latter observation was particularly true for HR-HPV infections in which the risk of clearance among women in the highest quartile of β + γ -tocopherol was five-fold that of women in the lowest quartile. The risk of clearance of LR-HPV infection was decreased significantly with higher levels of circulating *trans*-zeaxanthin, total *cis*-lutein/zeaxanthin, total lutein/zeaxanthin, and *cis*- β -cryptoxanthin; and increased significantly with higher levels of β + γ -tocopherol.

A total of 132 infections with 617 person-months of follow-up were included in the analysis of transient ($\leq$ 150 days) HPV infections; and 85 infections with 580 person-months of follow-up were included in the analysis of persistent ($>$ 150 days) infections (Table 4; Supp. Table 3). Few associations of circulating micronutrient levels with clearance of transient anal HPV infections were observed. Clearance of persistent anal HPV infection was 60% to 73% lower among women with the highest compared to the lowest quartile levels of *trans*-lutein, *trans*-zeaxanthin, total *trans*-lutein/zeaxanthin, total lutein/zeaxanthin, *trans*-, *cis*-, and total β -cryptoxanthin, and total cryptoxanthin. In contrast to the carotenoids, clearance of persistent anal HPV infections was significantly enhanced in women with higher circulating levels of β + γ -tocopherol.

DISCUSSION

Our results suggest that high levels of several carotenoids are associated with reduced risk of anal HPV acquisition and with delayed clearance of persistent HPV infection, while high

levels of $\beta + \gamma$ -tocopherol are associated with faster clearance of anal infections. The reduction in the risk of incident anal HPV infection was considerable for some of the carotenoids: up to 50% among women with the highest compared to women with the lowest circulating levels of total β -carotene, a common carotenoid that is readily convertible to vitamin A. Although only *cis*- β -carotene was associated with a significant inverse trend in the acquisition of HR anal HPV infection, results were generally consistent for oncogenic and non-oncogenic HPV types.

The multifactorial nature of viral infections complicates studies of the nutritional etiology of human disease (35). Factors, such as socioeconomic status (22), that are inversely associated with incident anal HPV infection may also influence diet and nutritional endpoints. Furthermore, correlation between micronutrients hampered our ability to distinguish the role of particular micronutrients in the acquisition or clearance of HPV infection. Although we adjusted for age, tobacco smoking, and other potential confounders of the association of circulating micronutrients with anal HPV acquisition, other unrecognized or unmeasured correlates of these nutritional markers may be the true mediators of initial infection.

In contrast to anal HPV acquisition, carotenoids such as lutein/zeaxanthin and cryptoxanthin delayed clearance of persistent HPV infection, although we found little or no association of these micronutrients with the clearance of transient infections. On the other hand, $\beta + \gamma$ -tocopherol tended to shorten the time to clearance of anal infections. In an earlier analysis of this cohort (21), we reported that clearance of transient (≤ 120 days), but not persistent (> 120 days), cervical HR-HPV infection was enhanced by higher circulating levels of the *trans* form of lutein and zeaxanthin, β - and total cryptoxanthin, lycopene, carotene, and total carotenoids. Although the difference in the time-related association of micronutrients with HPV persistence in the anus compared to the cervix may have resulted from distinct immune responses, we cannot rule out the possibility that it could be a chance finding or the result of different definitions of persistence (150 vs. 120 days).

Although this is the first study of the association of circulating antioxidant concentrations with clearance of anal HPV infection, longitudinal studies of blood micronutrient levels and cervical HPV persistence and clearance have reported mixed results. Giuliano et al. (16), in a study of two cervical samples collected 3 months apart from 123 women, found that women with persistent HPV infections (HPV-positive on both visits) had lower levels of β -carotene, β -cryptoxanthin, lutein, α - and γ -tocopherol compared to women with transient (positive on one visit) or no HPV infection. Sedjo et al. (17) studied serum antioxidant levels in 101 women with a persistent (positive on two or three visits) HR-HPV, compared to those in 58 women with a transient (positive on one visit) HR-HPV. They reported (nonsignificantly) higher levels of *cis*-lycopene associated with more transient infections. In a separate analysis of 84 women from the same study with a prevalent (detected at baseline) HR-HPV infection, Sedjo et al. (18) found that high levels of lycopene facilitated clearance of oncogenic HPV. In contrast, Palan et al. (19) and Siegel et al. (20) found no association of cervical HPV persistence with circulating micronutrient levels.

The natural history of anal malignancy has close parallels to cervical malignancy through the initial development of a precursor dysplastic condition, 'anal intraepithelial neoplasia', followed by cancer (9). In our cohort, the acquisition rate for anal HPV infection was higher than the acquisition rate for cervical HPV infection, but clearance was more rapid, suggesting more efficient innate and adaptive immune responses in the anus than the cervix (36). The immunomodulatory effects of vitamin E and the carotenoids are well-documented (reviewed in reference 37), and include an influence on soluble immune mediators, cytokine production and lymphocyte counts. Vitamin E supplementation has been reported to enhance macrophage function and CD4 T cell activity, and down-regulate inflammatory

cytokines (e.g., IL-1, IL-6, TNF). Through its antioxidant function, vitamin E may protect lipid membranes of immune cells in the presence of an inflammatory environment, preserving cellular function (37). However, large doses of vitamin E may also have harmful effects on the immune system, including depressing the bactericidal activity of phagocytes (38,39) and increasing the risk of pneumonia in some subgroups of smokers (40). Our findings were specific to γ -tocopherol: we found no influence of α -tocopherol on anal HPV clearance. The anti-inflammatory activity of γ -tocopherol, including trapping of reactive nitrogen species, and the inhibition of cyclooxygenase activity and eicosanoid production, is thought to be greater than that of α -tocopherol, providing a potential explanation for our findings regarding this form of tocopherol (41).

Our finding that carotenoids reduced the risk of both HPV acquisition and HPV clearance was unexpected. Similar to the tocopherols, carotenoids may protect immune cells from reactive oxygen species associated with the cellular respiratory burst (37), and thus might enhance cellular protection against viral infection. Unfortunately, studies regarding the influence of carotenoids on immune function have generally been limited to β -carotene. In contrast to antioxidant activity, a pro-oxidant effect of carotenoids has been observed. β -carotene acts as a scavenger of nitrogen oxides in cigarette smoke (42). Interestingly, β -carotene and α -tocopherol supplemented smokers engaging in heavy exercise in the Alpha Tocopherol Beta Carotene Cancer Prevention study had a greater risk of the common cold than sedentary smokers (43), in accordance with the observed inverse association of several carotenoids with anal HPV clearance. It is possible that excessive carotenoid cleavage products triggered by smoking and exercise may have pro-oxidant activity (44), stimulating the production of reactive oxygen species.

Strengths of the present study include multiple measurements of micronutrient levels over a period of time, which provided us with a better understanding of variation in participants' micronutrient levels over time. A number of investigators (27,29,45) report substantial intra-individual variability in circulating micronutrient levels, suggesting that more than one blood sample is required to accurately assess micronutrient concentrations. A further advantage of our study design is simultaneous collection of cervical and anal samples, which allowed us to account for the presence of cervical HPV infection in our analyses of anal HPV acquisition and clearance.

Potential limitations of this study include a modest sample size, which limited the power for some analyses. Our definition of a transient infection was limited by the possibility that an incident HPV 'infection' detected at one visit may not represent a true infection, but rather the simple presence of HPV in the cervix. A weakness of this and other HPV natural history studies is the inability to distinguish between persistent HPV infection and a repeat infection with the same genotype. We defined clearance as a single HPV genotype-specific negative anal infection following a positive anal infection with the same genotype. Some investigators have conservatively defined clearance by two or more consecutive negative results, reducing the possibility that a negative result could represent a latent infection, rather than true clearance. In our cohort, only 13 out of 189 type-specific incident anal HPV infections were repeatedly detected following a single negative result. We conducted statistical analyses including such repeat infections and stratifying on the event number (data not shown), and observed little or no change in the direction and magnitude of the estimated effects. Finally, circulating micronutrients may not be representative of levels of these chemicals in target tissues, such as the anus. Thus, the relevance of our findings to local infections is unclear.

In conclusion, little is known regarding the natural history of anal HPV infection, particularly the correlates of anal HPV infection among younger women at high risk for

sexually-transmitted infections. Results from the present study suggest that plasma micronutrients may have a significant influence on early events in anal HPV infection. Although available prophylactic HPV vaccines have the potential to prevent up to 70% of anal cancers (46), given the presence of non-HPV-16 and -18 related anal cancer, the lag-time for the vaccinated population to realize a reduced risk of anal cancer, and delays in global vaccine uptake, the identification of nutritional co-factors in viral carcinogenesis continues to be important.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Joseph DA, Miller JW, Wu X, et al. Understanding the burden of human papillomavirus-associated anal cancers in the US. *Cancer* 2008;113:2892–900. [PubMed: 18980293]
2. Frisch M, Melbye M, Moller H. Trends in incidence of anal cancer in Denmark. *BMJ* 1993;306:419–22. [PubMed: 8461721]
3. Brewster DH, Bhatti LA. Increasing incidence of squamous cell carcinoma of the anus in Scotland, 1975–2002. *Br J Cancer* 2006;95:87–90. [PubMed: 16721368]
4. American Cancer Society. Cancer facts and figures 2009. American Cancer Society; Atlanta: 2009.
5. Hoots BE, Palefsky JM, Pimenta JM, Smith JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer* 2009;124:2375–83. [PubMed: 19189402]
6. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2008. Atlanta: U.S. Department of Health and Human Services; 2009.
7. Moscicki AB, Hills NK, Shiboski S, et al. Risk factors for abnormal anal cytology in young heterosexual women. *Cancer Epidemiol Biomarkers Prev* 1999;8:173–8. [PubMed: 10067816]
8. Edgren G, Sparen P. Risk of anogenital cancer after diagnosis of cervical intraepithelial neoplasia: a prospective population-based study. *Lancet Oncol* 2007;8:311–6. [PubMed: 17395104]
9. Holly EA, Ralston ML, Darragh TM, et al. Prevalence and risk factors for anal squamous intraepithelial lesions in women. *J Natl Cancer Inst* 2001;93:843–9. [PubMed: 11390533]
10. Daling JR, Madeleine MM, Johnson LG, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer* 2004;101:270–80. [PubMed: 15241823]
11. Frisch M, Glimelius B, van den Brule AJ, et al. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med* 1997;337:1350–8. [PubMed: 9358129]
12. Hernandez BY, McDuffie K, Zhu X, et al. Anal human papillomavirus infection in women and its relationship with cervical infection. *Cancer Epidemiol Biomarkers Prev* 2005;14:2550–6. [PubMed: 16284377]
13. Palefsky JM, Holly EA, Ralston ML, Da CM, Greenblatt RM. Prevalence and risk factors for anal human papillomavirus infection in human immunodeficiency virus (HIV)-positive and high-risk HIV-negative women. *J Infect Dis* 2001;183:383–91. [PubMed: 11133369]
14. Melbye M, Smith E, Wohlfahrt J, et al. Anal and cervical abnormality in women—prediction by human papillomavirus tests. *Int J Cancer* 1996;68:559–64. [PubMed: 8938134]

15. Shvetsov YB, Hernandez BY, McDuffie K, et al. Duration and clearance of anal human papillomavirus (HPV) infection among women: the Hawaii HPV cohort study. *Clin Infect Dis* 2009;48:536–46. [PubMed: 19191636]
16. Giuliano AR, Papenfuss M, Nour M, et al. Antioxidant nutrients: associations with persistent human papillomavirus infection. *Cancer Epidemiol Biomarkers Prev* 1997;6:917–23. [PubMed: 9367065]
17. 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. [PubMed: 12223432]
18. 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. [PubMed: 12846362]
19. Palan PR, Chang CJ, Mikhail MS, et al. Plasma concentrations of micronutrients during a nine-month clinical trial of beta-carotene in women with precursor cervical cancer lesions. *Nutr Cancer* 1998;30:46–52. [PubMed: 9507512]
20. Siegel EM, Craft NE, Duarte-Franco E, et al. Associations between serum carotenoids and tocopherols and type-specific HPV persistence: the Ludwig-McGill cohort study. *Int J Cancer* 2007;120:672–80. [PubMed: 17096322]
21. Goodman MT, Shvetsov YB, McDuffie K, et al. Hawaii cohort study of serum micronutrient concentrations and clearance of incident oncogenic human papillomavirus infection of the cervix. *Cancer Res* 2007;67:5987–96. [PubMed: 17553901]
22. Goodman MT, Shvetsov YB, McDuffie K, et al. Acquisition of anal human papillomavirus infection among women: the Hawaii HPV cohort study. *J Infect Dis* 2008;197:957–66. [PubMed: 18429348]
23. Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol* 2000;38:357–61. [PubMed: 10618116]
24. Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. *J Clin Microbiol* 1998;36:3020–7. [PubMed: 9738060]
25. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur HH. Classification of papillomaviruses. *Virology* 2004;324:17–27. [PubMed: 15183049]
26. Castle PE. The evolving definition of carcinogenic human papillomavirus. *Infect Agent Cancer* 2009;4:7. [PubMed: 19432962]
27. Cooney RV, Franke AA, Hankin JH, et al. Seasonal variations in plasma micronutrients and antioxidants. *Cancer Epidemiol Biomarkers Prev* 1995;4:207–15. [PubMed: 7606195]
28. Franke AA, Custer LJ, Cooney RV. Quantitation of lipid-phase micronutrients in plasma by HPLC with synthetic carotenoids as internal standards. *J Chromatogr B Biomed Appl* 1993;614:43–57.
29. Shvetsov YB, Hernandez BY, Wong SH, et al. Intraindividual variability in serum micronutrients: effects on reliability of estimated parameters. *Epidemiology* 2009;20:36–43. [PubMed: 18813024]
30. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol* 1989;129:125–37. [PubMed: 2910056]
31. Lin DY, Wei LJ. The robust inference for the Cox proportional hazards model. *J Am Stat Assoc* 1989;84:1074–8.
32. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 1994;81:515–26.
33. Quandt RE. The estimation of the parameter of a linear regression system obeying two separate regimes. *Journal of the American Statistical Association* 1958;53:873–80.
34. Hinkley DV. Inference in two-phase regression. *Journal of the American Statistical Association* 1971;66:736–43.
35. 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. [PubMed: 15912536]
36. de Paula PR, Matos D, Franco M, et al. Why do anal wounds heal adequately? A study of the local immunoinflammatory defense mechanisms. *Dis Colon Rectum* 2004;47:1861–7. [PubMed: 15622578]

37. Webb AL, Villamor E. Update: effects of antioxidant and non-antioxidant vitamin supplementation on immune function. *Nutr Rev* 2007;65:181–217. [PubMed: 17566547]
38. Garg R, Singh N, Dube A. Intake of nutrient supplements affects multiplication of *Leishmania donovani* in hamsters. *Parasitology* 2004;129:685–91. [PubMed: 15648691]
39. Baehner RL, Boxer LA, Allen JM, Davis J. Autooxidation as a basis for altered function by polymorphonuclear leukocytes. *Blood* 1977;50:327–35. [PubMed: 871528]
40. Hemila H, Kaprio J. Vitamin E supplementation and pneumonia risk in males who initiated smoking at an early age: effect modification by body weight and dietary vitamin C. *Nutr J* 2008;7:33. [PubMed: 19019244]
41. Ju J, Picinich SC, Yang Z, et al. Cancer Preventive Activities of Tocopherols and Tocotrienols. *Carcinogenesis*. 2009
42. Eiserich JP, van der Vliet A, Handelman GJ, Halliwell B, Cross CE. Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction. *Am J Clin Nutr* 1995;62:1490S–1500S. [PubMed: 7495250]
43. Hemila H, Virtamo J, Albanes D, Kaprio J. Physical activity and the common cold in men administered vitamin E and beta-carotene. *Med Sci Sports Exerc* 2003;35:1815–20. [PubMed: 14600543]
44. Siems W, Salerno C, Crifo C, Sommerburg O, Wiswedel I. Beta-carotene degradation products - formation, toxicity and prevention of toxicity. *Forum Nutr* 2009;61:75–86. [PubMed: 19367112]
45. Block G, Dietrich M, Norkus E, et al. Intraindividual variability of plasma antioxidants, markers of oxidative stress, C-reactive protein, cotinine, and other biomarkers. *Epidemiology* 2006;17:404–12. [PubMed: 16755268]
46. Hoots BE, Palefsky JM, Pimenta JM, Smith JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer* 2009;124:2375–83. [PubMed: 19189402]

Figure 1.

Distribution of incident anal HPV infections, by genotype and oncogenic risk (Hawaii HPV Cohort Study of women, 1998-2003).

Note: HR, high-risk HPV genotypes; LR, low-risk HPV genotypes.

Table 1

Participant characteristics (Hawaii HPV Cohort Study of women, 1998-2003)

Number of participants with 2+ visits	279
Total number of visits	1090
Mean number of visits (range)	3.9 (2-12)
Mean length of follow-up in days (range)	490 (89-1667)
Mean age at first visit (range)	37.7 (18-71)
Median number of lifetime sexual partners (IQR)	7 (4-13)
Mean age at first sexual intercourse (yrs)	17.6
Ethnicity	%
Caucasian	41.6
Japanese	11.8
Chinese	3.9
Filipino	5.7
Hawaiian or part Hawaiian	14.7
Other or mixed	22.3
Tobacco smoking at baseline	
Current smoker	14.0
Current nonsmoker	86.0
Alcohol drinking at baseline	
Current drinker	32.6
Current nondrinker	67.4
History of anal sex at baseline	
Never	75.5
Past practice	17.3
Current practice	7.2
Pregnancy history at baseline	
Never pregnant	36.2
Ever been pregnant	63.8

Table 2

Hazard ratios and 95% confidence intervals for acquisition of HPV infection by levels of circulating plasma micronutrients (Hawaii HPV Cohort Study of women, 1998-2003).

Micronutrient		Number of participants		Number of infections		Hazard ratio (95% CI)	
Vitamin A		n		n		HR (95% CI)	
Lowest quartile		1,000		100		1.0	
Second quartile		1,000		100		1.1 (0.8-1.5)	
Third quartile		1,000		100		1.2 (0.9-1.6)	
Highest quartile		1,000		100		1.3 (1.0-1.7)	
Trend						P = 0.001	
Vitamin E		n		n		HR (95% CI)	
Lowest quartile		1,000		100		1.0	
Second quartile		1,000		100		1.1 (0.8-1.5)	
Third quartile		1,000		100		1.2 (0.9-1.6)	
Highest quartile		1,000		100		1.3 (1.0-1.7)	
Trend						P = 0.001	
Vitamin C		n		n		HR (95% CI)	
Lowest quartile		1,000		100		1.0	
Second quartile		1,000		100		1.1 (0.8-1.5)	
Third quartile		1,000		100		1.2 (0.9-1.6)	
Highest quartile		1,000		100		1.3 (1.0-1.7)	
Trend						P = 0.001	
Folate		n		n		HR (95% CI)	
Lowest quartile		1,000		100		1.0	
Second quartile		1,000		100		1.1 (0.8-1.5)	
Third quartile		1,000		100		1.2 (0.9-1.6)	
Highest quartile		1,000		100		1.3 (1.0-1.7)	
Trend						P = 0.001	

* Number of study participants who had a specified micronutrient levels within the specified range at any clinic visit.

† Number of acquired infections across all participants who had the specified micronutrient within the specified range.

‡ Adjusted for age at first visit; current tobacco smoking status; current practice of anal sex; history of pregnancy over lifetime; period of time the blood was kept frozen, from blood draw until laboratory analysis; coinfection in the anus with other HPV genotypes; cervical infection with the index HPV type.

§ CI, confidence interval.

// The P for trend is based on a Wald statistic for a trend variable assigned the median for the appropriate quartile.

Table 3

Hazard ratios and 95% confidence intervals for clearance of incident HPV infection by levels of circulating plasma micronutrients (Hawaii HPV Cohort Study of women, 1998-2003).

* Quartile range (ng/mL).

[†] Cumulative infection length in months, from first positive to first negative visit, across infections in that quartile.

[‡] Adjusted for age at first visit; current tobacco smoking status; current practice of anal sex; history of pregnancy over lifetime; period of time the blood was kept frozen, from blood draw until laboratory analysis; coinfection in the anus with other HPV genotypes; cervical infection with the index HPV type.

[§] CI, confidence interval.

// The *P* for trend is based on a Wald statistic for a trend variable assigned the median for the appropriate quartile.

** Statistically significant after Bonferroni correction for multiple comparisons.

Table 4

Hazard ratios and 95% confidence intervals for clearance of persistent incident HPV infection by levels of circulating plasma micronutrients (Hawaii HPV Cohort Study of women, 1998-2003).

Micronutrient	Quartile range (ng/mL)	No. cleared	Follow-up (woman-months)	Hazard ratio [†] (95% CI) [‡]	P for trend [§]
<i>Trans</i> -zeaxanthin	3.7-49.0	16	128.8	1.00	0.02
	>49.0-62.1	22	169.6	1.03 (0.53-2.01)	
	>62.1-82.6	16	127.2	0.75 (0.34-1.67)	
	>82.6-281.1	17	154.5	0.32 (0.11-0.89)	
Total lutein/zeaxanthin	91.9-269.8	25	166.6	1.00	0.04
	>269.8-369.8	8	90.3	0.62 (0.27-1.43)	
	>369.8-491.0	25	214.2	0.57 (0.25-1.26)	
	>491.0-1287.6	13	109.1	0.39 (0.16-0.92)	
Total β -cryptoxanthin	17.5-115.4	20	121.6	1.00	0.03
	>115.4-172.5	20	133.0	0.79 (0.38-1.64)	
	>172.5-258.1	17	187.1	0.30 (0.11-0.80)	
	>258.1-1994.7	14	138.4	0.27 (0.09-0.77)	
α -carotene	0.1-29.8	11	107.4	1.00	0.21
	>29.8-50.9	30	172.7	2.22 (1.09-4.55)	
	>50.9-78.9	12	138.1	0.82 (0.31-2.16)	
	>78.9-436.6	18	162.0	0.98 (0.40-2.42)	
Total β -carotene	2.5-109.2	15	137.7	1.00	0.01
	>109.2-192.5	25	119.3	2.55 (1.40-4.64)	
	>192.5-364.8	22	198.6	0.54 (0.22-1.36)	
	>364.8-1762.1	9	124.6	0.58 (0.23-1.44)	
Total carotenoids	198.9-968.3	16	138.6	1.00	0.01
	>968.3-1331.8	23	124.3	1.52 (0.85-2.73)	
	>1331.8-1785.5	15	157.2	0.39 (0.15-1.00)	
	>1785.5-5581.3	17	160.0	0.49 (0.25-0.97)	
α -tocopherol	4339.0-8560.2	16	102.3	1.00	0.31
	>8560.2-10969.8	23	222.4	0.39 (0.15-0.99)	
	>10969.8-14641.5	20	121.3	0.78 (0.30-2.05)	
	>14641.5-67919.8	12	134.2	0.40 (0.13-1.22)	
β - + γ -tocopherol	4.34-549.4	17	187.5	1.00	0.0003**
	>549.4-991.4	17	150.4	1.13 (0.60-2.16)	
	>991.4-1520.6	17	157.6	1.57 (0.85-2.89)	
	>1520.6-6390.9	20	84.5	3.67 (1.82-7.39)**	

* Cumulative infection length in months, from day 151 until first negative result.

[†] Adjusted for age at first visit; current tobacco smoking status; current practice of anal sex; history of pregnancy over lifetime; period of time the blood was kept frozen, from blood draw until laboratory analysis; coinfection in the anus with other HPV genotypes; cervical infection with the index HPV type.

[‡] CI, confidence interval.

[§] The *P* for trend is based on a Wald statistic for a trend variable assigned the median for the appropriate quartile.

** Statistically significant after Bonferroni correction for multiple comparisons.