Pretreatment serum xanthophyll concentrations as predictors of head and neck cancer recurrence and survival

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ABSTRACT: *Background.* The purpose of this study was to examine associations of pretreatment serum carotenoids, tocopherols, and quercetin with prognosis in 154 patients newly diagnosed with head and neck cancer.

Methods. Pretreatment blood and health surveys were collected. Serum micronutrients were measured by high performance liquid chromatography. Data on recurrence and death were collected annually. Cox proportional hazards models measured associations of serum nutrient concentrations with recurrence and overall survival.

Results. During a median follow-up time of 37 months, there were 32 recurrences and 27 deaths. After controlling for covariates, subjects with high versus low serum xanthophyll and total carotenoid concentrations had significantly longer recurrence-free time ($p = .002$ and $p = .02$, respectively). Overall survival time was significantly longer in patients with high versus low serum xanthophyll concentrations ($p = .02$).

Conclusion. Future research should evaluate the possible benefits of interventions to increase intakes of rich food sources of xanthophylls in this patient population. © 2015 Wiley Periodicals, Inc. *Head Neck* **38**: E1591–E1597, 2016

KEY WORDS: serum micronutrients, xanthophylls, quercetin, head and neck cancer, recurrence, survival

INTRODUCTION

Higher fruit and vegetable intake is associated with decreased risk of head and neck cancer development and mortality.[1–6] We recently reported that a whole foods pattern, characterized by high intakes of vegetables, fruits, whole grains, poultry, and fish, was associated with lower recurrence and mortality rates in a prospective cohort of patients newly diagnosed with head and neck cancer.[7] This same dietary pattern was associated with lower levels of proinflammatory cytokines that have been hypothesized to facilitate tumor growth and dissemination in patients with head and neck cancer.[8]

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Although our previous work provides evidence that consuming a healthy diet before head and neck cancer treatment is beneficial to clinical and biological head and neck cancer outcomes, a limitation of these studies was the use of a self-reported diet via food frequency questionnaire, which is susceptible to measurement error. Examining biomarkers of dietary intake in relation to head and neck cancer disease outcomes is important, as consistency across results will reduce the likelihood that bias or confounding was responsible for previously observed associations with the reported diet.[9]

Blood carotenoids have been established as valid biomarkers of fruit and vegetable intake.[10] Tocopherols are found mainly in vegetable oils and high fat foods like nuts. The roles of carotenoids and tocopherols in cancer chemoprevention have been of great interest over the past 2 decades because of their antioxidant properties.[11] Quercetin is a flavonol that accounts for about 75% of typical U.S. intake of flavonoids.[12] It is widely distributed in many fruits and vegetables; tea, onions, allium vegetables, and apples with skin are major food sources of quercetin in the United States, and these foods are low in the highly pigmented carotenoids.[12–15] Dietary intake of β-carotene and quercetin have been reported to be moderately correlated ($r = 0.27$ and $r = 0.32$ for women and men, respectively),[12] suggesting measurement of quercetin may capture fruit and vegetable intakes that are not well

represented by carotenoids.[16] Like carotenoids and tocopherols, quercetin has a potential role in cancer prevention[17,18] because of its anti-inflammatory effects and promotion of apoptosis and autophagy in cancer cells.[19–23] Few studies have examined associations of serum carotenoids and tocopherols with head and neck cancer outcomes,[3,6,24] and quercetin concentrations have never been reported in relation to head and neck cancer mortality.

This study evaluated the associations of pretreatment serum carotenoids, tocopherols, and quercetin with recurrence-free and overall survival time in a cohort of patients newly diagnosed with head and neck cancer. The hypothesis was that a protective relationship would be observed between the examined serum micronutrients and outcomes.

MATERIALS AND METHODS

Subjects

This prospective cohort study utilized data collected from 154 patients newly diagnosed with head and neck cancer enrolled in the University of Michigan Head and Neck Specialized Program of Research Excellence (HN-SPORE). All study activities were approved by the internal Institutional Review Board at the University of Michigan and study participants gave signed, informed consent to participate. Recruitment and data collection methods have been described elsewhere.[8] Briefly, subjects were recruited between November 2008 and August 2012. Exclusion criteria included being <18 years of age, pregnant, non-English speaking, diagnosed as mentally unstable (eg, presence of schizophrenia, severe, unmanaged depression, or any other state or condition that study personnel feel would make active study participation difficult or impossible), diagnosed with another non-upper aerodigestive tract cancer, or diagnosed with any other head and neck primary within the past 5 years.

Enrolled participants completed a self-administered epidemiologic health questionnaire before treatment that collected data on demographics, tobacco use, alcohol consumption, weight status, and comorbidities, as well as a food frequency questionnaire.[9] Clinical data were recorded from the medical records. Pretreatment peripheral blood samples (30 mL) were collected using routine venipuncture. Sera were collected after centrifugation of blood and stored in the HN-SPORE Tissue Core in 0.5 mL aliquots at −80°C until testing. All serum samples were barcoded and analyzed blinded to subject identifiers or outcomes.

During the study period, a total of 520 evaluable subjects were recruited, yielding a 92% participation rate. For the current analysis, we analyzed serum nutrient values for those subjects for whom pretreatment serum samples, epidemiologic health questionnaires, and food frequency questionnaires were available. Two hundred nineteen subjects (42.1%) completed baseline health questionnaires and food frequency questionnaires with valid data. Food frequency questionnaire data were considered valid according to standard criteria (ie, no complete pages missing, no more than 70 missing items, and total energy intake ≤5000 kcals/day and ≥200 kcals per day).[9] Sixty-five subjects did not have baseline blood collected or adequate samples available for measurement, leaving a final sample size of 154.

Measures

Predictors — serum micronutrients, chemicals, and instrumentation. Quercetin, morin, citric acid, ammonium acetate, hydrochloric acid, and enzymes were obtained from Sigma Aldrich (St. Louis, MO). High performance liquid chromatography grade hexane, acetonitrile, water, methyl tert-butyl ether and methanol were Burdick and Jackson brand, high performance liquid chromatography-grade (Honeywell, Morristown, NJ). Tocol was a gift of Hoffman La Roche (Basel, Switzerland). High performance liquid chromatography was performed using a Shimadzu system consisting of 2 LC-20AT pumps, a CTO-20A autosampler, a CBM-20A controller, and 2 detectors in tandem: SPD-20AV UV/visible detector and a 5600A ESA CoulArray Multi-Electrode Detector (ESA Biosciences, Chelmsford, MA). Chromatograms were analyzed using ESA CoulArray Software (1.1.2.0, 2002).

Analysis of serum carotenoids, tocopherols, and quercetin. Analysis of serum carotenoids and tocopherols has been described elsewhere.[8] For quercetin, serum aliquots, 200 μL, were buffered with 21 μL 0.55 M sodium acetate buffer (pH 5.0) followed by addition of 800 units β-glucuronidase from Helix pomatia, that contains sulfatase activity, and incubated at 37°C for 17 hours to release conjugates, based on the methods of Erlund et al.[25] The internal standard morin, 10 μL of 10 μg/mL in methanol, was then added along with 0.1 M hydrochloric acid (25 μL) followed by vortexing. Extraction with ethyl acetate (500 μL) was performed twice. The organic extracts were dried in the SpeedVac and reconstituted in 100 μL of high performance liquid chromatography mobile phases A and B mixed in a 1:1 ratio before high performance liquid chromatography analysis. Detection was with the ESA detector set at 50 mV, 100 mV, and 150 mV.

The separations were performed with a reversed phased YMC-Pack Pro C18 column (150 × 4.6 mm, catalog #AS12SO3–1546WT; YMC, Allentown, PA). The mobile phases used were: (A) 10% acetonitrile, 80% water, and 10% buffer (250 m*M* ammonium acetate, 750 m*M* citric acid, and pH 2.7), and (B) 50% acetonitrile, 40% water, and 10% buffer. The gradient began at 40% B and then increased to 100% B over 9 minutes. There was a 3-minute final hold, and then a 5-minute re-equilibration period. The flow rate was 1.0 mL/min. The serum samples were analyzed in 6 batches, and aliquots of the same standards were used for calibration with each batch. The inter-day coefficients of variation for the individual carotenoids were between 3% and 11%. Our laboratory participated in the National Institutes of Standards Technology Round Robin for carotenoids, and the values obtained by our laboratory were within 2 SDs for all the samples analyzed.

Covariates

Sociodemographic variables included age and sex. Tobacco and alcohol use data were categorized as current,

former, or never, whereas "current" reflected use in the 12 months before cancer diagnosis. Body mass index (BMI) (kg/m^2) was calculated at diagnosis based on self-reported weight and height. Comorbidities were recorded using the Adult Comorbidity Evaluation-27 and categorized into none, mild, moderate, or severe comorbidities.[26] As previously described, tumor human papillomavirus (HPV) status was determined by an ultrasensitive method using real-time competitive polymerase chain reaction and matrix-assisted laser desorption/ionization time of flight mass spectroscopy with separation of products on a matrix loaded silicon chip array.[27] As a result of having equivocal HPV status data for 34 participants (22.1%), tumor site was recorded from the medical records and categorized into 4 groups, which allowed for consideration of known HPV status: (1) oral cavity; (2) oropharyngeal HPV-positive; (3) oropharyngeal HPV-negative or unknown; and (4) larynx. To increase statistical power, cancer stage was categorized a priori into 2 groups, with stages 1 and 2 collapsed, and stages 3 and 4 collapsed to increase statistical power. The method used to determine HPV status has been previously described.[8,27]

Outcomes: Recurrence and survival

Patients were followed according to National Comprehensive Cancer Network guideline intervals. New tumor events and status (recurrence, residual disease, persistent disease, and second primary) were updated at each visit and annually via medical record review. For subjects who did not return to our institution for surveillance after their treatment, information on tumor events and status was obtained through self-report and contact with local physicians. Deaths were confirmed through the Social Security Death Index, yearly survey updates, notification from family, and medical record reviews. Survival time was censored to February 1, 2014. Recurrence events were censored to the last date of each subject's annual medical record review. Persistent disease was assigned a follow-up time of 1 day for calculation of time to recurrence. Subjects were considered to be lost to follow-up if they had not returned for care, and there was a failure to contact the subject or to collect updates when contacting the referring physician. Subjects lost to follow-up were censored until their last known status.

Statistical analyses

Descriptive statistics (means and frequencies) were generated for demographic, epidemiologic, and clinical variables. To assess relationships between serum nutrient concentrations (categorized into tertiles) and demographic, clinical, and behavioral variables, chi-square tests were used for categorical variables and Wilcoxon rank-sum tests were used for continuous variables. To examine whether degradation of serum nutrient concentrations may have occurred over time, Spearman correlations were computed between sample storage time (number of days between date of assay and date blood samples were collected) and serum nutrient concentrations.

Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations of recurrence-free and overall survival time with serum nutrient concentrations. To arrive at a parsimonious final multivariable model, variables were considered for inclusion using a backward selection strategy based on a priori knowledge and input from treating physicians of this study population. Final multivariable models were adjusted for age, BMI, tumor site, cancer stage, and tobacco use. Sex, alcohol use, comorbidities, and sample storage time were considered as covariates but excluded from final models as they did not substantially alter parameter estimates. Treatment modality was not considered as a covariate in this analysis because of the high collinearity of treatment and disease site ($p < .001$ for chi-square test with the p value approximated by Monte–Carlo simulation). Other behavioral variables (eg, physical activity and red meat intake) were not considered as covariates based on previous findings that showed a lack of association of these variables with mortality in this study population.[1,7] All serum nutrient concentrations were categorized into tertiles (low, medium, and high concentrations) for use in models and display in Kaplan–Meier plots. HRs and 95% CIs were

TABLE 1. Pretreatment characteristics of patients newly diagnosed with head and neck cancer ($n = 154$).

Characteristics	Mean, SD, range, or number (%)
Age, y	59 (11); range, 25–93
Female	33 (21)
Non-Hispanic white	147 (95.4)
Death events	27 (17.5)
Recurrence events	32 (27.8)
BMI, kg/m^2	27.5 (6.2); range, 13.0–54.3
Underweight, <18.5	6 (4)
Normal, 18.5–24.9	45 (29)
Overweight, 25–29.9	66 (43)
Obese, 30+	37 (24)
Tobacco status	
Never	30 (19)
Current, within 12 mo	57 (37)
Former, quit >12 mo	67 (44)
Alcohol status	
Never	7 (5)
Current, within 12 mo	110 (71)
Former, quit >12 mo	37 (24)
Disease site	
Oral cavity	52 (34)
Oropharynx HPV-positive	46 (30)
Oropharynx HPV-negative or unknown	22 (14)
Larynx	32 (21)
Hypopharynx	2 (1)
Clinical stage	
Stage IV	102 (66)
Stage III	26 (17)
Stage II	13 (8)
Stage I	13 (8)
Comorbidities	
None	41 (27)
Mild	71 (46)
Moderate	29 (19)
Severe	13 (8)

Abbreviations: BMI, body mass index; HPV, human papillomavirus.

TABLE 2. Patient characteristics of subjects by serum nutrient levels ($n = 154$).*

Characteristic	Total carotenoids			Total xanthophylls			Quercetin			α-Tocopherols and γ-tocopherols		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Median concentration, μg/dL	0.13	0.25	0.47	0.07	0.15	0.28	80.4	137.7	222.1	6.4	10.1	15.7
Mean age, y	57.7	60.9	60.5	57.6	59.5	62.2	60	59.9	58.5	56.9	59.2	63.2†
Mean BMI, kg/m^2	27.3	27.5	26.3	26.2	28.2	26.6	25.8	27.8	28.8†	26.8	27.9	26.3
Female, %	25.6	25.6	13.9	23.3	22.7	18.6	21.6	17.3	25.5	16.3	29.5	18.6
Stage III or IV, %	90.7	74.4	81.4	95.3	77.3	74.4†	84.3	82.7	82.3	86	77.3	83.7
Current smokers, %	62.3	32.6	16.3‡	60.5	36.4	13.9‡	47.1	32.7	31.4	18.6	15.9	27.9
No comorbidities, %	20.9	23.3	34.9	20.9	27.3	30.2	21.6	26.9	31.4	23.3	25	30.2
Leafy green vegetables, servings/d	0.36	0.32	0.46	0.28	0.36	0.51†	0.32	0.35	0.44	0.33	0.46	0.36
Cruciferous vegetables, servings/d	0.26	0.20	0.28	0.16	0.24	0.21	0.28	0.16	0.25	0.25	0.25	0.23
Dark yellow and orange vegetables, servings/d	0.16	0.18	0.37‡	0.16	0.19	0.35‡	0.26	0.18	0.23	0.21	0.23	0.26
Fruits, servings/d	0.80	0.94	1.21†	0.72	0.95	1.28‡	0.91	0.87	1.02	0.78	1.21	0.95

Abbreviation: BMI, body mass index.
* Comparisons between tertiles were derived by using a chi-square test for categorical variables and a Wilcoxon rank-sum test for continuous variables.
† $p < .05$.
‡ $p < .01$.

estimated for each tertile of serum nutrient concentration and compared with the lowest, tertile 1. The SAS system version 9.4 (SAS Institute, Cary, NC) was used for all analyses. The p values of .05 or less were considered statistically significant and no multiplicity adjustments were performed.

RESULTS

During a median longitudinal follow-up time of 37 months, there were 32 (20.8%) recurrence events and 27 deaths (17.5%). Epidemiological characteristics of the study population are given in Table 1. The mean age of study participants was 59 years. The majority of participants were men (79%). The mean BMI at diagnosis was 27.2 kg/m^2, with 67% of the population considered overweight or obese. Only 19% of the participants reported never smoking, whereas 37% reported smoking in the last 12 months. Tumors were most commonly of the oral cavity (37%) or HPV-positive oropharynx (30%) and diagnosed at an advanced stage (66% stage IV).

Select patient characteristics across tertiles of serum total carotenoids, xanthophylls, quercetin, α-tocopherol, and γ-tocopherol are shown in Table 2. Age was positively associated with tocopherols. Mean BMI increased significantly across increasing tertiles of quercetin. A significantly higher proportion of patients with stage III or IV cancers had low serum xanthophyll concentrations. Participants with the highest serum xanthophyll and total carotenoid concentrations were significantly less likely to be current smokers. Patients self-reporting higher intake of leafy green vegetables, dark yellow and orange vegetables, and fruit on the food frequency questionnaire had significantly higher serum xanthophyll concentrations. Serum total carotenoid concentrations were significantly positively associated with intake of dark yellow and orange vegetables and fruit on the food frequency questionnaire. We previously reported serum total carotenoids, α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and α-tocopherol to be significantly correlated with reported dietary intake of each micronutrient in this study population.[8] Significant, negative Spearman correlations were found between sample days and β-carotene ($\rho = -0.29$; $p = .001$), α-carotene ($\rho = -0.28$; $p = .001$), and total carotenoids ($\rho = -0.22$; $p = .01$).

Kaplan–Meier curves and associated p values for the associations between each serum nutrient (categorized into tertiles) with time-to-recurrence and time-to-death (any cause) are shown in Figure 1. HRs and 95% CIs for recurrence and mortality by serum micronutrient levels are shown in Table 3. After controlling for covariates, participants with high serum xanthophylls and total carotenoids were significantly less likely to experience a recurrence than those with low concentrations. Participants with high serum xanthophylls also had significantly lower mortality than those with low concentrations. Although not statistically significant, there seemed to be trends toward lower recurrence with higher serum β-carotene, lycopene, and α-tocopherol levels, and lower mortality with high serum γ-tocopherol levels. There were no apparent associations between recurrence and α-carotene, quercetin, and γ-tocopherol, or between mortality and α-carotene, β-carotene, lycopene, total carotenoids, quercetin, and α-tocopherol.

DISCUSSION

This study of pretreatment serum micronutrient concentrations in patients newly diagnosed with head and neck cancer showed that high pretreatment serum xanthophyll concentrations were associated with a lower risk of recurrence and mortality, independent of other factors known to influence these outcomes. Results also showed that high pretreatment total carotenoid concentrations were associated with decreased risk of recurrence. The results of this study are of particular importance because they are consistent with the previous findings of our group that a whole foods dietary pattern, derived from principal components analysis, was associated with lower risk of head and neck cancer mortality and recurrence.[7] Foods that were among the most significant contributors to this dietary pattern (ie, had the highest factor loadings)

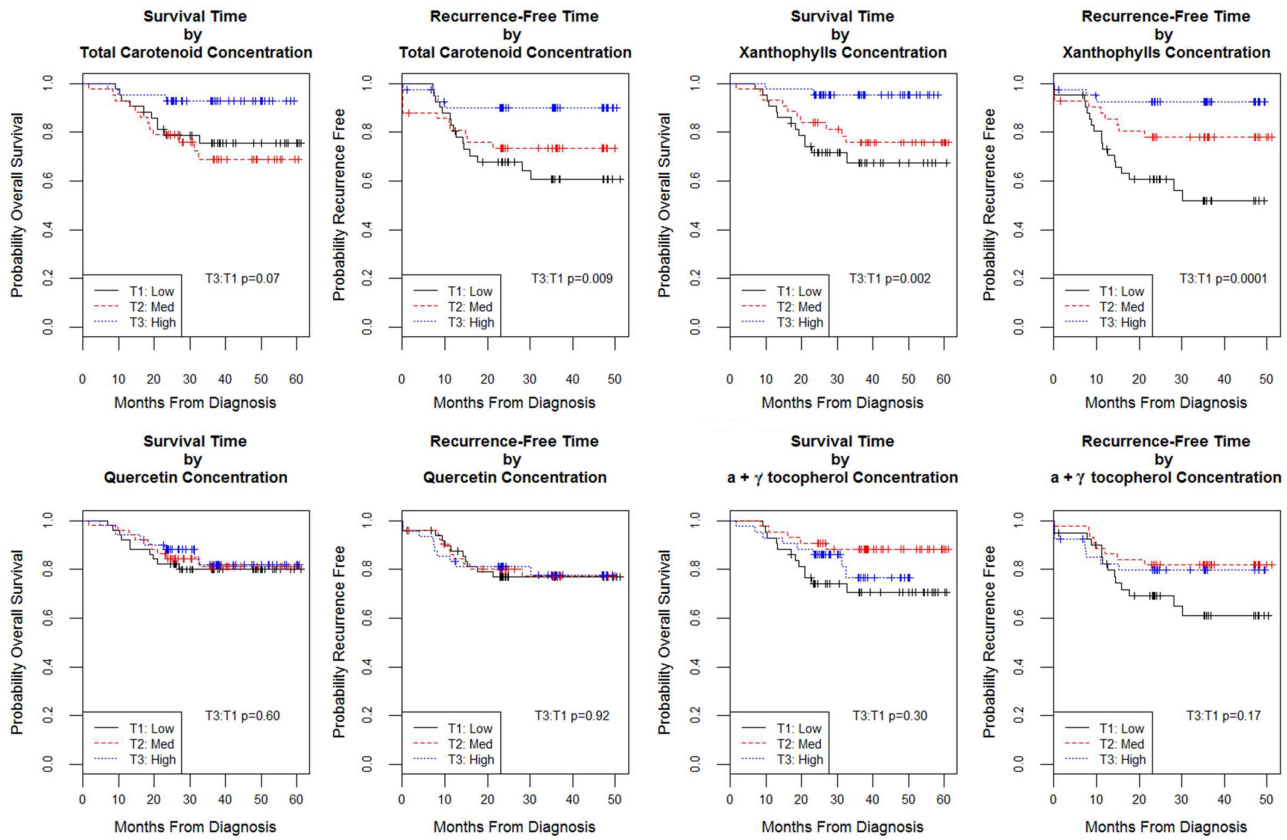

FIGURE 1. Kaplan–Meier plots for recurrence and survival from head and neck cancer by tertiles of serum micronutrient concentrations, given in months from diagnosis. Xanthophylls were the sum of lutein, zeaxanthin, and β-cryptoxanthin. Total carotenoids were the sum of xanthophylls, lycopene, α-carotene, and β-carotene. Total tocopherols were the sum of α-tocopherols and γ-tocopherols.

included leafy green vegetables, dark-yellow and orange vegetables, and fruit – all rich food sources of xanthophylls and total carotenoids. Therefore, it is less likely that bias or confounding were responsible for these previously observed associations based on the reported diet.

Similar to our results, Sakhi et al[6] found that higher pretreatment serum carotenoids measured in 60 patients, predicted better progression-free survival. However, other studies suggest the relationship between head and neck cancer mortality and carotenoids and tocopherols may be modified by smoking status. As has been previously reported, it is possible that carotenoids and tocopherols have a detrimental effect, especially in smokers, when consumed in high doses from supplements that are unlikely to be attained through dietary intake alone.[28,29] A randomized controlled trial carried out in a sample of 540 patients newly diagnosed with head and neck cancer showed that smokers randomized to the α-tocopherol and β-carotene supplementation group had increased recurrence, mortality, and second primary cancers compared to nonsmokers and placebo.[30] Interestingly, however, higher reported β-carotene dietary intake (ie, not from supplements) before randomization and subsequent radiotherapy was associated with reduced risk of local recurrence, regardless of smoking status.[24] In another study, Mayne et al[3] reported protective effects of total serum carotenoids on mortality, but increased mortality with higher α-tocopherol in posttreatment of patients with head and neck cancer who were smokers and disease-free at the time of blood sampling. We did not have the statistical power to assess effect modification by smoking status in the current study, but this issue warrants further investigation in the future.

We found that xanthophylls were strongly associated with better head and neck cancer outcomes. Consistent with our findings, Hughes et al[31] found that xanthophylls, either individually or grouped, were inversely associated with a urinary marker of oxidative stress. The xanthophylls include the oxygenated carotenoids lutein, β-cryptoxanthin, and zeaxanthin. It is unclear if their protective effects in cells are the result of chemical or biochemical properties of these carotenoids or of some other aspect of foods that are high in xanthophylls. Although α-carotenes and β-carotenes are distributed in a wider variety of fruits and vegetables than the other carotenoids measured, lutein and zeaxanthin are high mainly in leafy green vegetables that are typically low in the U.S. diet.[32] Men tend to eat less fruit than women,[33] and β-cryptoxanthin is especially high in dark orange fruits. More heterogeneity in dietary intakes and blood concentrations may be present for xanthophylls than the other carotenoids because of their distribution in a smaller variety of fruits and vegetables. Few supplements containing xanthophylls are currently available and so it is unknown

TABLE 3. Multivariate hazard ratios and 95% confidence intervals from Cox proportional hazards models according to level of serum nutrient concentration for recurrence and overall survival.*

Serum nutrient	Recurrence OR (95% CI)	Overall survival OR (95% CI)
α-Carotene		
Low	1.0	1.0
Medium	1.04 (0.44–2.46)	1.82 (0.73–4.58)
High	0.43 (0.14–1.32)	0.78 (0.22–2.73)
$P_{\text{contrast T3:T1}}$	.14	.69
β-Carotene		
Low	1.0	1.0
Medium	0.75 (0.29–1.92)	0.8 (0.28–2.27)
High	0.47 (0.17–1.29)	0.79 (0.29–2.15)
$P_{\text{contrast T3:T1}}$	.14	.64
Xanthophylls		
Low	1.0	1.0
Medium	0.42 (0.17–1.00)	0.94 (0.39–2.24)
High	0.11 (0.03–0.43)	0.13 (0.02–0.77)
$P_{\text{contrast T3:T1}}$	.002†	.02†
Lycopene		
Low	1.0	1.0
Medium	0.76 (0.32–1.81)	0.27 (0.08–0.90)
High	0.59 (0.21–1.61)	0.84 (0.30–2.33)
$P_{\text{contrast T3:T1}}$	.3	.74
Total carotenoids		
Low	1.0	1.0
Medium	0.7 (0.31–1.63)	1.33 (0.54–3.29)
High	0.21 (0.06–0.79)	0.31 (0.07–1.44)
$P_{\text{contrast T3:T1}}$	.02†	.14
Quercetin		
Low	1.0	1.0
Medium	1.19 (0.50–2.83)	0.93 (0.37–2.37)
High	1.01 (0.40–2.57)	0.92 (0.34–2.47)
$P_{\text{contrast T3:T1}}$	.97	.86
α-Tocopherol		
Low	1.0	1.0
Medium	0.69 (0.28–1.72)	0.37 (0.12–1.10)
High	0.53 (0.20–1.37)	0.48 (0.18–1.27)
$P_{\text{contrast T3:T1}}$	.19	.14
γ-Tocopherol		
Low	1.0	1.0
Medium	0.62 (0.24–1.58)	0.53 (0.20–1.42)
High	0.75 (0.30–1.89)	0.37 (0.12–1.13)
$P_{\text{contrast T3:T1}}$	.54	.08

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
* Adjusted for age, body mass index, tumor site, cancer stage, and tobacco use.
† $p < .05$ was considered statistically significant.

whether supplemental xanthophylls would behave the same way as β-carotene and tocopherols and interact with smoking and/or increase cancer risk and mortality.

Unlike carotenoids, higher quercetin concentrations were not protective. Concentrations of quercetin, kaempferol, naringenin, and hesperitin in fasting plasma have been shown to be reasonable biomarkers of recent dietary intakes with a short half-life.[34] Carotenoids are highly non-polar with much longer half-lives.[35–37] Quercetin is more polar and has both poor and highly variable bioavailability.[38] Therefore, the findings that quercetin was not associated with survival may not negate the potential protective effects of foods that contain quercetin.

The results of this study should be interpreted in light of several strengths and limitations. Strengths include the prospective study design, ability to adjust for some of the most significant potential confounding factors, and the ability to account for HPV status of the tumor. Numerous studies have reported a survival advantage in patients with head and neck cancer with HPV-positive tumors compared to those with HPV-negative tumors.[39] Our group previously reported that HPV-positivity was associated with significantly higher intakes of chemoprotective micronutrients.[40] In the current study, we were able to account for HPV status when adjusting for tumor site, decreasing the potential for confounding by this characteristic. Because of the small sample size and incomplete data on posttreatment smoking status on 30.6% of the study population, we did not have the statistical power to consider tobacco use as a time-dependent variable in statistical models, which was a limitation. Another limitation was that we did not have the power to evaluate patients by type of cancer treatment received. In patients with stage IV disease, which were the majority of the study group, the efficacy of chemotherapy and radiation therapy is variable. We also found significant, inverse correlations between sample storage time and α-carotene, β-carotene, and total carotenoids, suggesting there may have been some degradation of these nutrients in the older serum samples that were used in this analysis. However, we hypothesize that any degradation of nutrients that may have occurred in our samples over time likely resulted in an attenuation of their associations with recurrence-free and overall survival time. Finally, the prospective study design only allowed for the determination of associations, and we cannot rule out the possibility of reverse causality and potential unmeasured and residual confounding.

In summary, we found strong protective associations of serum xanthophyll and total carotenoid concentrations on overall survival time and protective associations of serum xanthophyll concentrations with recurrence-free time in patients with head and neck cancer. This indicates that, in this population, dietary intake of other micronutrients with putative preventive properties was either already sufficiently high for maximal benefit and/or that intake of foods high in xanthophylls and carotenoids was deficient. Future research could evaluate the possible benefits of an intervention to increase intakes of foods that are rich sources of xanthophylls in patients with head and neck cancer or in persons at increased risk for head and neck cancer.

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