

## SERUM CAROTENOIDS ARE ASSOCIATED WITH INCREASED LUNG CANCER RISK AMONG ALCOHOL DRINKERS, BUT NOT AMONG NON-DRINKERS IN A COHORT OF TIN MINERS

DUMINDA RATNASINGHE\*, MICHELE R. FORMAN, JOSEPH A. TANGREA, YOU LIN QIAO<sup>1</sup>, SHU-XIANG YAO<sup>2</sup>, ELAINE W. GUNTER<sup>3</sup>, MICHAEL J. BARRETT<sup>4</sup>, CAROL A. GIFFEN<sup>4</sup>, YENER EROZAN<sup>5</sup>, MELVYN S. TOCKMAN<sup>6</sup> and PHILIP R. TAYLOR

Division of Clinical Sciences, National Cancer Institute, NIH, Bethesda, MD, USA, <sup>1</sup>CICAMS, Beijing, <sup>2</sup>Yunnan Tin Corporation, Gejiu, Yunnan Province, People's Republic of China, <sup>3</sup>NHANES Laboratory of Biochemical Analyses at the Centers for Disease Prevention and Control, Atlanta, GA, <sup>4</sup>Information Management Services, Inc., Silver Spring MD, <sup>5</sup>Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD and <sup>6</sup>H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

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**Abstract** — To examine the association between pre-diagnostic serum carotenoid levels and lung cancer risk and the effects of alcohol intake on the carotenoid–lung cancer relationship, we conducted a case-control study in an occupational cohort from the Yunnan Tin Corporation in China. During 6 years of follow-up, 339 cases of confirmed lung cancer were diagnosed. Among these cases, those who donated pre-diagnostic blood ( $n = 108$ ) were eligible for this study. For each case, two individuals alive and free of cancer at the time of case diagnosis, matched on age, sex, and date of blood collection, were selected as controls. Serum  $\beta$ -carotene (odds ratios (ORs) for tertiles: 1, 1.3, 2.0) and  $\beta$ -cryptoxanthin (ORs for tertiles: 1, 1.8, 2.9) levels were positively associated with lung cancer risk after adjustment for tobacco use and radon exposure. Among alcohol drinkers, higher serum carotenoid levels were significantly associated with increased lung cancer risk ( $\alpha$ -carotene OR 2.2, 95% confidence interval (CI) 1.1–4.4,  $\beta$ -carotene OR 7.6, 95% CI 3.1–18.6, lutein/zeaxanthin OR 2.3, 95% CI 1.2–6.6 and  $\beta$ -cryptoxanthin OR 7.6, 95% CI 2.7–21.5). Conversely, risk estimates among non-drinkers suggest a possible protective association for higher carotenoid levels.

### INTRODUCTION

Lung cancer is a leading cause of death worldwide. Whereas cigarette smoking is clearly the major risk factor for lung cancer, exposures to dusts containing carcinogens, such as arsenic, and to gases such as radon also contribute to increased risk (Doll and Peto, 1981). The tin miners of Yunnan province in southern China are at high risk for lung cancer by virtue of their smoking habits and occupational exposures to radon and arsenic (Qiao *et al.*, 1997). In addition, approximately 50% of the tin miners consume large amounts of alcohol (170 g/day) (Forman *et al.*, 1999). Observational studies have investigated the relationship between elevated serum carotenoid levels and reduced risk of lung cancer. The carotenoids assayed include  $\alpha$ - and  $\beta$ -carotene, lutein and zeaxanthin, and  $\beta$ -cryptoxanthin among others. The potential anti-cancer properties of carotenoids have been attributed to their antioxidant properties and/or provitamin-A activity. Carotenoids can function as scavengers of oxidative free radicals and also stimulate the immune system and boost defences against cancer cells (Krinsky, 1989). The inverse association between intake of carotenoid-rich fruits and vegetables and the incidence of lung cancer has been well documented in numerous epidemiologic studies (Ziegler *et al.*, 1986; Paganini-Hill *et al.*, 1987; Ziegler, 1989; Fraser *et al.*, 1991; Forman *et al.*, 1992; Le Marchand *et al.*, 1993; Comstock *et al.*, 1997). The consistency of this observed inverse association between carotenoid intake and lung cancer was part of the scientific rationale behind the implementation of two large cancer prevention trials of pharmaceutical doses of  $\beta$ -carotene

(ATBC Cancer Prevention Study Group, 1994; Omenn *et al.*, 1996).

The Alpha-Tocopherol Beta-Carotene (ATBC) cancer prevention study (ATBC Cancer Prevention Study Group, 1994) and Beta-Carotene and Retinol Efficacy Trial (CARET) (Omenn *et al.*, 1996) were randomized double-blind placebo-controlled trials conducted among smokers and/or occupationally exposed men (and women in CARET) to determine the effects of supplemental doses of  $\beta$ -carotene (and/or  $\alpha$ -tocopherol and retinol palmitate) on lung cancer incidence and mortality. Both studies demonstrated an increased incidence of lung cancer among the  $\beta$ -carotene-supplemented individuals, compared to those taking placebo. Also in both studies, as suggested by Leo and colleagues, alcohol intake potentiated the adverse effect of  $\beta$ -carotene supplementation on lung cancer risk (Leo and Lieber, 1994; Albanes *et al.*, 1996; Omenn *et al.*, 1996).

In the present study, we examined the association of pre-diagnostic serum carotenoid levels and risk of lung cancer among Chinese tin miners. Based on the finding of the ATBC and CARET studies, we also examined whether the association between pre-diagnostic serum carotenoid levels and subsequent risk of lung cancer was modified by alcohol intake.

### MATERIALS AND METHODS

#### Study cohort

The subjects in this study were miners in the Yunnan Tin Corporation (YTC), China. The incidence rates of lung cancer are extraordinarily high in this population. Males >40 years old with underground mining experience have a crude annual incidence rate of over 1%. Miners aged 60–64 years have an incidence rate in excess of 2.5% annually. Lung cancer represents about 80% of all cancers seen annually among YTC

\*Author to whom correspondence should be addressed at: Cancer Prevention Studies Branch, DCS, NCI, 6006 Executive Blvd, Suite 321, Bethesda, MD 20892–7058, USA.

employees and mortality from this cancer is 10-fold higher in this area than in the rest of China (Qiao *et al.*, 1989). For males over 50 years old, the lung cancer incidence rate is three to seven times higher than SEER (Surveillance, Epidemiology and End Results) rates for US males (SEER, 1994). This population has been exposed to a number of known carcinogens, including tobacco smoke, radon, and arsenic (Yu *et al.*, 1990).

A longitudinal, prospective cohort study of high risk miners of the YTC was established in 1992 with annual follow-up through 1999. Eligible high risk miners were aged  $\geq 40$  years, with  $\geq 10$  years of underground mining and/or smelting experience, and who were free of cancer (except for non-melanoma skin cancer) at baseline in 1992. The baseline and follow-up activities were added to an annual YTC screening of the miners ongoing since 1973. These activities included: an interview about demographic, dietary, residential, occupational, and medical histories; a 24-h food recall, X-rays, physical examinations, and sputum collections. The initial cohort established in 1992 had 6508 miners, and with each annual screen more miners entered the cohort as they reached the eligibility criteria, resulting in 9142 cohort members by 1997. During the annual screenings in 1993 and 1994, miners were asked to provide a fasting blood specimen. The subcohort who gave blood specimens comprised 40% of the screened population and were representative of the larger high risk cohort. Lung cancer cases were ascertained by reports to the Cancer Registry of the Labour Protection Institute of the YTC or from the annual screens. Over two-thirds of the cases were classified as squamous cell carcinomas of the lung.

#### *Definition of exposures*

Cumulative radon exposure for each subject was obtained by summing across the estimated working level months (WLM) for each job held at the YTC prior to baseline screening of the high risk cohort. The cumulative individual arsenic exposure for each job was estimated by using an index for arsenic exposure (index of arsenic exposure months or IAEM), which was calculated as a time weighted average of arsenic concentration ( $\text{mg}/\text{m}^3$ ) times exposure months ( $\text{mg}/\text{m}^3 \times \text{months}$ ). Individuals who had smoked cigarettes and/or pipes (water pipes or Chinese long-stem pipes) regularly for 6 months or longer at any time in their life were classified as ever smokers and were asked for information on a variety of smoking-related issues. Pack year equivalents ( $\text{g}/\text{day} \times \text{years} \div 20$ ) were used to measure cumulative tobacco consumption, which was calculated separately for cigarettes (1 cigarette = 1 g) water pipe, and long-stem pipe and for total tobacco use (Qiao *et al.*, 1997).

Trained interviewers administered a culture-specific 109-item dietary food frequency questionnaire (FFQ) in the 1992, 1993, and 1995 annual screens. Miners reported the frequency of intake of foods including alcohol from grain liquor, wine, and other spirits during the past year, with specification of month/season of intake when appropriate. Dietary and alcohol intake information was also obtained by a single 24-h food and beverage recall questionnaire, which was the primary source of dietary data presented in the current study (Forman *et al.*, 1999). The data from the single 24-h food and beverage recall questionnaire and the dietary FFQ were quite concordant. In order to further assure the reproducibility of the questionnaire data, a sample of the participants (2%) were re-interviewed

daily by the supervisory staff. In a random subsample of the cohort, a diet validation study was conducted in 1992–1993 and again in 1995–1996 in order to compare the diet history FFQ to 28 days of food recalls that were collected over seven consecutive days in each of four seasons. De-attenuated Pearson correlation coefficients of the frequency of food intake between the FFQ and food recalls were in the range of  $-4.0$  to  $0.72$  for both validation studies (Forman *et al.*, 1999).

#### *Selection of cases and controls*

The cases consisted of 106 men and two women, aged 41–79 years, diagnosed with primary lung cancer (ICD-9: 162) during the years 1992–1997 among those who gave blood at either the 1993 or 1994 screening. Using incidence density sampling, the controls were selected from cohort participants who were alive and free of cancer at the time the case was diagnosed. Controls were matched to cases on age ( $\pm 2$  years), sex and date of blood draw ( $\pm 2$  weeks) in a 2 : 1 ratio for a total of 108 cases and 216 controls. Selection of cases and controls was independent of assessment of exposure status.

#### *Assessment of micronutrient levels*

Serum was prospectively collected (on the average 2.5 years prior to case diagnosis) and was analysed in case-control triplet sets. Interspersed throughout the sets were 48 masked quality control (QC) samples (13% of study group) composed of pooled sera arranged also in sets of three to include one set per assay batch. Laboratory personnel were blind to the case-control status and identity of QC samples. The intra-set coefficients of variation (based on masked reference serum assays) were 1.0, 7.1, 4.6, and 2.1 for  $\alpha$ -carotene,  $\beta$ -carotene, lutein/zeaxanthin, and  $\beta$ -cryptoxanthin, respectively. The inter-batch coefficients of variation were 1.1, 8.4, 3.2, and 5.1 for  $\alpha$ -carotene,  $\beta$ -carotene, lutein/zeaxanthin, and  $\beta$ -cryptoxanthin, respectively. Lycopene was not included in the analyses, because the serum levels were below the detectable limits and had higher coefficients of variation than for the other carotenoids.

Serum levels of carotenoids were measured by isocratic high-performance liquid chromatography with UV/VIS detection at three different wavelengths. Serum was mixed with an ethanol solution of the internal standard nonapreno- $\beta$ -carotene. The analytes were extracted into hexane, which was removed under vacuum. The extract was redissolved in ethanol; an equal volume of acetonitrile was then added. The extract was filtered to remove insoluble material. An aliquot of the filtrate was injected onto a C18 reversed-phase column and eluted with a 50% ethanol:50% acetonitrile solution containing 100  $\mu\text{l}$  of diethylamine/l. Chromatograms at 300, 325, and 450 nm were recorded. Quantification was accomplished by comparing the peak height of the analyte in the unknown with the peak height of a known amount of the same analyte in a standard solution. A correction based on the peak height of an internal standard was used. The carotenoids were compared with nonapreno- $\beta$ -carotene at 450 nm (Gunter *et al.*, 1996).

#### *Statistical analyses*

The Wilcoxon rank sum test was used to test the hypothesis that the distribution of serum carotenoid concentrations was the same for cases and controls. Conditional logistic regression analysis was used to examine the association between serum

Table 1. Selected baseline characteristics of lung cancer cases and controls<sup>a</sup>

Characteristic	Cases (n = 108)	Controls (n = 216)	P-value <sup>b</sup>
Age (years)	63.0 (6.5)	62.8 (6.5)	0.88
BMI (kg/m <sup>2</sup> )	21.9 (2.9)	22 (3.2)	0.83
Schooling completed (years)	2.4 (3.2)	3.3 (4.0)	0.05
Monthly salary (Yuan)	303.8 (212)	304.2 (170)	0.66
Age started work (years)	16.6 (4.9)	17.8 (5.6)	0.09
Years mining/smelting jobs	29.9 (9.1)	27.5 (9.6)	0.04
All tobacco use (g/day)	19.2 (10.4)	16.3 (9.8)	0.02
Cumulative radon exposure (WLM)	591.6 (442.5)	512.2 (448.0)	0.04
Cumulative arsenic exposure (IAEM) <sup>c</sup>	10 702	9038	0.30
Retired	99 (91.7%)	194 (89.8%)	0.59
Total alcohol (g/day)			
All participants	87 (111)	81 (121)	0.18
Drinkers (48%)	174 (98)	171 (124)	0.39
Diet (g/day)			
Total fruit	83 (138)	79 (149)	0.49
Total vegetables	227 (130)	260 (194)	0.46
Total meat	116 (84)	119 (123)	0.37
Serum			
$\alpha$ -Carotene ( $\mu$ g/dl)	2.0 (1.8)	1.9 (1.5)	0.83
$\beta$ -Carotene ( $\mu$ g/dl)	18.6 (14)	16.5 (12)	0.14
Lutein and zeaxanthin ( $\mu$ g/dl)	55.0 (22)	54.8 (20)	0.92
$\beta$ -Cryptoxanthin ( $\mu$ g/dl)	9.0 (8)	8.0 (5)	0.07

BMI, body mass index; WLM, working level months; IAEM, index of arsenic exposure months. <sup>a</sup>Based on unmatched data with continuous variables expressed as the mean (SD); <sup>b</sup>P-values as determined by Wilcoxon rank-sum tests; <sup>c</sup>median shown due to skewed data.

carotenoids and risk of lung cancer. Modification of the effect of carotenoids by age, season, tobacco, radon, arsenic, and alcohol consumption on lung cancer risk was examined by statistical tests of the first-order interaction term in the logistic regression models. Statistical analyses stratified by alcohol status (drinkers vs non-drinkers, respectively) were conducted by breaking the case-control match to avoid the loss of subjects due to splitting of matched sets that fell into different strata and using unconditional logistic regression adjusted for age, date of blood draw and sex, the original matching criteria, and other potential confounders. Serum carotenoid levels were categorized into tertiles (one lowest and three being the highest) or median splits (one lowest and two being the highest) based on the distribution of serum carotenoids among the controls. In order to conduct linear trend analyses, variables were created using exposure scores based on the median values of each metabolite for the first to third tertiles among the controls and including them in the logistic regression models. Probabilities of differences at the level of  $P < 0.05$  were regarded as statistically significant.

Potential confounding of the association between serum carotenoids and cancer risk by other related risk factors was explored using Spearman correlation analysis and multivariate logistic regression models. Potential confounding variables were entered in stepwise regression models. If the potential confounder had a significant effect on the log likelihood estimate ( $P < 0.05$ ) and a greater than 10% change in the serum carotenoid  $\beta$ -coefficient, it was kept in the model for further multivariate analyses. Exclusion of early cases (blood drawn within 1 year of case diagnosis) did not materially alter the risk estimates of any of the serum carotenoids. All analyses were performed using the statistical software package STATA (Texas).

## RESULTS

Cases and controls were matched closely on age and date of blood draw. Comparison of anthropometric, dietary, and life-style variables that could be related to cancer risk demonstrated some differences between cases and controls (Table 1). As expected, tobacco use and radon exposure were significantly higher in the cases compared to the controls. In addition, the cases also worked longer in mining/smelting jobs than did the controls.

Pre-diagnostic serum carotenoid levels by case status are also shown in Table 1. All the carotenoid levels were marginally higher among the cases than controls, but differences were not statistically significant. The serum carotenoid levels ranged from 0–10, 1–64, 10–123, and 0–41  $\mu$ g/dl for  $\alpha$ -carotene,  $\beta$ -carotene, lutein/zeaxanthin, and  $\beta$ -cryptoxanthin, respectively among the controls, whereas the corresponding serum carotenoid levels ranged from 0–12, 1–90, 14–147, and 1–80  $\mu$ g/dl respectively among the cases.

Significant positive relationships with lung cancer risk were observed for both serum  $\beta$ -carotene [odds ratio (OR) 2.0, 95% confidence interval (CI) 1.1–3.8 for individuals in the highest tertile compared to the lowest tertile] and  $\beta$ -cryptoxanthin (OR 2.9, 95% CI 1.4–5.8) (Table 2). Excluding cases that were diagnosed with lung cancer within 1 year of blood collection had no effect on the risk estimates for the association between each carotenoid and lung cancer. Adjustment for baseline covariates, such as serum cholesterol, total vegetable, total fruit, meat, and alcohol consumption, arsenic, radon or tobacco exposure, that were correlated with any of the carotenoids and were possibly associated with lung cancer, did not materially change the risk estimates. The risk estimates shown in Table 2 were adjusted for tobacco use (g/day) and radon exposure,

Table 2. Association between serum carotenoids and lung cancer (univariate)<sup>a</sup>

Carotenoid <sup>b</sup>	OR (c/c)	95% CI	P-trend
$\alpha$ -Carotene ( $\mu\text{g}/\text{dl}$ ) <sup>c</sup>			
M1: <1	1.0 (63/129)		
M2: >1	1.2 (45/87)	0.7–2.0	
$\beta$ -Carotene ( $\mu\text{g}/\text{dl}$ )			
T1: <9	1.0 (31/82)		
T2: 10–18	1.3 (34/67)	0.7–2.5	
T3: >19	2.0 (43/67)	1.1–3.8	0.08
Lutein and zeaxanthin ( $\mu\text{g}/\text{dl}$ )			
T1: <44	1.0 (39/75)		
T2: 45–60	1.0 (30/67)	0.5–2.0	
T3: >61	1.3 (39/74)	0.7–2.4	0.96
$\beta$ -Cryptoxanthin ( $\mu\text{g}/\text{dl}$ )			
T1: <4	1.0 (25/71)		
T2: 5–7	1.8 (34/70)	0.9–3.8	
T3: >8	2.9 (49/75)	1.4–5.8	0.03

OR, odds ratios; CI, confidence intervals; c/c, cases/controls. <sup>a</sup>Adjusted for radon and tobacco exposure in conditional logistic regression models; <sup>b</sup>first tertile used as reference group, <sup>c</sup>median split shown due to narrow range of values; median split (M) and tertiles (T).

because they were significantly associated with lung cancer risk. Furthermore, there was no association between fruit and vegetable intake based on 24-h recalls and lung cancer risk.

Mean serum carotenoid levels among cases and controls stratified by alcohol consumption are shown in Table 3. Among cases and controls in our study, 48% were alcohol drinkers (50% cases and 48% controls), and almost all the alcohol consumed was in the form of grain liquor. Alcohol consumption is stratified only as drinker or non-drinker, because our case-control sample set had a limited sample size and because alcohol intake was based on one 24-h estimate, which is less representative of usual intake than data from a FFQ. Therefore, we felt that the 24-h recall data gave a better estimate of drinking status rather than actual consumption. Among drinkers, the intake of alcohol ranged from 10 g/day to 600 g/day. Among the reported alcohol drinkers, serum  $\alpha$ -carotene,  $\beta$ -carotene,

Table 3. Mean serum carotenoid levels among cases and controls by alcohol status<sup>a</sup>

Drinking status	Cases	Controls	P-value <sup>b</sup>
$\alpha$ -Carotene ( $\mu\text{dl}$ )			
Non-drinkers	1 (54)	1 (114)	0.27
Drinkers	1 (54)	1 (102)	<0.10
$\beta$ -Carotene ( $\mu\text{g}/\text{dl}$ )			
Non-drinkers	13 (54)	16 (114)	0.10
Drinkers	19 (54)	9 (102)	<0.01
Lutein and zeaxanthin ( $\mu\text{g}/\text{dl}$ )			
Non-drinkers	44 (54)	52 (114)	<0.05
Drinkers	59 (54)	52 (102)	<0.05
$\beta$ -Cryptoxanthin ( $\mu\text{g}/\text{dl}$ )			
Non-drinkers	6 (54)	6 (114)	0.70
Drinkers	8 (54)	6 (102)	<0.01

<sup>a</sup>Based on unmatched data with continuous variables expressed as the median (*n*); <sup>b</sup>P-values as determined by Wilcoxon rank-sum tests.

lutein/zeaxanthin, and  $\beta$ -cryptoxanthin levels were higher (by 25, 55, 14 and 36%, respectively) among the cases compared to controls, with significant differences in serum  $\beta$ -carotene, lutein/zeaxanthin, and  $\beta$ -cryptoxanthin. Moreover, higher serum  $\alpha$ -carotene,  $\beta$ -carotene, lutein/zeaxanthin, and  $\beta$ -cryptoxanthin levels were associated with an increased risk of lung cancer, with ORs ranging from 2.2 to 7.6 comparing individuals in the highest carotenoid level group to the lowest group (Table 4). Interestingly, among the non-drinkers, we observed the opposite effect for all the carotenoids, albeit non-significantly. Adjustment for age at baseline, date of blood draw, tobacco use (g/day and/or pack-years), and radon exposure did not materially alter the risk estimates. However, the risk estimates shown were adjusted for tobacco use (g/day) and radon exposure in addition to matching variables, because they were significantly associated with the risk of lung cancer. The age-adjusted relative risk estimates for lung cancer due to tobacco smoking and radon exposure were 3.57 (95% CI 1.6–8.0) and 2.9 (95% CI 1.3–6.8) comparing those in the highest quartile of exposure to the lowest quartile, respectively.

Table 4. Association between serum carotenoids and lung cancer stratified by alcohol drinking status<sup>a</sup>

Carotenoid <sup>b</sup>	Non-drinkers			Drinkers		
	OR (c/c)	95% CI	P-trend <sup>c</sup>	OR (c/c)	95% CI	P-trend
$\alpha$ -Carotene ( $\mu\text{g}/\text{dl}$ ) <sup>c</sup>						
M1	1.0 (33/59)			1.0 (30/70)		
M2	0.6 (21/55)	0.3–1.3		2.2 (24/32)	1.1–4.4	
$\beta$ -Carotene ( $\mu\text{g}/\text{dl}$ )						
T1	1.0 (17/29)			1.0 (14/53)		
T2	1.1 (21/36)	0.4–2.9		1.7 (13/31)	0.7–4.2	
T3	0.5 (16/49)	0.2–1.3	0.13	7.6 (27/18)	3.1–18.6	<0.001
Lutein and zeaxanthin ( $\mu\text{g}/\text{dl}$ )						
T1	1.0 (27/40)			1.0 (12/35)		
T2	0.6 (14/33)	0.3–1.6		1.6 (16/34)	0.7–4.2	
T3	0.4 (13/41)	0.2–1.1	0.04	2.3 (26/33)	1.2–6.6	0.04
$\beta$ -Cryptoxanthin ( $\mu\text{g}/\text{dl}$ )						
T1	1.0 (18/34)			1.0 (7/37)		
T2	0.8 (17/39)	0.3–1.9		3.7 (17/31)	1.3–10.7	
T3	0.8 (19/41)	0.3–2.0	0.70	7.6 (30/34)	2.7–21.5	<0.001

OR, odds ratios; CI, confidence intervals; c/c, cases/controls. <sup>a</sup>Adjusted for age, sex, date of blood draw, radon, and tobacco exposure in unconditional logistic regression models; <sup>b</sup>first tertile used as reference group; <sup>c</sup>median split shown due to narrow range of values; Median split (M) and tertiles (T).

## DISCUSSION

The YTC tin miners provide a unique population in which to explore the associations between serum carotenoid levels and lung cancer risk due to their markedly elevated lung cancer incidence rates. In addition to occupational exposures that are known causes of lung cancer, this population is also marginally deficient in many carotenoids (Forman *et al.*, 1999). Serum levels of  $\alpha$ -carotene among these miners were lower than other populations, except for a northern Chinese population in Linxian province (Stryker *et al.*, 1988; Zheng *et al.*, 1989; Rimm and Cojdtz, 1993; Ross *et al.*, 1995). Serum  $\beta$ -carotene and  $\beta$ -cryptoxanthin concentrations were similar to other populations of smokers in Scotland (Ross *et al.*, 1995) and higher than in Linxian (Zheng *et al.*, 1989). On the other hand, serum lutein/zeaxanthin levels were much higher than other populations, such as those in the USA (Comstock *et al.*, 1997).

In this population, increased serum  $\beta$ -carotene and  $\beta$ -cryptoxanthin levels were apparently associated with increased risk of lung cancer. In our study, there was essentially no association between  $\alpha$ -carotene or lutein/zeaxanthin levels and lung cancer risk.

Many studies have explored the association between dietary intake or serum levels of carotenoids and lung cancer risk. A study among Finnish men found a moderately strong negative association between dietary  $\alpha$ -carotene and lung cancer risk (Knekt *et al.*, 1991). The Finnish study and the Iowa Women's Health Study (Steinmetz *et al.*, 1993) found no association between dietary lutein and lung cancer risk. Two case-control studies of dietary intake of  $\beta$ -cryptoxanthin and lung cancer (Candelora *et al.*, 1992; Le Marchand *et al.*, 1993), one among Florida women and the other among men and women in Hawaii, described a protective effect from dietary cryptoxanthin intake on the odds of developing lung cancer. Most prospective serologic studies of  $\beta$ -carotene have shown a consistent protective effect against lung cancer. For example, a serologic study in Washington County, MD, USA (Comstock *et al.*, 1997) reported an inverse association between  $\alpha$ -carotene,  $\beta$ -carotene, lutein, cryptoxanthin and lung cancer. In contrast,  $\beta$ -carotene intervention trials among smokers in Finland and the USA showed an increase in lung cancer incidence among the  $\beta$ -carotene-supplemented individuals, compared to placebo (ATBC Cancer Prevention Study Group, 1994; Omenn *et al.*, 1996). Results of these intervention studies among heavy smokers suggest a possible promoting effect of  $\beta$ -carotene on lung cancer. Both studies also showed an adverse interaction between  $\beta$ -carotene and alcohol intake that was associated with a further increase in lung cancer incidence in the  $\beta$ -carotene-supplemented individuals in the highest quartile of alcohol intake (Albanes *et al.*, 1996; Omenn *et al.*, 1996).

In the current study, the strong adverse association between serum  $\alpha$ -carotene,  $\beta$ -carotene, lutein/zeaxanthin,  $\beta$ -cryptoxanthin and lung cancer appears among alcohol drinkers only. The consumption of alcohol among the YTC study men is extremely high compared to, for example, drinkers among the ATBC study in Finland (84 vs 20 g/day respectively). Tobacco use among the YTC miners on the other hand was quite similar to the ATBC participants in Finland.

The apparent protective association of carotenoids among non-drinkers and the adverse association among drinkers is

intriguing. Alcohol intake has been reported to alter plasma carotenoid concentrations and therefore potentially modify or confound the plasma carotenoid-cancer relation (Rimm and Cojdtz, 1993). The difficulty in assessing the role of alcohol in the plasma carotenoid-cancer relation is further complicated by the strong positive correlation between alcohol intake and smoking (Subar and Harlan, 1993). In addition, smokers who drink have a different profile of dietary characteristics than non-smokers who drink (Nebeling *et al.*, 1997). These factors have complicated the alcohol-serum carotenoid relation and in turn, its relation to lung cancer.

Alcohol is also known to disrupt lipoproteins, alter liver function and the bioavailability of carotenoids. Ahmed *et al.*, (1994) reported that alcoholics had lower plasma  $\beta$ -carotene concentrations than control subjects, but heavy drinkers (>200 g/day) had about twice the  $\beta$ -carotene of those drinking less, with a significant correlation between plasma  $\beta$ -carotene and alcohol intake. In a controlled diet study, pre-menopausal women had higher plasma  $\alpha$ - and  $\beta$ -carotene concentrations and lower lutein concentrations when fed 30 g of alcohol/day for 3 months, compared to 3 months without alcohol intake (Forman *et al.*, 1995). It has also been shown in a series of studies in baboons that alcohol raises the level of carotenoids in the plasma and delays its clearance (Leo *et al.*, 1992). In addition, alcohol consumption may also increase the intestinal absorption of carotenoids by improving solubility and aiding in its transport across the mucosal surface. However, these findings do not explain the differential carotenoid levels seen in the current study comparing cases to controls by alcohol status. What we seem to observe is an alcohol- and carotenoid-derived interaction that possibly enhances the mechanisms of lung cancer development. This observation is very similar to the results of the two  $\beta$ -carotene cancer prevention trials (Albanes *et al.*, 1996; Omenn *et al.*, 1996).

One of the strengths of this study is its prospective design. The collection of blood specimens and covariate data before case diagnosis minimized the potential for recall bias and for disease to affect serum carotenoid measurements. However, the average time from blood collection to diagnosis was short (2.5 years). Nonetheless, exclusion of early cases altered neither the risk estimates nor their statistical significance. The biological relevance of examining the relation between a few carotenoids and subsequent lung cancer incidence is a limitation of this and most similar observational studies. The serum carotenoid levels measured are also probably not indicative of lifetime serum carotenoid status. But, serum levels in controls were of a similar range and mean to serum levels observed among a random subcohort of these miners in 1995 and 1996. In addition, the generalizability of these results may be somewhat restricted, because the study only included individuals exposed to mining-associated occupational pollutants.

In summary, this is the first observational study to report a significant adverse association between prospectively collected serum  $\beta$ -cryptoxanthin and lung cancer. In addition, our data also suggest a modest positive relation between pre-diagnostic serum levels of  $\beta$ -carotene and lung cancer risk. Furthermore, alcohol seems to potentiate this adverse affect of serum carotenoids with the observed risk increasing as much as sevenfold for serum  $\beta$ -carotene and  $\beta$ -cryptoxanthin levels. Paradoxically, there was a suggestion of a protective association for these analytes among the non-drinkers. Future observational studies

need to evaluate the effects of alcohol consumption on the carotenoid–cancer association.

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