

## Serum Micronutrients and Upper Aerodigestive Tract Cancer<sup>1</sup>

Abraham M. Y. Nomura,<sup>2</sup> Regina G. Ziegler,  
Grant N. Stemmermann, Po-Huang Chyou,  
and Neal E. Craft

Japan-Hawaii Cancer Study, Kuakini Medical Center, Honolulu, Hawaii 96817 [A. M. Y. N.]; Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland 20892 [R. G. Z.]; Department of Pathology, University of Cincinnati Medical Center, Cincinnati, Ohio 45267 [G. N. S.]; Department of Epidemiology and Biostatistics, Marshfield Medical Research Foundation, Marshfield, Wisconsin 54449 [P.-H. C.]; and Craft Technologies, Wilson, North Carolina 27893 [N. E. C.]

### Abstract

Numerous dietary studies have found that vegetables and fruits protect against upper aerodigestive tract cancer. To evaluate the role of  $\beta$ -carotene and other specific carotenoids, a nested case-control study using prediagnostic serum was conducted among 6832 American men of Japanese ancestry examined from 1971 to 1975. During a surveillance period of 20 years, the study identified 28 esophageal, 23 laryngeal, and 16 oral-pharyngeal cancer cases in this cohort. The 69 cases were matched to 138 controls. A liquid chromatography technique, designed to optimize recovery and separation of the individual carotenoids, was used to measure serum levels of lutein, zeaxanthin,  $\beta$ -cryptoxanthin, lycopene,  $\alpha$ -carotene,  $\beta$ -carotene, retinol, retinyl palmitate, and  $\alpha$ -,  $\delta$ -, and  $\gamma$ -tocopherol.

With adjustment for cigarette smoking and alcohol intake, we found that  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, total carotenoids and  $\gamma$ -tocopherol levels were significantly lower in the 69 upper aerodigestive tract cancer patients than in their controls. Trends in risk by tertile of serum level were significant for these five micronutrients. These significant trends persisted in cases diagnosed 10 or more years after phlebotomy for the three individual carotenoids and total carotenoid measurements. The odds ratios for the highest tertile were 0.19 (95% confidence interval, 0.05-0.75) for  $\alpha$ -carotene, 0.10 (0.02-0.46) for  $\beta$ -carotene, 0.25 (0.06-1.04) for  $\beta$ -cryptoxanthin, and 0.22 (0.05-0.88) for total carotenoids. When the cases were separated into esophageal, laryngeal, and oral-pharyngeal cancer, both

$\alpha$ -carotene and  $\beta$ -carotene were consistently and strongly associated with reduced risk at each site. The findings suggest that  $\alpha$ -carotene and other carotenoids, as well as  $\beta$ -carotene, may be involved in the etiology of upper aerodigestive tract cancer.

### Introduction

A substantial number of dietary studies have found that the frequent intake of vegetables and fruits, especially those containing  $\beta$ -carotene and other carotenoids, has a protective effect against upper aerodigestive tract cancer involving the oral-pharyngeal, esophageal, and laryngeal sites (1-15). A few studies, however, have not reported a similar dietary effect (16, 17). Carotenoids are red and yellow compounds found in vegetables and fruits and are postulated to inhibit carcinogenesis by functioning in humans as they function in plants, as singlet oxygen quenchers and antioxidants (18). Vitamin E, an intracellular antioxidant, has also been reported to have an inverse association with upper aerodigestive tract cancer (3, 4). It protects polyunsaturated fats and other lipids from oxidation (19), which may promote tumor development (20).

In contrast to dietary studies, there have been very few serum studies of upper aerodigestive tract cancer (21, 22). This is partly due to the difficulty of obtaining prediagnostic samples and to the relative infrequency of this cancer. One study involved 40 cases of upper aerodigestive tract cancer (21), whereas the other included just 28 oral/pharyngeal cancer cases (22).

We conducted a prospective study in which serum samples were obtained on a large cohort of men who were then followed for 20 years to identify 69 incident cases of upper aerodigestive tract cancer (18 oral-pharyngeal, 28 esophageal, and 23 laryngeal). We investigated the relationships of serum levels of individual and total carotenoids, retinol and total retinoids, and individual and total tocopherols to subsequent development of this cancer. To measure these micronutrients simultaneously, we used a HPLC<sup>3</sup> method that was developed recently at the National Institute of Standards and Technology to optimize the separation and recovery of the individual carotenoids (23, 24).

### Materials and Methods

The study population consisted of Japanese American men born from 1900 to 1919 who were identified by the Honolulu Heart Program in 1965 with use of the comprehensive 1942 Selective Service registration files (25). Of 11,148 eligible men on the Hawaiian island of Oahu, 8,006 (72%) were interviewed and examined from 1965 to 1968, 180 (2%) died before they could be examined, and 2,962 (26%) did not participate in the program.

Approximately 6 years later, from 1971 to 1975, 6860 (85.7%) of the 8006 examined men returned for another round

Received 1/14/97; accepted 3/12/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> Supported in part by Grant CA-33644, National Cancer Institute, National Institutes of Health, Bethesda, MD. Micronutrient analyses for this project were performed in the Organic Analytical Research Division of the Chemical Science and Technology Laboratory at the National Institute of Standards and Technology and were partially supported by Interagency Agreement No. Y01-CP9-0513 with the National Cancer Institute.

<sup>2</sup> To whom requests for reprints should be addressed, at Japan-Hawaii Cancer Study, Kuakini Medical Center, 347 North Kuakini Street, Honolulu, HI 96817.

<sup>3</sup> The abbreviation used is: HPLC, high performance liquid chromatography.

of examinations. At that time, a nonfasting venous blood sample was obtained from 6832 (99.6%) of the men and centrifuged; then the separated serum was stored at  $-75^{\circ}\text{C}$ . There were 10 prevalent cases of upper aerodigestive tract cancer; therefore, there were excluded from this analysis. The subsequent diagnosis of upper aerodigestive tract cancer among the remaining men was recorded by continuous surveillance of all general hospitals on Oahu. To reduce the possibility of missing diagnosed cases, a computer linkage file was established with the Hawaii Tumor Registry, a member of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute. The identification of incident cases should be nearly complete, because only 1.3% of the men could not be located on Oahu during a mail survey conducted 15 years later.

There were 69 cases of upper aerodigestive cancer initially diagnosed from 1971 to 1991 and confirmed by examination of tissue obtained surgically or by biopsy. Two other cases were excluded because they had not been confirmed histologically. All of the histologically confirmed cases had squamous cell cancer. The distribution of the newly diagnosed cases of cancer was as follows: 28 esophageal, 23 laryngeal, and 18 oral-pharyngeal.

Each of the 69 cases was matched with two control subjects from the study cohort with the same age at examination (except for 6 controls with a median difference of 1.2 years) and with the same month and year of examination (except for 31 controls with a median difference of 2 months). If a potential control had been diagnosed with any cancer (except nonmelanoma skin cancer), he was excluded from the analysis. Each control subject was alive at the time the corresponding case patient was diagnosed with upper aerodigestive tract cancer. Because an earlier study of this cohort had shown strong associations of cigarette smoking and alcohol intake with these cancers (26), the controls were also matched to cases according to cigarette smoking history (nonsmoker, past smoker, current smoker) and alcohol intake history (nondrinker, drank less than 20 ounces per month, drank 20 or more ounces per month) at time of examination from 1971–1975.

Individual carotenoids, retinoids, and tocopherols were measured in the stored sera from the cases and controls using the extraction and HPLC procedures described by Epler *et al.* (23). After thawing, 200- $\mu\text{l}$  aliquots were deproteinized by vortexing with an equal volume of ethanol containing B-apo-10'-carotenol oxime and tocol as internal standards and butylated hydroxytoluene as an antioxidant. The samples were then extracted twice with 1 ml of hexane; the combined supernatant was evaporated and redissolved in 50/50 (v/v) ethanol/ethyl acetate containing butylated hydroxytoluene.

The HPLC system consisted of a refrigerated autosampler at  $15^{\circ}\text{C}$ , a Bakerbond  $\text{C}_{18}$  column maintained at  $20^{\circ}\text{C}$ , an UV/visible detector to measure the carotenoids and retinoids, a fluorescence detector to measure the tocopherols, and a computer data system. The programmed mobile phase was based on three solvents, acetonitrile, methanol with 0.05 M ammonium acetate, and ethyl acetate, each containing 0.05% triethylamine. Calibration solutions at three concentrations, which spanned physiological levels of the micronutrients in serum, were prepared for lutein, zeaxanthin,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, retinol, retinyl palmitate, and  $\alpha$ -,  $\delta$ -, and  $\gamma$ -tocopherol. Internal standards were added to the calibration solutions at the same concentrations as used for the samples. The analyte area:internal standard area ratio was plotted against the analyte concentration:internal standard concentration ratio to generate linear calibration curves.

At the beginning and end of each day, National Institute of

Table 1 Percentage of distribution of cases and controls according to matching and nonmatching factors

Factor	Cases (n = 69)	Controls (n = 138)
Matching factors		
Age at exam (yr)		
<55	10.1	10.1
55–59	42.0	42.0
60–64	21.8	22.5
$\geq 65$	26.1	25.4
Cigarette smoking status		
Never smoked	14.5	14.5
Past smoker	29.0	29.0
Current smoker	56.5	56.5
<20 cigarettes/day	13.0	15.2
$\geq 20$ cigarettes/day	43.5	41.3
Alcohol consumption (oz"/mo)		
None	7.2	7.2
<20	29.0	29.0
$\geq 20$	63.8	63.8
20–39	16.0	29.0
$\geq 40$	47.8	34.8
Nonmatching factors		
Education		
Grades 0–6	44.1	50.8
Grades 7–12	39.7	41.6
Beyond high school	16.2	7.6
Religion		
Buddhist/Shintoist	78.1	81.3
All others	21.9	18.7
Marital status		
Never married	4.4	1.5
Married once	86.8	89.4
Married more than once	8.8	9.1

<sup>a</sup>oz, ounces.

Standards and Technology quality control sera were analyzed to ensure that the HPLC method was in control. In addition, quality control samples prepared at low and typical concentrations by the National Cancer Institute were interspersed with the prediagnostic sera prior to shipment to the laboratory. The relative SD for these blinded samples were 3–5% for all micronutrients, except  $\gamma$ -tocopherol.

Lycopene,  $\alpha$ -carotene, and  $\beta$ -carotene include both the *trans* and *cis* stereoisomers. Total carotenoids combine lutein, zeaxanthin,  $\beta$ -cryptoxanthin, lycopene,  $\alpha$ -carotene,  $\beta$ -carotene, and unidentified carotenoids. Total retinoids includes retinol and retinyl palmitate. Total tocopherols combine  $\alpha$ -,  $\delta$ -, and  $\gamma$ -tocopherol.

Because of the skewed frequency distributions of several micronutrients, Spearman's correlation coefficients were used to examine the interrelationship between serum micronutrient levels. One-way unbalanced analysis of covariance (27) was used to calculate the age-, smoking-, and alcohol-adjusted arithmetic mean serum micronutrient levels ( $P$ s based on  $\log_e$  transformation) in upper aerodigestive tract cancer cases and controls. Conditional logistic regression analysis (28) for a matched case-control study design was used to obtain estimates of covariate-adjusted odds ratios and corresponding 95% confidence intervals for upper aerodigestive tract cancer by tertile of serum micronutrient levels and time interval from phlebotomy to diagnosis. In addition, the dose-response trend in risk for cancer with increasing levels for specific serum micronutrients was evaluated by a method of the likelihood ratio test ( $P \leq 0.05$  was considered as statistically significant). This

Table 2 Spearman's correlation coefficients of serum micronutrients among controls

	Lutein	Zeaxanthin	$\beta$ -Cryptoxanthin	Lycopene	$\alpha$ -Carotene	$\beta$ -Carotene	Total carotenoids	Retinol	Total retinoids	$\alpha$ -Tocopherol	$\delta$ -Tocopherol	$\gamma$ -Tocopherol	Total tocopherols
Lutein	1.00	0.72	0.21	0.23	0.40	0.36	0.63	-0.16	-0.09	0.27	0.09	0.07	0.29
Zeaxanthin		1.00	0.38	0.27	0.32	0.40	0.67	-0.09	-0.02	0.26	0.14	0.15	0.28
$\beta$ -Cryptoxanthin			1.00	0.00	0.37	0.56	0.68	-0.02	0.00	0.13	-0.09	-0.05	0.10
Lycopene				1.00	0.26	0.28	0.40	0.16	0.19	0.29	0.16	0.27	0.32
$\alpha$ -Carotene					1.00	0.68	0.67	-0.02	0.02	0.11	0.03	-0.03	0.10
$\beta$ -Carotene						1.00	0.82	-0.05	0.02	0.14	0.07	0.00	0.12
Total carotenoids							1.00	-0.06	0.02	0.23	0.06	0.04	0.23
Retinol								1.00	0.95	0.40	0.30	0.16	0.38
Total retinoids									1.00	0.48	0.32	0.16	0.46
$\alpha$ -Tocopherol										1.00	0.40	0.26	0.98
$\delta$ -Tocopherol											1.00	0.67	0.49
$\gamma$ -Tocopherol												1.00	0.41
Total tocopherols													1.00

conditional logistic regression modeling approach was also used to assess the combined effects of specific micronutrients on the risk for cancer of the upper aerodigestive tract.

## Results

The 69 cases and 138 controls were matched on age at examination, date of examination, cigarette smoking history, and alcohol intake history. The distribution according to these factors was thus similar, as shown in Table 1. However, there were some differences between cases and controls in the number of cigarettes smoked per day among current smokers ( $P = 0.24$ , based on  $\chi^2$  test) and amount of alcohol consumed for those who drank 20 or more ounces per month ( $P = 0.04$ , based on  $\chi^2$  test). For this reason, statistical adjustment for detailed smoking and drinking history was carried out in the subsequent analyses.

Table 1 also presents the distribution of cases and control subjects according to other factors not matched in the study design, including education, religion, and marital status. No statistically significant differences were found.

Spearman's correlation coefficients for the 13 micronutrient measurements among controls are shown in Table 2. Total carotenoids, which includes lutein, zeaxanthin,  $\beta$ -cryptoxanthin, lycopene,  $\alpha$ -carotene and  $\beta$ -carotene, were moderately correlated ( $r = 0.6-0.8$ ) with all of the individual carotenoids except lycopene ( $r = 0.4$ ). Intercorrelation was moderate ( $r = 0.7$ ) between  $\alpha$ -carotene and  $\beta$ -carotene and between lutein and zeaxanthin. Intercorrelation between total retinoids and retinol and between total tocopherols and  $\alpha$ -tocopherol was close to 1.0, whereas  $\delta$ -tocopherol and  $\gamma$ -tocopherol were moderately correlated ( $r = 0.7$ ).

Table 3 compares the adjusted mean levels of serum micronutrients between the 69 upper aerodigestive tract cancer cases and their 138 matched controls. Statistically significant differences were found for total carotenoids,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, and  $\gamma$ -tocopherol with the cases having lower levels than controls for each of these micronutrients.

The cases also had a lower mean serum cholesterol level compared with controls (200.3 mg/dl versus 215.7 mg/dl;  $P = 0.005$ ). Further adjustment by analysis of covariance for serum cholesterol levels did not substantially change the results in Table 3, except the difference in total carotenoids was reduced (84.4  $\mu$ g/dl for cases and 95.2  $\mu$ g/dl for controls,  $P = 0.053$ ).

The odds ratios by tertile (based on the distribution of controls) were computed for the five serum micronutrients that were significantly different between cases and controls. The

Table 3 Adjusted<sup>a</sup> mean serum micronutrient levels in upper aerodigestive tract cancer cases and controls

Micronutrients	Cases (mean $\pm$ SE) <i>n</i> = 69	Controls (mean $\pm$ SE) <i>n</i> = 138	<i>P</i> <sup>b</sup>
Total carotenoids ( $\mu$ g/dl)	82.9 $\pm$ 5.3	96.7 $\pm$ 3.8	0.004
Lutein	15.4 $\pm$ 0.9	16.1 $\pm$ 0.6	0.327
Zeaxanthin	3.0 $\pm$ 0.2	3.3 $\pm$ 0.1	0.069
$\beta$ -Cryptoxanthin	11.3 $\pm$ 1.9	14.2 $\pm$ 1.3	0.021
Lycopene	19.1 $\pm$ 1.4	21.1 $\pm$ 1.0	0.266
$\alpha$ -Carotene	3.5 $\pm$ 0.4	4.9 $\pm$ 0.3	<0.001
$\beta$ -Carotene	12.5 $\pm$ 1.6	16.6 $\pm$ 1.2	<0.001
Total Retinoids ( $\mu$ g/dl)	74.5 $\pm$ 3.4	68.0 $\pm$ 2.4	0.421
Retinol ( $\mu$ g/dl)	66.4 $\pm$ 2.1	62.3 $\pm$ 1.5	0.497
Total tocopherols ( $\mu$ g/ml)	13.75 $\pm$ 1.18	14.26 $\pm$ 0.80	0.811
$\alpha$ -Tocopherol ( $\mu$ g/ml)	12.46 $\pm$ 1.08	12.78 $\pm$ 0.77	0.981
$\delta$ -Tocopherol ( $\mu$ g/ml)	0.11 $\pm$ 0.01	0.11 $\pm$ 0.01	0.632
$\gamma$ -Tocopherol ( $\mu$ g/ml)	1.14 $\pm$ 0.10	1.33 $\pm$ 0.07	0.001

<sup>a</sup> Adjusted for age, smoking status, number of cigarettes/day for past smokers, years smoked for past smokers, age started smoking for current smokers, number of cigarettes/day for current smokers, drinking status (yes/no), and number of ounces/month for drinkers.

<sup>b</sup> *P*s are based on  $\log_e$  transformation of serum micronutrient values.

trend was statistically significant for each of the micronutrients (Table 4; time interval of 0-20 years).

The importance of the time interval from phlebotomy to diagnosis was also evaluated for these five micronutrients. Because the subclinical presence of cancer may affect serum values, cases diagnosed within 5 years of phlebotomy were excluded from the analysis. Of the remaining cases, 17 were diagnosed from 5 to 10 years after examination, whereas 33 were diagnosed more than 10 years after examination. As shown in Table 4, inverse associations persisted for cases diagnosed more than 10 years after examination for  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and total carotenoids but were weakened in the same cases for  $\gamma$ -tocopherol.

The cases were then evaluated by specific subsite of cancer in Table 5, which shows the relative case-control difference [ $100 \times (\text{mean of cases} - \text{mean of controls}) / \text{mean of controls}$ ] for each micronutrient. Oral-pharyngeal cancer showed a significant inverse association with  $\alpha$ -carotene and  $\gamma$ -tocopherol; esophageal cancer with total carotenoids,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene, and  $\beta$ -carotene; and laryngeal cancer with  $\alpha$ -carotene,  $\beta$ -carotene, and  $\gamma$ -tocopherol. Thus,  $\alpha$ -carotene was significantly associated with all three cancer sites, and  $\beta$ -carotene

Table 4 Adjusted<sup>a</sup> odds ratios and 95% confidence intervals for upper aerodigestive tract cancer by tertile of serum micronutrient levels and time interval from phlebotomy to diagnosis

Micronutrients and time interval (yr)	Tertile of serum level			P for trend
	1	2	3	
<b><math>\alpha</math>-Carotene</b>				
0-20	1.00	0.27 (0.12-0.61) <sup>b</sup>	0.20 (0.08-0.50)	<0.01
5-10	1.00	0.03 (0.00-1.02)	0.02 (0.00-1.32)	0.02
11-20	1.00	0.33 (0.10-1.09)	0.19 (0.05-0.75)	<0.01
<b><math>\beta</math>-Carotene</b>				
0-20	1.00	0.22 (0.09-0.55)	0.11 (0.04-0.31)	<0.01
5-10	1.00	0.09 (0.01-1.40)	0.03 (0.00-0.87)	<0.01
11-20	1.00	0.37 (0.12-1.22)	0.10 (0.02-0.46)	<0.01
<b><math>\beta</math>-Cryptoxanthin</b>				
0-20	1.00	0.52 (0.26-1.05)	0.37 (0.16-0.86)	0.01
5-10	1.00	1.63 (0.30-8.78)	2.18 (0.20-23.36)	0.46
11-20	1.00	0.38 (0.13-1.10)	0.25 (0.06-1.04)	0.02
<b>Total carotenoids</b>				
0-20	1.00	0.26 (0.11-0.59)	0.36 (0.16-0.85)	<0.01
5-10	1.00	0.31 (0.04-2.66)	0.22 (0.02-2.37)	0.13
11-20	1.00	0.53 (0.16-1.83)	0.22 (0.05-0.88)	0.01
<b><math>\gamma</math>-Tocopherol</b>				
0-20	1.00	0.32 (0.15-0.71)	0.39 (0.19-0.80)	<0.01
5-10	1.00	0.72 (0.10-5.40)	0.04 (0.00-0.96)	0.03
11-20	1.00	0.26 (0.07-1.02)	0.69 (0.26-1.87)	0.27

<sup>a</sup> Adjusted for age, smoking status, number of cigarettes/day for past smokers, years smoked for past smokers, age started smoking for current smokers, number of cigarettes/day for current smokers, drinking status (yes/no), and number of ounces/month for drinkers.

<sup>b</sup> 95% confidence interval is in parentheses.

was significantly associated with two and almost ( $P = 0.07$  for oral cancer) three sites. Further adjustment for serum cholesterol reduced the association of esophageal cancer with total carotenoids ( $P = 0.17$ ) and  $\beta$ -cryptoxanthin ( $P = 0.07$ ).

The interrelationship between  $\alpha$ - and  $\beta$ -carotene was then explored, as shown in Table 6. The cutpoints for the analysis were medians, based on the distribution of controls. Subjects with high serum levels for both carotenes had an odds ratio of 0.17 (95% confidence interval, 0.06-0.45) for upper aerodigestive tract cancer compared with subjects who had low levels for both micronutrients. Similarly, persons with high levels for  $\alpha$ -carotene and  $\beta$ -cryptoxanthin had an odds ratio of 0.14 (95% confidence interval, 0.05-0.39) compared with those who had low levels for both micronutrients (data not shown). For subjects with high levels of  $\beta$ -carotene and  $\beta$ -cryptoxanthin, the odds ratio was 0.16 (95% confidence interval, 0.06-0.45).

## Discussion

This investigation used a nested case-control study design to analyze prospective data on the risk of upper aerodigestive tract

cancer in a cohort of Japanese-American men in Hawaii. After a follow-up period of twenty years, 69 study participants were diagnosed with histologically confirmed (squamous cell) cancer arising from this site. We found serum levels of total carotenoids,  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and  $\gamma$ -tocopherol were significantly reduced in men who developed this cancer. Except for  $\gamma$ -tocopherol, the association persisted even in cases diagnosed more than 10 years after their serum samples were obtained. As a result, it seems unlikely that the cancers themselves or preclinical conditions affected the serum micronutrient levels.

$\alpha$ -Carotene,  $\beta$ -carotene, and  $\beta$ -cryptoxanthin, the carotenoids that were significantly low in cases, can be cleaved to form retinal, which is reduced to retinol or oxidized to retinoic acid. Retinoic acid, which activates gene expression by binding to nuclear receptors, is critical in the development and maintenance of many tissues (18). Thus, it is conceivable that these three carotenoids could generate retinoids that inhibit carcinogenesis. Lutein, lycopene, and zeaxanthin were also lower in cases than controls, but the differences were not remarkable. These carotenoids cannot be converted to retinol or retinoic acid.

To lessen the potential confounding effects of established risk factors, the controls were matched to cases based on alcohol intake and smoking history. These exposures were strongly associated with the risk of cancers of the upper aerodigestive tract in an earlier study of this cohort (26). Statistical adjustment by levels of alcohol and cigarette use was made in the analysis to reduce further their confounding effects.

In a previous study, we found that serum cholesterol levels were inversely associated with risk of cancer of the oropharynx and esophagus (29). Statistical adjustment for serum cholesterol levels had only marginal effects on our results.

When the cancers were separated into oral-pharyngeal, esophageal, and laryngeal cancer, inverse associations were seen for  $\alpha$ -carotene and  $\beta$ -carotene for each site. However, such consistency was not observed for total carotenoids,  $\beta$ -cryptoxanthin and  $\gamma$ -tocopherol. The limited numbers of cases for each cancer site may have contributed to this instability in the serum results.

Only two earlier studies have examined the association of prediagnostic serum micronutrient levels with oral-pharyngeal cancer (21, 22). The Finland study included 20 patients and found no significant association, although serum levels of  $\beta$ -carotene and retinol were lower and  $\alpha$ -tocopherol was higher in cases than in controls (21). This study did not test for other carotenoids or tocopherols. The Maryland study reported that 28 oral-pharyngeal cancer patients had lower levels of all carotenoid compounds compared with controls, especially  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin (22).  $\alpha$ -tocopherol levels were lower and  $\gamma$ -tocopherol levels were higher in cases than controls, but there were no differences in serum retinol levels. We observed an inverse association for individual carotenoids, which included  $\alpha$ -carotene,  $\beta$ -carotene, and  $\beta$ -cryptoxanthin. We found an inverse association for  $\gamma$ -tocopherol, but no significant association was present for total tocopherol levels in the serum. Except for total tocopherols (vitamin E), our results are consistent with the findings from dietary case-control studies that recruited substantially larger numbers of cases than serum studies. They have reported that provitamin A carotenoids (1, 4) and fruits and vegetables (2, 4, 5) are consumed less often by oral-pharyngeal cancer cases compared with controls. Two dietary studies also had an inverse association with vitamin E intake (3, 4).

Esophageal cancer was also inversely associated with

Table 5 Differences in adjusted<sup>a</sup> mean serum micronutrient levels between oral-pharyngeal, esophageal, and laryngeal cancer cases and their respective controls

Micronutrients	Oral-pharyngeal cancer (18 cases)			Esophageal cancer (28 cases)			Laryngeal cancer (23 cases)		
	Mean of cases	Relative difference <sup>b</sup>	P <sup>c</sup>	Mean of cases	Relative difference	P	Mean of cases	Relative difference	P
Total carotenoids (μg/dl)	88.9	-3.4	0.20	79.5	-23.3	0.05	81.6	-11.5	0.07
Lutein	16.8	-1.2	0.76	15.1	-6.2	0.74	13.9	-12.0	0.12
Zeaxanthin	3.6	5.9	0.72	2.7	-20.6	0.13	2.9	-12.1	0.12
β-Cryptoxanthin	10.3	-23.7	0.06	11.1	-35.5	0.03	12.3	11.8	0.94
Lycopene	20.2	3.6	0.88	19.7	-6.6	0.95	18.4	-16.4	0.08
α-Carotene	3.7	-15.9	0.02	3.1	-41.5	0.01	3.7	-22.9	0.02
β-Carotene	14.8	-4.5	0.07	10.7	-44.2	<0.01	12.4	-15.1	0.03
Total retinoids (μg/dl)	64.9	-3.1	0.37	80.7	15.9	0.13	76.7	16.9	0.17
Retinol	62.2	-2.5	0.42	72.5	15.1	0.12	64.7	9.5	0.08
Total tocopherols (μg/ml)	14.27	10.9	0.84	13.52	0.2	0.86	13.42	-20.7	0.52
α-Tocopherol	12.70	11.3	0.81	11.86	-1.0	0.92	12.93	-12.9	0.97
δ-Tocopherol	0.12	3.3	0.23	0.10	-6.3	0.85	0.11	0.9	0.65
γ-Tocopherol	1.20	-16.7	0.03	1.21	-0.8	0.58	0.99	-29.8	<0.01

<sup>a</sup> Adjusted for age, smoking status, number of cigarettes/day for past smokers, years smoked for past smokers, age started smoking for current smokers, number of cigarettes/day for current smokers, drinking status (yes/no), and number of ounces/month for drinkers.

<sup>b</sup> [(mean of cases - mean of controls)/mean of controls] expressed as a percentage.

<sup>c</sup> Ps are based on log<sub>e</sub> transformation of serum micronutrient values.

Table 6 Adjusted<sup>a</sup> odds ratios and number of upper aerodigestive tract cancer cases and controls by serum α-carotene and β-carotene levels

Serum β-carotene (μg/dl)	Serum α-carotene (μg/dl)	
	≤4.2	≥4.2
12.9	1.00	0.37 (0.13-1.07) <sup>b</sup>
	46/51 <sup>c</sup>	5/18
>12.9	0.31 (0.10-0.97)	0.17 (0.06-0.45)
	6/18	12/50

<sup>a</sup> Adjusted for age, smoking status, number of cigarettes/day for past smokers, years smoked for past smokers, age started smoking for current smokers, number of cigarettes/day for current smokers, drinking status (yes/no), and number of ounces/month for drinkers.

<sup>b</sup> 95% confidence interval.

<sup>c</sup> Cases/controls.

α-carotene, β-carotene, and β-cryptoxanthin in our study. Only the Finnish study of nine cases used prediagnostic sera and found no association with serum β-carotene, α-tocopherol, or retinol (21). Dietary case-control studies have usually found that esophageal cancer cases have a low consumption of fruits and vegetables, especially raw and leafy green vegetables, as compared with controls (6-11). Our results are consistent with the view that a high intake of carotenoids reduces the risk of this cancer.

In the present study, laryngeal cancer was inversely associated not only with α- and β-carotene but also with γ-tocopherol. No other serum micronutrient study has been done for laryngeal cancer, except for the Finnish study (21). It included 11 cases and found that serum β-carotene, retinol, and α-tocopherol were low in laryngeal cancer cases, but the differences were not statistically significant. Dietary studies have usually shown an inverse association between laryngeal cancer and the consumption of provitamin A carotenoids (12, 13), green/yellow vegetables, and various fruits (14, 15). One dietary study showed no association with vitamin E intake (13).

Because of the consistent, strong inverse association of α- and β-carotene for oral-pharyngeal, esophageal, and laryngeal cancer in our study, we looked at their joint effects with regard to upper aerodigestive tract cancer (Table 6). The results showed that subjects with a high level of both α-carotene and β-carotene have a substantially lower risk than subjects with

low serum levels for one or both carotenes. The correlation coefficient of α- and β-carotene in the serum was 0.68, which reflects the fact that certain foods are high in both compounds, such as carrots and pumpkin (30), and that people who eat more vegetables and fruits tend to eat a variety of vegetables and fruits. Other foods are rich in β-carotene but not α-carotene, such as broccoli, Chinese cabbage, romaine lettuce, spinach, and sweet potato. Persons with high levels of β-cryptoxanthin and β-carotene or of β-cryptoxanthin and α-carotene also had a low risk for upper aerodigestive tract cancer. Papaya and peach contain more β-cryptoxanthin than β-carotene, but they are low in α-carotene. Taken together, our findings suggest that in addition to β-carotene, other carotenoids, such as α-carotene and β-cryptoxanthin, may inhibit the development of upper aerodigestive tract cancer, and that the risk of these tumors may be lowered with a diet rich in a variety of fruits and vegetables.

#### Acknowledgments

We thank Castle Medical Center, Kaiser Medical Center, Kuakini Medical Center, Queen's Medical Center, St. Francis Medical Center, Straub Clinic and Hospital, Wahiawa General Hospital, and the Hawaii Tumor Registry for support of this study.

#### References

- Negri, E., La Vecchia, C., Franceschi, S., and Tavani, A. Attributable risk for oral cancer in northern Italy. *Cancer Epidemiol., Biomarkers & Prev.*, 2: 189-193, 1993.
- Zheng, W., Blot, W. J., Shu, X.-O., Diamond, E. L., Gao, Y.-T., Ji, B.-T., and Fraumeni, J. F., Jr. Risk factors for oral and pharyngeal cancer in Shanghai, with emphasis on diet. *Cancer Epidemiol., Biomarkers & Prev.*, 1: 441-448, 1992.
- Gridley, G., McLaughlin, J. K., Block, G., Blot, W. J., Gluch, M., and Fraumeni, J. F., Jr. Vitamin supplement use and reduced risk of oral and pharyngeal cancer. *Am. J. Epidemiol.*, 135: 1083-1092, 1992.
- Gridley, G., McLaughlin, J. K., Block, G., Blot, W. J., Winn, D. M., Greenberg, R. S., Schoenberg, J. B., Preston-Martin, S., Austin, D. F., and Fraumeni, J. F., Jr. Diet and oral and pharyngeal cancer among Blacks. *Nutr. Cancer*, 14: 219-225, 1990.
- Winn, D. M., Ziegler, R. G., and Pickle, L. W. Carcinoma of the esophagus. *Cancer Res.*, 48: 3843-3848, 1988.
- Cheng, K. K., Day, N. E., Duffy, S. W., Lam, T. H., Fok, M., and Wong, J. Pickled vegetables in the aetiology of oesophageal cancer in Hong Kong Chinese. *Lancet*, 339: 1314-1318, 1992.
- Yu, M. C., Garabrant, D. H., Peters, J. M., and Mack, T. M. Tobacco, alcohol, diet, occupation, and carcinoma of the esophagus. *Cancer Res.*, 48: 3843-1988.

8. Brown, L. M., Blot, W. J., Shuman, S. H., Smith, V. M., Ershow, A. G., Marks, R. D., and Fraumeni, J. F., Jr. Environmental factors and high risk of esophageal cancer among men in coastal South Carolina. *J. Natl. Cancer Inst.*, 80: 1620-1625, 1988.
9. Notani, P. N., and Jayant, K. Role of diet in upper aerodigestive tract cancers. *Nutr. Cancer*, 10: 103-113, 1987.
10. Decarli, A., Liati, P., Negri, E., Franceschi, S., and La Vecchia, C. Vitamin A and other dietary factors in the etiology of esophageal cancer. *Nutr. Cancer*, 10: 29-37, 1987.
11. Ziegler, R. G., Morris, L. E., Blot, W. I., Pottern, L. M., Hoover, R., and Fraumeni, J. F., Jr. Esophageal cancer in black men in Washington, D.C. II. Role of nutrition. *J. Natl. Cancer Inst.*, 67: 1199-1206, 1981.
12. Tavani, A., Negri, E., Franceschi, S., Barbone, F., and La Vecchia, C. Attributable risk for laryngeal cancer in northern Italy. *Cancer Epidemiol., Biomarkers & Prev.*, 3: 121-125, 1994.
13. Freudenheim, J. L., Graham, S., Byers, T. E., Marshall, J. R., Haughey, B. P., Swanson, M. K., and Wilkinson, G. Diet, smoking, and alcohol in cancer of the larynx: a case-control study. *Nutr. Cancer*, 17: 33-45, 1992.
14. Zheng, W., Blot, W. J., Shu, X.-O., Gao, Y.-T., Ji, B.-T., Ziegler, R. G., and Fraumeni, J. F., Jr. Diet and other risk factors of laryngeal cancer in Shanghai, China. *Am. J. Epidemiol.*, 136: 178-191, 1992.
15. La Vecchia, C., Negri, E., D'Avanzo, B., Franceschi, S., Decarli, A., and Boyle, P. Dietary indicators of laryngeal cancer risk. *Cancer Res.*, 50: 4497-4500, 1990.
16. Graham, S., Marshall, J., Haughey, B., Brasure, J., Freudenheim, J., Zielezny, M., Wilkinson, G., and Nolan, J. Nutritional epidemiology of cancer of the esophagus. *Am. J. Epidemiol.*, 131: 454-467, 1990.
17. Li, J.-Y., Ershow, A. G., Chen, Z.-J., Wacholder, S., Li, G.-Y., Guo, W., Li, B., and Blot, W. J. A case-control study of cancer of the esophagus and gastric cardia in Linxian. *Int. J. Cancer*, 43: 755-761, 1989.
18. Olson, J. A. Vitamin A, retinoids, and carotenoids. In: M. E. Shils, J. A. Olson, and M. Shike (eds.), *Modern Nutrition in Health and Disease*, Ed. 8, pp. 287-307. Philadelphia: Lea and Febiger, 1994.
19. Bendich, A., and Machlin, L. J. Safety of oral intake of vitamin E. *Am. J. Clin. Nutr.*, 48: 612-619, 1988.
20. Cerutti, P. A. Prooxidant states and tumor promotion. *Science (Washington DC)*, 2: 375-381, 1985.
21. Knekt, P., Aromaa, A., Maatela, J., Alfthan, G., Aaran, R.-K., Nikkari, T., Nakama, M., Halulinen, T., and Teppo, L. Serum micronutrients and risk of cancers of low incidence in Finland. *Am. J. Epidemiol.*, 134: 356-361, 1991.
22. Zheng, W., Blot, W. J., Diamond, E. L., Norkus, E. P., Spate, V., Morris, J. S., and Comstock, G. W. Serum micronutrients and the subsequent risk of oral and pharyngeal cancer. *Cancer Res.*, 53: 795-798, 1993.
23. Epler, K. S., Ziegler, R. G., and Craft, N. E. Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in food. *J. Chromatogr.*, 619: 37-48, 1993.
24. Epler, K. S., Sander, L. C., Ziegler, R. G., Wise, S. A., and Craft, N. E. Evaluation of reversed-phase liquid chromatographic columns for recovery and selectivity of selected carotenoids. *J. Chromatogr.*, 595: 89-101, 1992.
25. Worth, R. M. and Kagan, A. Ascertainment of men of Japanese ancestry in Hawaii through World War II Selective Service registration. *J. Chronic Dis.*, 23: 389-397, 1970.
26. Chyou, P.-H., Nomura, A. M. Y., and Stemmermann, G. N. Diet, alcohol, smoking and cancer of the upper aerodigestive tract: a prospective study among Hawaii Japanese men. *Int. J. Cancer*, 60: 616-621, 1995.
27. Freund, R. J., and Littell, R. C. *Statistical Analysis System (SAS) for Linear Models*. Cary, NC: SAS Institute, Inc., 1981.
28. Breslow, N. E., and Day, N. E. *Statistical Models in Cancer Research*, Vol. 1, pp. 164-166, 247-276. Lyon, France: IARC, 1980.
29. Chyou, P.-H., Nomura, A. M. Y., Stemmermann, G. N., and Kato, I. Prospective study of serum cholesterol and site-specific cancers. *J. Clin. Epidemiol.*, 45: 287-292, 1992.
30. Mangels, A. R., Holden, J. M., Beecher, G. R., Forman, M. R., and Lanza, E. Carotenoid content of fruits and vegetables: an evaluation of analytic data. *J. Am. Diet Assoc.*, 93: 284-296, 1993.