

Serum Carotenoids and α -Tocopherol and Risk of Nonmelanoma Skin Cancer

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Abstract

Background: Carotenoids and tocopherols have been hypothesized to protect against cancer. **Methods:** We prospectively evaluated associations of several carotenoids and α -tocopherol with risk of nonmelanoma skin cancer using serum collected at baseline from 302 subjects in the Isotretinoin-Basal Cell Carcinoma Prevention Trial. All subjects had at least two BCCs in the 5 years prior to randomization. During 5 years of follow-up, 70 subjects did not develop a nonmelanoma skin cancer, 221 developed a BCC, and 85 developed a squamous cell carcinoma (SCC). Cox proportional hazards models were used to estimate risk ratios. Models were stratified by clinical center and gender and adjusted for age, solar damage, skin type, number of prior BCCs and/or SCCs, treatment group, body mass index, and serum low-density lipoprotein-cholesterol and high-density

lipoprotein-cholesterol. **Results:** Risk of developing a subsequent BCC was not related to serum levels of any of the carotenoids measured or to α -tocopherol. Serum levels of α -carotene, β -carotene, lycopene, and α -tocopherol also were not independently related to risk of a subsequent SCC. However, serum lutein, zeaxanthin, and β -cryptoxanthin were positively related to SCC risk; risk ratios for subjects in the highest versus lowest tertiles of these micronutrients were 1.63 [95% confidence interval (95% CI) 0.88-3.01; *P* for trend = 0.01], 2.40 (95% CI 1.30-4.42; *P* for trend = 0.01), and 2.15 (95% CI 1.21-3.83; *P* for trend = 0.09), respectively. **Conclusion:** Additional research is needed on the relationship of carotenoids to SCC risk in the general population and in subsets of the population who are at increased risk. (Cancer Epidemiol Biomarkers Prev 2004;13(8):1276-82)

Introduction

Nonmelanoma skin cancers (NMSC) including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common malignancies in the United States, and their incidence is rising (1). While rarely fatal, NMSCs can cause considerable morbidity (2), and because of their high incidence, treatment-associated societal costs are substantial (3). Exposure to UV radiation is the major risk factor for NMSC (4). Melanin is the primary UV-absorbing pigment in the skin, and individuals with higher concentrations of melanin are at a reduced risk of NMSC (4). Carotenoids are another class of pigments that are accumulated in the skin and s.c. fat (5). Carotenoids could potentially prevent NMSC by acting as antioxidants to prevent UV-induced free radicals from damaging the skin (6). α -Tocopherol also accumulates in the skin (5) and could protect against NMSC by scavenging UVB-induced oxidants (7).

There are over 600 carotenoids in nature (8). β -Carotene, α -carotene, lycopene, lutein, zeaxanthin, and

β -cryptoxanthin are distributed widely in human tissues including skin (5, 9). β -carotene is the carotenoid that has been studied most extensively in relationship to NMSC, and research generally does not support a protective effect (10-20). Less is known about the relationships of the other carotenoids with risk of NMSC. We prospectively evaluated associations of serum α -carotene, β -carotene, lycopene, lutein, zeaxanthin, β -cryptoxanthin, and α -tocopherol with risk of developing NMSC using serum collected at baseline in the Isotretinoin-BCC Prevention Trial (ISO-BCC).

Methods

The ISO-BCC was a randomized double-blind, placebo-controlled clinical trial to test the efficacy of low-dose isotretinoin to reduce BCC incidence in high-risk populations and to evaluate its safety. The study has been described in detail (21). Briefly, the trial was conducted at eight clinical centers around the United States. Caucasian males and females ages 40 to 75 years old who had two or more biopsy-proven BCCs during the 5 years before randomization in 1984 to 1987 were eligible to participate if they were free of serious illness and had normal liver and renal function; their entire skin surface could be evaluated; and they were willing to

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seek appropriate definitive treatment for BCCs that arose during the trial, were willing and able to participate for the 5-year duration of the trial, agreed not to take high-dose vitamin A (>5,000 units/d) during the 3-year treatment phase of the trial, and, for women only, were incapable of childbearing. Individuals were excluded if they had a history of basal cell nevus syndrome, xeroderma pigmentosum, or psoriasis; used topical 5-fluorouracil or tretinoin within 6 months or isotretinoin within 1 year of randomization; had cancer other than NMSC within 5 years of randomization; and were hyperlipidemic or allergic to retinoids or parabens (preservatives used in the drug capsule). All subjects gave written informed consent before trial entry, and an independent Data and Safety Monitoring Committee monitored the study.

Prior to randomization, dermatologic examinations of the entire skin surface were done, and suspicious skin lesions were removed and biopsied. Overall actinic damage was evaluated as an indication of cumulative solar damage using a reference set of standardized photographs as a guide. Additionally, the dermatologist assessed each subject's skin type using the Fitzpatrick skin grading criteria (22). Subjects' reports of NMSC in the prior 5 years were confirmed by medical records and pathology reports. Blood for biochemical measurements was collected in the morning after a 12-hour fast, and at three clinical centers (Northwestern University, University of Arkansas, and RPMI), additional blood was collected for long-term storage of serum at -70°C .

Trial medication, either isotretinoin (5 mg/d) or placebo, was dispensed at 6-month intervals over the first 3 years of the trial. The last 2 years consisted of follow-up only. Follow-up clinic visits to monitor skin cancer, potential treatment toxicity, and compliance were scheduled at 2 weeks, 3 months, 6 months, and 12 months and every 6 months thereafter for the remaining 4 years of the trial. The dermatologist did a detailed dermatologic examination of the entire skin surface for each subject at each visit. Any suspicious lesions were biopsied. Definitive treatment was provided, and the precise location of the biopsied lesion was recorded to avoid double counting at subsequent exams. At the end of the trial, there were no significant treatment group differences in the percentage of subjects with a new BCC or the annual rate of BCC formation (23).

Serum collected at baseline for long-term storage from 302 subjects at Northwestern University, University of Arkansas, and RPMI clinical centers was used for the current analysis of serum carotenoids and α -tocopherol in relationship to subsequent NMSC. All assays were done by the Inorganic Toxicology and Nutrition Branch, Division of Laboratory Sciences, Centers for Disease Control and Prevention (Atlanta, GA). Serum fat-soluble micronutrient determinations were done using a modification of the National Health and Nutrition Examination Survey III high-pressure liquid chromatography method (24). The major modifications were the use of a Phenomenex (Torrance, CA) 150×4.6 mm Ultracarb 3 C18 column, gradient elution of the column using 15:85 to 50:50 ethanol/acetonitrile, and use of apo-8'-carotenal as an internal standard for the carotenoids. Quality control was established using duplicate analysis of three in-house serum pools with target values determined over

multiple runs. Quality control pools were linked to a National Institute of Standards and Technology-certified standard reference material and to previous quality control pools. The laboratory participates and scores well on National Institute of Standards and Technology round robin exercises for fat-soluble micronutrients. The samples were assayed in 12 separate runs on different days over the course of 2 months. Assay coefficients of variation for blinded external quality control samples included with study subjects' samples were 10.3% for α -carotene, 9.9% for β -carotene, 10.5% for lycopene, 8.9% for lutein, 9.0% for zeaxanthin, 8.7% for β -cryptoxanthin, and 9.4% for α -tocopherol.

We evaluated relationships of carotenoid and α -tocopherol concentrations in serum collected at baseline in ISO-BCC with risk of subsequent NMSC using Cox proportional hazards models with time to first NMSC as the dependent variable (25). Analyses were done separately for BCCs and SCCs. All analyses were stratified by sex and clinic. Because females tend to have higher concentrations of most carotenoids than males (26), we created sex-specific tertiles using distributions of micronutrients for all males and separately for all females. Final models were adjusted for age, overall solar damage at baseline, skin type, treatment group, body mass index [weight (kg)/height (m^2)], and serum high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol concentrations. Models to evaluate associations of micronutrients with risk of BCC were also adjusted for number of prior BCCs, and models for risk of SCC were adjusted for number of prior BCCs and number of prior SCCs. Time since randomization was used as the underlying time metric in all models, because the risk of disease changed more with time since randomization than with age. Proportionality of hazards was evaluated by testing the statistical significance of time-by-micronutrient interactions. All interaction *P* values were >0.05 . Statistical analyses were conducted using SAS version 8 (SAS Institute, Inc., Cary, NC).

Results

Of the 302 ISO-BCC subjects included in the current analysis, 70 did not develop any NMSC during follow-up, 147 developed only a BCC, 11 developed only an SCC, and 74 developed a BCC and a SCC. Characteristics of subjects by NMSC diagnosis during follow-up are shown in Table 1. Males were significantly more likely to be diagnosed with NMSC, particularly SCC, during follow-up compared with females. Older individuals were also more likely to be diagnosed with SCC compared with younger individuals. Moderate-to-severe solar damage and a higher number of BCCs and SCCs during the 5 years prior to randomization were also strongly related to diagnosis of NMSC during follow-up.

Unadjusted mean concentrations of carotenoids and α -tocopherol tended to be lower in subjects who developed a NMSC during follow-up compared with those who did not develop a NMSC (Table 2). Serum concentrations of carotenoids were significantly correlated. The magnitude of the correlation varied from 0.71 for α -carotene and β -carotene to 0.29 for α -carotene and

Table 1. Characteristics of ISOBCC participants by NMSC diagnosis during follow-up who were included in analyses of baseline serum micronutrient concentrations and NMSC risk

	No NMSC		<i>P</i> [‡]	SCC [†]	
	<i>n</i> (%)	BCC* <i>n</i> (%)		<i>n</i> (%)	<i>P</i> [‡]
Gender					
Male	38 (54.3)	174 (78.7)	<0.0001	81 (95.3)	<0.0001
Female	32 (45.7)	47 (21.3)		4 (4.7)	
Age (y)			0.12		0.004
40-49	12 (17.1)	21 (9.5)		3 (3.5)	
50-59	24 (34.3)	60 (27.2)		20 (23.5)	
60-69	27 (38.6)	104 (47.0)		46 (54.1)	
70+	7 (10.0)	36 (16.3)		16 (18.8)	
Clinic			<0.0001		<0.0001
University of Arkansas	12 (17.1)	82 (37.1)		52 (61.2)	
Northwestern University	45 (64.3)	77 (34.8)		20 (23.5)	
RPMI	13 (18.6)	62 (28.0)		13 (15.3)	
Skin type			0.71		0.59
Never tans, always burns	28 (40.0)	92 (41.6)		37 (43.5)	
Usually tans, always burns	20 (28.6)	68 (30.8)		20 (23.5)	
Always tans, sometimes burns	18 (25.7)	55 (24.9)		26 (30.6)	
Always tans, rarely burns	4 (5.7)	6 (2.7)		2 (2.4)	
Overall solar damage			<0.0001		<0.0001
None or mild	45 (64.3)	80 (36.2)		15 (17.6)	
Moderate	24 (34.3)	108 (48.9)		52 (61.2)	
Severe	1 (1.4)	33 (14.9)		18 (21.2)	
No. of BCCs in prior 5 years			<0.0001		<0.0001
1-2	52 (74.3)	65 (29.4)		22 (25.9)	
3-4	15 (21.4)	81 (36.6)		27 (31.8)	
5-6	2 (2.9)	29 (13.1)		20 (23.5)	
7+	1 (1.4)	46 (20.8)		16 (18.8)	
No. of SCCs in prior 5 years			0.002		<0.0001
None	66 (94.3)	178 (80.5)		55 (64.7)	
1	4 (5.7)	25 (11.3)		14 (16.5)	
2+	0.0	18 (8.1)		16 (18.8)	
Treatment group			0.23		0.22
Group A	39 (55.7)	105 (47.5)		39 (45.9)	
Group B	31 (44.3)	116 (52.5)		46 (54.1)	

*Includes 147 subjects with only a BCC and 74 subjects with a BCC and a SCC.

[†]Includes 11 subjects with only a SCC and 74 subjects with a SCC and a BCC.

[‡]*P* values from two-sided χ^2 tests for comparisons with no NMSC (data column 1).

zeaxanthin. α -Tocopherol concentration was also significantly correlated with the concentration of each of the carotenoids.

Serum levels of carotenoids and α -tocopherol were not related to risk of a subsequent BCC in our subjects (Table 3). Lycopene and α -tocopherol also were not re-

lated to risk of a subsequent SCC. Lutein, zeaxanthin, and β -cryptoxanthin, however, were positively associated with SCC risk in our subjects, all of whom had a history of multiple prior BCCs. The risk ratio (RR) for subjects in the highest versus lowest tertile of zeaxanthin was 2.40 [95% confidence interval (95% CI) 1.30-4.42],

Table 2. Baseline serum concentrations of carotenoids and α -tocopherol and subsequent NMSC in ISO-BCC participants

Micronutrients (μ g/dL)	No NMSC (<i>n</i> = 70)		BCC* (<i>n</i> = 221)			SCC [†] (<i>n</i> = 85)		
	Mean	SE	Mean	SE	<i>P</i> [‡]	Mean	SE	<i>P</i> [‡]
α -Carotene	4.59	0.42	4.12	0.39	0.52	4.03	0.84	0.58
β -Carotene	23.07	1.86	19.65	1.08	0.12	19.94	1.84	0.24
Lycopene [§]	46.37	2.69	42.03	1.41	0.14	39.56	2.12	0.05
Lutein	16.42	1.02	15.27	0.54	0.30	16.07	1.05	0.81
Zeaxanthin	5.94	0.39	5.41	0.16	0.15	5.65	0.31	0.57
β -Cryptoxanthin	7.41	0.56	7.01	0.32	0.54	6.91	0.48	0.49
α -Tocopherol	1,454.62	79.45	1,376.71	34.48	0.30	1,359.23	53.74	0.31

*Includes 147 subjects with only a BCC and 74 subjects with a BCC and a SCC.

[†]Includes 11 subjects with only a SCC and 74 subjects with a SCC and a BCC.

[‡]*P* values from two-sided χ^2 tests for comparisons with no NMSC (data column 1).

[§]Lycopene data were not available for one subject with no subsequent NMSC, two subjects with a subsequent BCC, and two subjects with a subsequent SCC.

Table 3. RRs for BCC and SCC associated with serum carotenoid and α -tocopherol levels

Micronutrients	Tertile*	BCC [†]					SCC [‡]				
		Cases (n)	Noncases (n)	RR	95% CI	P for trend	Cases (n)	Noncases (n)	RR	95% CI	P for trend
α -Carotene	1	74	26	1.00		0.56	27	73	1.00		0.04
	2	76	26	1.21	0.87-1.70		31	71	1.48	0.85-2.56	
	3	71	29	1.06	0.74-1.51		27	73	1.45	0.92-3.06	
β -Carotene	1	77	23	1.00		0.94	26	74	1.00		0.06
	2	69	33	0.77	0.55-1.09		30	72	1.09	0.62-1.93	
	3	75	25	1.01	0.71-1.44		29	71	1.47	0.81-2.68	
Lycopene	1	79	20	1.00		0.56	31	68	1.00		0.74
	2	66	34	0.72	0.50-1.03		29	71	1.06	0.59-1.89	
	3	74	26	1.01	0.70-1.45		23	77	0.99	0.58-2.01	
Lutein	1	76	24	1.00		0.68	23	77	1.00		0.01
	2	74	28	0.81	0.58-1.14		34	68	1.50	0.85-2.68	
	3	71	29	1.04	0.72-1.50		28	72	1.63	0.88-3.01	
Zeaxanthin	1	75	25	1.00		0.37	25	75	1.00		0.01
	2	76	26	0.94	0.66-1.32		30	72	1.75	0.99-3.08	
	3	70	30	0.97	0.64-1.39		30	70	2.40	1.30-4.42	
β -Cryptoxanthin	1	78	22	1.00		0.42	25	75	1.00		0.09
	2	68	34	0.84	0.59-1.19		26	76	1.45	0.80-2.65	
	3	75	25	1.06	0.75-1.50		34	66	2.15	1.21-3.83	
α -Tocopherol	1	73	27	1.00		0.53	30	70	1.00		0.14
	2	76	26	0.97	0.69-1.38		25	77	1.24	0.67-2.19	
	3	72	28	1.15	0.78-1.71		30	70	1.60	0.87-3.08	

*Tertile cut points for males ($\mu\text{g}/\text{dL}$): α -carotene: 1 (<1.81), 2 (1.81-3.51), 3 (3.52+); β -carotene: 1 (<10.50), 2 (10.50-20.37), 3 (20.38+); lycopene: 1 (<30.88), 2 (30.88-49.60), 3 (49.61+); lutein: 1 (<10.95), 2 (10.95-16.91), 3 (16.92+); zeaxanthin: 1 (<4.11), 2 (4.11-6.13), 3 (6.14+); β -cryptoxanthin: 1 (<4.42), 2 (4.42-7.34), 3 (7.35+); α -tocopherol: 1 (<1,088.95), 2 (1,088.95-1,382.37), 3 (1,382.38+). Tertile cut points for females ($\mu\text{g}/\text{dL}$): α -carotene: 1 (<3.23), 2 (3.23-6.08), 3 (6.09+); β -carotene: 1 (<14.65), 2 (14.65-26.57), 3 (26.58+); lycopene: 1 (<34.87), 2 (34.87-54.40), 3 (54.41+); lutein: 1 (<13.31), 2 (13.31-20.73), 3 (20.74+); zeaxanthin: 1 (<4.26), 2 (4.26-6.28), 3 (6.29+); β -cryptoxanthin: 1 (<5.26), 2 (5.26-9.50), 3 (9.51+); α -tocopherol: 1 (<1,258.73), 2 (1,258.73-1,518.30), 3 (1,518.31+).

[†]Stratified by clinic and gender and adjusted for baseline age, solar damage, skin type, number of prior BCCs, body mass index, treatment group, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol.

[‡]Stratified by clinic and gender and adjusted for baseline age, solar damage, skin type, number of prior BCCs, number of prior SCCs, body mass index, treatment group, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol.

and the trend of increasing risk with increasing concentration was statistically significant ($P = 0.01$). The comparable RR for lutein was 1.63 (95% CI 0.88-3.01; P for trend = 0.01). For β -cryptoxanthin, the RR for subjects in the highest versus lowest tertile was 2.15 (95% CI 1.21-3.83). Furthermore, although the trend based on continuous data was not significant ($P = 0.09$), a pattern of increasing risk was observed across increasing tertiles of β -cryptoxanthin. α -Carotene and β -carotene also were positively associated with SCC when modeled separately. However, after adjustment for lutein and zeaxanthin, associations were no longer statistically significant (Table 4), which suggests that associations of α -carotene and β -carotene with SCC were likely due to their correlation with other carotenoids in serum.

Of the 221 subjects who developed a BCC during follow-up, 74 also developed a SCC. When we restricted analysis to the 147 subjects who developed only a BCC during follow-up, associations of serum micronutrients with risk of a subsequent BCC were not markedly different from those shown in Table 3 for risk of developing a BCC regardless of SCC status.

As expected, smokers had significantly lower serum concentrations of carotenoids and α -tocopherol compared with nonsmokers. However, adjusting for the number of cigarettes smoked per day did not substantially change results shown in Table 3 for associations of micronutrients with subsequent BCC and SCC. Adjusting for alcohol ingestion also did not alter these results.

Discussion

Carotenoids and α -tocopherol have been hypothesized to protect against cancer. β -Carotene is the carotenoid that has been studied most extensively in relationship to NMSC, with little support for an anticancer effect emerging from this research. Serum lycopene concentration also was not related to NMSC in one earlier prospective study (10). This is the first study to prospectively evaluate associations of serum levels of the carotenoids α -carotene, lutein, zeaxanthin, and β -cryptoxanthin with NMSC risk. Results suggest no effect of serum carotenoids and α -tocopherol on risk of a subsequent BCC in individuals with two or more prior BCCs. α -Carotene, β -carotene, lycopene, and α -tocopherol also were not independently related to risk of a subsequent SCC. In contrast, our findings suggest an increased risk of subsequent SCC associated with higher levels of lutein, zeaxanthin, and β -cryptoxanthin among individuals at high risk of SCC because of multiple prior NMSCs.

Our study had several strengths. Complete dermatologic exams of the entire skin surface were done at baseline and at 6-month or more frequent intervals to identify and remove skin cancers. All exams were done by board-certified dermatologists who attended a central orientation session to improve consistency of skin tumor surveillance across the participating clinical centers. All cancers were histologically confirmed, and incident and

recurrent skin cancers were distinguished. Serum was frozen at -70°C , and concentrations of carotenoids and α -tocopherol are stable for many years when specimens are maintained under appropriate conditions (27, 28). Additionally, established risk factors for NMSC including male gender, age, moderate-to-severe solar skin damage, and number of prior NMSCs (4, 29) were associated with risk of a subsequent NMSC in our subjects. Although it would have been ideal to measure micronutrients in multiple blood samples from each subject, this was not feasible; instead, we measured micronutrients in a single blood sample collected from each subject. Concentrations of carotenoids and α -tocopherol are reported to be reasonably stable over time in the same individual in some studies (30, 31) although more variable in others (32, 33). Profiles of carotenoids and tocopherols are similar in plasma and skin; Spearman correlations range from 0.62 for zeaxanthin to 0.83 for β -cryptoxanthin (5). Therefore, serum levels of these micronutrients provide a reasonable approximation of skin levels, which are probably more relevant to skin cancer etiology.

Limitations of our study include our relatively small sample size, fairly short 5-year follow-up period, and

lack of generalizability of results to individuals without a history of prior NMSC. All of our subjects had at least two prior NMSCs in the 5 years before randomization, and many had more than two NMSCs. Associations of serum micronutrients with NMSCs in individuals with multiple prior NMSCs could differ from other individuals as a consequence of higher exposures to environmental risk factors such as UV radiation and/or increased genetic susceptibility. Therefore, inferences from our results should not be made to individuals without a positive history of NMSC. Half of the subjects in our study received supplemental isotretinoin (5 mg/d) for 3 years. In a separate study, individuals supplemented with 25,000 IU retinol for 4 to 5 years had significantly lower serum lutein and significantly higher serum α -tocopherol levels compared with the control group, but serum concentrations of β -carotene, α -carotene, lycopene, zeaxanthin, and β -cryptoxanthin did not differ between the two groups (5). Furthermore, skin concentrations of all the carotenoids and α -tocopherol were similar in the two treatment groups. We adjusted for treatment group in our analysis, and tests for interaction did not suggest significant effect modification by treatment group of observed associations between serum micronutrients and NMSC risk.

Individuals who eat a diet rich in carotenoids may also be more physically active and spend more time outdoors exposed to the sun. SCC is more directly dependent on high doses of UV exposure compared with BCC (34), and the higher risk of SCC associated with elevated carotenoids that we observed could also possibly be explained by greater UV exposure in a particularly susceptible segment of the population. Although we adjusted for solar skin damage and skin type in our analysis, residual confounding cannot be ruled out.

β -Carotene exhibits an anticancer effect for NMSC in some animal models (35, 36). However, observational studies in humans generally do not support a protective effect for BCC (10-14, 20, 37) or SCC (10, 15, 16). Furthermore, results of several clinical trials do not provide evidence for a protective effect of β -carotene for BCC or SCC (17-19). Data are scant on associations of carotenoids other than β -carotene with NMSC risk. Similar to us, Breslow et al. (10) did not observe an association of serum lycopene concentration with BCC or SCC in a small prospective nested case-control study, and dietary lycopene was not associated with BCC risk in the Health Professionals Follow-up Study (20). Although dietary α -carotene intake was weakly inversely associated with men's risk of developing BCC in the Health Professionals Follow-up Study (20), it was not associated with women's risk of BCC in the Nurses' Health Study (14) or with SCC risk in either cohort (16). Dietary lutein/zeaxanthin intake was positively associated with BCC and SCC risk in the Nurses' Health Study but not in the Health Professionals Follow-up Study (14, 16, 20). β -Cryptoxanthin intake also exhibited a slight positive association with risk of SCC in the Nurses' Health Study (16), but it was not related to risk of SCC in the Health Professionals Follow-up Study or with BCC risk in either cohort (14, 16, 20).

Topical α -tocopherol inhibits UVB-induced photocarcinogenesis and DNA photodamage in mice *in vivo* (38). However, studies in humans generally have not

Table 4. RRs for SCC associated with serum carotenoid levels with adjustment for another carotenoid

Micronutrients	Tertile*	RR	95% CI	P for trend
α -Carotene and lutein				
	α -Carotene	1	1.00	0.22
		2	1.40	0.80-2.48
	3	1.54	0.81-2.91	
Lutein	1	1.00		0.03
	2	1.44	0.81-2.55	
	3	1.39	0.73-2.66	
α -Carotene and zeaxanthin				
	α -Carotene	1	1.00	0.06
		2	1.27	0.73-2.22
	3	1.46	0.79-2.71	
Zeaxanthin	1	1.00		0.02
	2	1.64	0.92-2.91	
	3	2.21	1.19-4.13	
β -Carotene and lutein				
	β -Carotene	1	1.00	0.32
		2	0.99	0.55-1.78
	3	1.24	0.65-2.38	
Lutein	1	1.00		0.04
	2	1.44	0.79-2.62	
	3	1.50	0.76-2.95	
β -Carotene and zeaxanthin				
	β -Carotene	1	1.00	0.25
		2	0.88	0.49-1.60
	3	1.06	0.56-2.03	
Zeaxanthin	1	1.00		0.03
	2	1.76	0.95-3.25	
	3	2.38	1.24-4.58	

NOTE: Stratified by clinic and gender and adjusted for baseline age, solar damage, skin type, number of prior BCCs, number of prior SCCs, body mass index, treatment group, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and other carotenoid.

*Tertile cut points for males ($\mu\text{g}/\text{dL}$): α -carotene: 1 (<1.81), 2 (1.81-3.51), 3 (3.52+); β -carotene: 1 (<10.50), 2 (10.50-20.37), 3 (20.38+); lutein: 1 (<10.95), 2 (10.95-16.86), 3 (16.87+); zeaxanthin: 1 (<4.08), 2 (4.08-6.13), 3 (6.14+). Tertile cut points for females ($\mu\text{g}/\text{dL}$): α -carotene: 1 (<3.18), 2 (3.18-6.08), 3 (6.09+); β -carotene: 1 (<14.65), 2 (14.65-29.59), 3 (29.60+); lutein: 1 (<13.31), 2 (13.31-20.73), 3 (20.74+); zeaxanthin: 1 (<4.26), 2 (4.26-6.28), 3 (6.29+).

observed a protective effect of α -tocopherol for NMSC. Serum α -tocopherol concentration was not significantly related to risk of BCC (10, 39) or SCC (10, 15) in three prospective serum studies. Dietary intake of α -tocopherol also is not related to SCC (16), and findings for BCC are inconsistent (11, 14, 20).

Both BCCs and SCCs arise from keratinocytes in the epidermis (40). Whereas SCCs originate via multiple steps of initiation, promotion, and progression, BCCs seem to arise *de novo* (41). Because of our relatively short 5-year follow-up, our findings may be more relevant to progression of SCCs than to initiation. Phorbol acetate promotes radiation induced SCCs but not BCCs in mice (42). Differences in associations of serum micronutrients that we observed for BCC and SCC could similarly be related to the different pathogenesis of these tumors.

The local environment strongly influences the chemical and physical properties of carotenoids. The electron-rich polyene chain characteristic of all carotenoids confers their susceptibility to oxidation (6). These reactions generate short-lived carotenoid radicals. *In vitro* carotenoids can act as antioxidants or prooxidants depending on experimental conditions including concentrations of the carotenoids and other antioxidants (e.g., α -tocopherol and vitamin C) and oxygen tension (43-47). The positive associations we observed for lutein, zeaxanthin, and β -cryptoxanthin in relation to subsequent SCC could possibly be due to prooxidant activities. Interactions with proteins and other molecules are critical for correct functioning of carotenoids, and damage to our subjects' skin, all of whom had at least two BCCs in the 5 years before randomization, could also have played a role by modifying the local environment.

Our data suggest that, in individuals with two or more BCCs, serum carotenoids and α -tocopherol are not related to risk of a subsequent BCC. Serum α -carotene, β -carotene, lycopene, and α -tocopherol also are not independently related to risk of a subsequent SCC. However, in these individuals, higher serum lutein, zeaxanthin, and β -cryptoxanthin levels are associated with an increased risk of a subsequent SCC. Additional research is needed to clarify the relationships of carotenoids to SCC risk in the general population and in subsets of the population who, because of genetic predisposition or environmental exposures, are at an increased risk.

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