Prediagnostic Levels of Serum β-Cryptoxanthin and Retinol Predict Smoking-related Lung Cancer Risk in Shanghai, China[1]

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Abstract

Higher blood levels of β-carotene have been found to be associated with reduced risk of lung cancer, but large intervention trials have failed to demonstrate reduced lung cancer incidence after prolonged high-dose β-carotene supplementation. Data on blood levels of specific carotenoids other than β-carotene in relation to lung cancer are scarce. Little is known about the relationship between prediagnostic serum levels of carotenoids, retinol, and tocopherols, and risk of lung cancer especially in non-Western populations. Between January 1986 and September 1989, 18,244 men ages 45–64 years participated in a prospective study of diet and cancer in Shanghai, China. Information on tobacco smoking and other lifestyle factors was obtained through in-person interviews. A serum sample was collected from each study participant at baseline. During the first 12 years of follow-up, 209 lung cancer cases, excluding those diagnosed within 2 years of enrollment, were identified. For each cancer case, three cancer-free control subjects were randomly selected from the cohort and matched to the index case by age (within 2 years), month and year of blood sample collection, and neighborhood of residence. Serum concentrations of retinol, α- and γ-tocopherols, and specific carotenoids including α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were determined on the 209 cases and 622 matched controls by high-performance liquid chromatography methods. A high prediagnostic serum level of β-cryptoxanthin was significantly associated with reduced risk of lung cancer; relative to the lowest quartile, the smoking-adjusted relative risks (95% confidence intervals) for the 2nd, 3rd, and 4th quartile categories were 0.72 (0.41–1.26), 0.42 (0.21–0.84), and 0.45 (0.22–0.92), respectively (*P* for trend = 0.02). Increased serum levels of other specific carotenoids including α-carotene, β-carotene, lycopene, and lutein/zeaxanthin were related to reduced risk of lung cancer although the inverse associations were no longer statistically significant after adjustment for smoking. A statistically significant 37% reduction in risk of lung cancer was noted in smokers with above *versus* below median level of total carotenoids. Serum retinol levels showed a threshold effect on lung cancer risk. Compared with the lowest quartile (<40 μg/dl), the smoking-adjusted relative risk (95% confidence interval) was 0.60 (0.39–0.92) for men in the 2nd-4th quartiles of retinol values combined; no additional decrease in risk was observed between individuals from the 2nd to 4th quartiles. There were no associations between prediagnostic serum levels of α- and γ-tocopherols and lung cancer (all *P*s for trend ≥0.4). The present data indicate that higher prediagnostic serum levels of total carotenoids and β-cryptoxanthin were associated with lower smoking-related lung cancer risk in middle-aged and older men in Shanghai, China. Low level of serum retinol (with a threshold effect) is associated with increased lung cancer risk in this oriental population.

Introduction

Cigarette smoking is by far the single most important etiological factor for lung cancer (1). About 90% of lung cancer in the United States is attributable to smoking (2). There are several likely mechanisms, but induction of oxidative damage to DNA and other cellular structures by carcinogens in tobacco smoke is believed to be an important mechanistic pathway to smoking-related lung carcinogenesis (3). Antioxidants such as carotenoids, retinol, and vitamin E can neutralize free radicals, thereby preventing such cell damage and subsequent malignant transformation. Observational studies have demonstrated that people who eat more carotenoid-rich vegetables and fruits, more carotenoids in general, and β-carotene in particular, are less likely to develop lung cancer (4). Prospective observational studies have consistently shown that persons with high baseline serum β-carotene concentrations exhibit reduced risk of lung cancer relative to those with lower β-carotene levels (5–9). However, three large, double-blind, placebo-controlled intervention trials have failed to demonstrate any observable reduction in lung cancer risk among subjects after prolonged (4–12 years) administration of high-dose β-carotene supplementation (10–12). In fact, the Alpha-Tocopherol Beta-Carotene Study among smokers in Finland and the Beta-Carotene and Retinol Efficacy Trial among smokers and asbestos-exposed workers in the United States both reported increased lung cancer incidence (18 and 28% above control rates, respectively) among subjects receiving such β-carotene supplements (10, 11). The third trial, the Physicians' Health Study, showed a nonsignificant lung

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cancer risk reduction of 8% among β-carotene-treated subjects (12).

About 600 carotenoids have been identified in vegetables and fruit (13). The predominant carotenoids circulating in human blood are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin (14). Other than β-carotene, dietary intakes of some of these carotenoids, including α-carotene, β-cryptoxanthin and lutein, have been found to be inversely related to lung cancer risk (15–18). However, data on serum concentrations of most of these specific carotenoids and lung cancer risk are scarce. Only one study has reported that prediagnostic serum concentrations of specific carotenoids other than β-carotene are inversely associated with risk of smoking-related lung cancer among United States whites (9).

Retinoids have powerful effects on cell differentiation and proliferation (19). Because carcinogenesis is fundamentally a disorder of cell differentiation and division, it is possible that the retinoid status of a cell significantly influences its potential for cancer development. Synthetic retinoids have been shown to be useful and effective in the prevention of carcinogenesis in experimental animals (20). However, a number of prospective studies have failed to observe an association between baseline serum retinol level and lung cancer risk (5, 6, 8, 21–24). All of these studies were conducted in well-nourished, Western populations with relatively high circulating levels of retinol.

α-Tocopherol (the predominant and most active form of vitamin E in humans) is the major chain-breaking antioxidant in lipid phase (25), and it is thought to inhibit carcinogenesis primarily through its antioxidant activity (26). Observational studies of the association between prediagnostic serum α-tocopherol concentration and lung cancer risk have yielded mixed results. An inverse association was observed in some (6, 27) but not in other studies (5, 9, 22, 28, 29).

The present study was designed to investigate the relationships between prospectively collected serum micronutrients and lung cancer incidence in a cohort of >18,000 middle-aged and older men in Shanghai, China. The micronutrients measured in prediagnostic serum were specific carotenoids (*i.e.*, α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin), retinol, and α- and γ-tocopherols.

Materials and Methods

The study design has been described previously in detail (30). Between January 1986 and September 1989, all of the men ages 45–64 years living in four small, geographically defined areas of the city of Shanghai were invited to participate in a prospective study of diet and cancer. At recruitment, each subject was interviewed in person using a structured questionnaire that included demographic information, history of tobacco and alcohol use, current diet (45 food items), and medical history. At the completion of interview, a 10-ml nonfasting blood sample was collected from each study participant. Blood samples were placed in an icebox (~4°C) immediately after they were collected and processed usually within 3–4 h. Different aliquots of serum (1–2 ml each) from each study subject were stored at −70°C and −20°C, respectively, until analysis. A total of 18,244 men (80% of eligible subjects) were enrolled in the study. Follow-up has been conducted by annual recontact with all of the surviving cohort members, routine reviews of cancer reports from the Shanghai Cancer Registry, and death certificates from the local vital statistics offices. Follow-up on the cohort is almost complete; to date, only 207 subjects have become lost to follow-up.

By September 1998, we identified 318 incident cases of lung cancer among cohort members. Of the 318 cases, 217 (68%) were histopathologically confirmed and 101 (32%) were based on radiological diagnosis including radiography or computer-assisted tomography with consistent clinical characteristics. We excluded all of the cases ($n = 100$) diagnosed within 2 years of enrollment because of the possibility that their dietary (hence, serum) profiles might have been altered because of disease symptoms. For each of the remaining lung cancer cases, three cancer-free controls matched to the index case by age (within 2 years), month and year of sample collection, and neighborhood of residence at recruitment were randomly chosen among all of the cohort members. Nine cases and an additional five control subjects not matched to these nine cases had missing values on at least one serum measurement (see below) and were excluded from the study. Thus, 209 cases and 622 controls were included in the present study.

Serum samples that had been continuously frozen at −70°C were used for measurements of serum micronutrients, including α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, retinol, and α- and γ-tocopherols. The serum concentrations of these micronutrients were determined by high-performance liquid chromatography using methods described previously (31, 32). The methods used were unable to quantify the lutein and zeaxanthin separately; therefore, these two carotenoids are combined in this report.

Serum samples were arranged in matched sets of four samples with each set containing serum samples from the case and the three matched controls of which disease status was blind to the laboratory personnel. For all of the laboratory measurements, samples within a given matched set were assayed in the same experiment, and triplicate measurements were made for each serum sample. The mean of the triplicate measurement was assigned as the sample value.

We summed α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin as "total" carotenoids and α- and γ-tocopherols as total tocopherols. The distributions of all of the serum markers under study were markedly skewed toward high values, which were corrected to a large extent by transformation to logarithmic values. Therefore, formal statistical testing was performed on logarithmically transformed values, and geometric (as opposed to arithmetic) means are presented. We used the ANOVA method (33) to examine the relationships between serum micronutrient levels and baseline cigarette smoking status among control subjects.

Data were analyzed by standard matched-set methods (34). Conditional logistic regression models were used to examine associations between serum micronutrient levels and lung cancer risk. The associations were measured by ORs[3] and their corresponding 95% CIs and *P*s. Study subjects were grouped into quartiles based on the distribution of values in control subjects (see Appendix A). The linear trend tests for exposure-disease associations were based on ordinal values (0–3) for the quartiles. We also examined the micronutrient-lung cancer associations in subgroups stratified by smoking status (ever and never). For these subgroup analyses, matched sets were constructed based on the batch status of the serum measurement under study. In other words, all of the cases and controls in a given matched set were tested within a single batch. The original matching factors (age, month and year of blood sample collection, and neighborhood of residence at

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; 8-OHdG, lymphocyte DNA 8-hydroxy-2′-deoxyguanosine.

Table 1 Cigarette smoking in relation to risk of lung cancer, Shanghai Cohort Study, 1986–1998

Smoking status at recruitment	Cases	Controls	Matched OR (95% CI)
Never	20	287	1.0
Ever	189	335	8.5 (5.1, 14.1)
Former smokers	14	60	3.7 (1.7, 7.8)
Current smokers	175	275	9.2 (5.6, 15.4)
No. of cigarettes/day			
<10	8	59	1.9 (0.8, 4.6)
10–19	49	120	5.8 (3.3, 10.4)
20–29	91	133	10.6 (6.1, 18.7)
≥30	41	23	27.0 (13.0, 55.8)
Age at starting to smoke (yrs)			
<20	78	74	15.8 (8.8, 28.3)
20–29	92	182	7.6 (4.5, 13.1)
≥30	19	79	3.7 (1.8, 7.4)

recruitment) were included in the conditional logistic regression model as covariates.

In all of the analyses, we adjusted for smoking by including covariate terms for age starting to smoke, average number of cigarettes smoked per day, and smoking status at the time of blood draw (nonsmoker, smoker). Statistical computing was conducted using the SAS version 6.12 (SAS Institute Inc., Cary, NC) and Epilog windows version 1.0 (Epicenter Software, Pasadena, CA) statistical software packages. All *P*s quoted are two-sided.

Results

The mean age (±SD) of cases at diagnosis of lung cancer was 64.8 (± 5.6) years. The corresponding age of controls at the time of cancer diagnosis of index cases was 64.7 (± 5.6) years. Among cases, the average time interval between blood sample collection and diagnosis of lung cancer was 7.2 (± 2.2) years (ranging from 2.7 to 12.3 years). At recruitment, 54% of controls and 90% of cases reported having smoked ≥1 cigarette/day for ≥6 months, and the majority of these were current smokers. Ever-smokers had an 8.5-fold excess risk of lung cancer compared with never-smokers. Risk of lung cancer increased monotonically with increasing number of cigarettes smoked per day. There was a 27-fold increase in risk among men who smoked ≥30 cigarettes/day relative to never-smokers. Men who began to smoke early in life experienced a higher risk of lung cancer than those who started later (Table 1).

Table 2 shows the associations between serum concentrations of various micronutrients and smoking status at recruitment among control subjects only. Ever-smokers (82% were current smokers) had lower serum concentrations of total and specific carotenoids than never-smokers. Values were especially low among heavy (≥20 cigarettes/day) smokers, and all of the linear trend tests were highly significant except lutein/zeaxanthin, which showed borderline statistical significance (Table 2). In contrast, serum concentrations of retinol and tocopherols were unrelated to smoking status at baseline.

Table 3 shows the relative risk of lung cancer in relation to quartile levels of serum micronutrients. Before adjustment for cigarette smoking, increased levels of total and individual carotenoids were significantly associated with reduced lung cancer risk (all *P*s for trend tests < 0.05). After adjustment for smoking, only β-cryptoxanthin showed a statistically significant inverse association with lung cancer risk (P for trend $= 0.02$). Compared with the lowest quartile, the smoking-adjusted ORs (95% CIs) for the 2nd, 3rd, and 4th quartile categories of serum β-cryptoxanthin concentration were 0.72 (0.41–1.26), 0.42 (0.21–0.84), and 0.45 (0.22–0.92), respectively. In this study population, the main food sources of β-cryptoxanthin are tangerines, oranges, peaches, and watercress. The smoking-adjusted associations of total and other individual carotenoids including α-carotene, β-carotene, lycopene, and lutein/zeaxanthin with lung cancer risk were all statistically nonsignificant. When the analyses were restricted to ever-smokers only, similar results were seen (Table 3).

Relative to the lowest quartile, individuals in the 2nd-4th quartiles of retinol values combined showed a 33% reduction in lung cancer risk (OR = 0.67; 95% CI = 0.47–0.94). The comparable OR after adjustment for cigarette smoking was 0.60 (95% CI = 0.39–0.92). No additional decrease in risk was observed between individuals from the 2nd to 4th quartiles of retinol values (Table 3). On the other hand, risk of lung cancer increased with decreasing levels of retinol within the lowest quartile range of retinol values. Compared with subjects in the 2nd-4th quartiles of retinol (>39.6 μg/dl), the smoking-adjusted OR (95% CI) of lung cancer was 1.43 (0.80–2.56) and 1.89 (1.12–3.20), respectively, for men in the upper half (34.3–39.6 μg/dl) and in the lower half (<34.3 μg/dl) of the lowest quartile of retinol values (P for trend = 0.01). Similar results were observed among ever-smokers only.

Levels of total and individual serum tocopherols were not associated with lung cancer risk, with or without adjustment for smoking. The null associations remained when analyses were restricted to ever-smokers only (Table 3).

There were only 20 lung cancer patients who had never smoked cigarettes. The associations between levels of serum micronutrients and lung cancer risk among never-smokers are presented in Table 4. None of the differences in risk of lung cancer between higher and lower levels of serum micronutrients were statistically significant. On the other hand, among ever-smokers, a statistically significant 35–40% reduction in risk of lung cancer was observed for elevated levels of total carotenoids, β-cryptoxanthin, and retinol. A high level of lutein/zeaxanthin was associated with a statistically borderline significant 30% reduction in risk of lung cancer ($P = 0.06$; Table 4).

Of the 209 lung cancers, 64 (31%) were squamous or small cell carcinomas, 64 (31%) were adenocarcinomas, and the remaining 81 (38%) were of unknown or other cell types. Risk estimates by histological classifications were relatively unstable because of small sample sizes upon stratification. Nonetheless, we examined the associations between serum micronutrients and risk of lung cancer according to histological categories and did not detect any meaningful variations in disease risk by histology (data not shown).

To examine the impact of duration of follow-up on the associations between prediagnostic serum micronutrients and lung cancer, we repeated all of the analyses after excluding lung cancer cases diagnosed within 5 years of study enrollment and their matched controls (171 cases and 510 controls remaining). Results were comparable with those based on the entire data (data not shown). We also repeated all of the analyses on only histopathologically confirmed cases and their matched controls (138 cases and 411 controls). Again, results were similar to those based on the whole data set (data not shown).

Discussion

To our knowledge, the present study is the first to prospectively examine the associations of serum total and specific carotenoids

Table 2 Geometric mean levels of serum micronutrients by cigarette smoking status among control subjects only, Shanghai Cohort Study, 1986–1998

	Never-smokers (n = 287)	Ever-smokers, <20 cigarettes/day (n = 179)	Ever-smokers, ≥20 cigarettes/day (n = 156)	2-sided P for linear trend
Total carotenoids (μg/dl)	52.66	52.36	46.60	0.002
α-Carotene (μg/dl)	1.20	1.14	1.03	0.006
β-Carotene (μg/dl)	11.27	11.10	9.60	0.009
β-Cryptoxanthin (μg/dl)	3.33	3.13	2.60	0.0003
Lycopene (μg/dl)	3.05	2.96	2.35	0.0002
Lutein/zeaxanthin (μg/dl)	31.58	32.16	29.14	0.051
Retinol (μg/dl)	46.45	47.90	46.74	0.83
Total tocopherols (mg/liter)	9.94	10.09	9.62	0.29
α-Tocopherol (mg/liter)	8.26	8.39	8.03	0.38
γ-Tocopherol (mg/liter)	1.63	1.63	1.55	0.28

with lung cancer in a non-Western population. Our study population had a mean level of serum total carotenoids 50% lower than United States whites (9). For specific carotenoids, mean values in our study population were 90% lower in lycopene, 60% lower in α-carotene, 50% lower in β-cryptoxanthin, and 20–50% lower in β-carotene but 50% higher in lutein/zeaxanthin relative to their counterparts in the United States (9) and northern Europe (7, 8).

The present study has demonstrated a statistically significant, inverse association between prediagnostic serum level of β-cryptoxanthin and lung cancer risk that is independent of cigarette smoking at the level of detail measured in this study. Only one prior study (conducted in the United States) has examined the associations between prediagnostic serum levels of multiple, specific carotenoids and lung cancer risk. After adjustment for cigarette smoking, there was an 11% lower mean baseline β-cryptoxanthin concentration among lung cancer patients than control subjects, whereas no difference was found for other individual carotenoids (9). Recently, Voorrips *et al.* (18) reported a statistically significant, inverse association between dietary intake of β-cryptoxanthin and risk of lung cancer, particularly among current smokers, in a cohort of 58,279 middle-aged and older men in the Netherlands. Haegele et al. (35) examined the relationships between plasma levels of various carotenoids (β-cryptoxanthin plus lutein, α- plus β-carotenes, and lycopene) and a marker of oxidative DNA damage (8-OhdG) among 47 subjects fed controlled diets for 14 days. Highly significant inverse associations between plasma level of β-cryptoxanthin plus lutein and 8-OHdG were observed in both pre- and postintervention blood samples. Furthermore, highly significant correlations were noted between changes in β-cryptoxanthin plus lutein and 8-OHdG levels among study subjects during the course of the intervention. Plasma carotenes, on the other hand, showed only a borderline significant (P = 0.048) inverse association with 8-OHdG among post-intervention samples, and no association was seen between changes in carotenes and 8-OHdG levels during the course of the study (35). Our study results along with those of others (9, 18, 35) are consistent with the notion that β-cryptoxanthin may be a chemopreventive agent for lung cancer.

Alternatively, β-cryptoxanthin might merely be a surrogate of fruit and vegetable intake. In fact, the present study also demonstrated a reduced risk of lung cancer in men with high levels of other specific carotenoids including α-carotene, β-carotenes, lycopene, and lutein/zeaxanthin, although none of the inverse carotenoid-lung cancer associations were statistically significant after adjustment for smoking. It is possible that the observed statistical associations of various carotenoids with lung cancer risk were markers of as-yet unidentified chemopreventive agents in fruits and vegetables.

Numerous observational studies have noted a statistically significant inverse association between dietary intake or serum level of β-carotene and lung cancer risk (5–9, 16, 36). Consistent with those reported previously, our data also demonstrated an inverse association between β-carotene and lung cancer risk. However, the inverse association was no longer statistically significant after adjustment for smoking. Three large, double-blind, placebo-controlled intervention trials have failed to show any protection from lung cancer development after prolonged high-dose β-carotene supplementation (10–12), suggesting that β-carotene may not be a chemopreventive agent for lung cancer prevention.

A number of epidemiological studies have examined the relationships between dietary intake of α-carotene, lycopene, and lutein/zeaxanthin and lung cancer risk. Some studies noted an inverse association of lung cancer risk with α-carotene (4, 15, 37) whereas others did not (17, 18, 38). None of these previous studies reported a significant association between dietary lycopene intake level and lung cancer risk (4, 15, 17, 18, 37, 38). Among the six studies that examined the association between dietary intake of lutein/zeaxanthin and lung cancer risk (4, 15, 17, 18, 37, 38), four reported an inverse association (4, 15, 18, 37). Only one prior prospective study has examined the associations between serum levels of α-carotene, lycopene, and lutein/and zeaxanthin and lung cancer risk. After adjustment for smoking, the level of lutein/zeaxanthin was lower in lung cancer cases than in controls although the difference was not statistically significant. No difference was found for α-carotene and lycopene in that study (9). Our results were generally consistent with those reported previously. A statistically borderline significant 30% reduction (P = 0.06) in risk of lung cancer was observed in smokers with higher *versus* lower than median levels of lutein/zeaxanthin. No differences were observed for α-carotene and lycopene.

An apparent threshold effect of serum retinol on lung cancer risk was observed in the present study population, of which average serum level is only 50–70% that of comparably aged Western populations (6, 8, 21). Compared with men with a serum retinol level <40 μg/dl, those with higher values experienced a statistically significant 40% decrease in risk of lung cancer. There was no additional decrease in risk among individuals with retinol values >40 μg/dl. On the other hand, there was increasing risk of lung cancer with decreasing retinol values among men with serum retinol levels <40 μg/dl. Six cohort studies have examined the

Table 3 Serum micronutrient levels in relation to risk of lung cancer, Shanghai Cohort Study, 1986–1998

Micronutrient	Quartile[a]				2-sided *P* for linear trend
	1st	2nd	3rd	4th	
Total carotenoids					
All subjects: OR (95% CI)[b]	1.00	0.84 (0.55–1.28)	0.43 (0.26–0.70)	0.58 (0.37–0.92)	0.003
All subjects: adjusted OR (95% CI)[c]	1.00	1.11 (0.67–1.86)	0.49 (0.27–0.89)	0.84 (0.48–1.47)	0.20
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	1.11 (0.67–1.85)	0.50 (0.28–0.90)	0.86 (0.49–1.50)	0.16
α-Carotene					
All subjects: OR (95% CI)[b]	1.00	1.18 (0.78–1.78)	0.51 (0.31–0.86)	0.74 (0.44–1.26)	0.04
All subjects: adjusted OR (95% CI)[c]	1.00	1.28 (0.78–2.09)	0.66 (0.37–1.19)	1.15 (0.62–2.15)	0.79
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	1.49 (0.92–2.43)	0.77 (0.41–1.43)	1.37 (0.75–2.50)	0.70
β-Carotene					
All subjects: OR (95% CI)[b]	1.00	0.71 (0.47–1.07)	0.52 (0.33–0.82)	0.51 (0.31–0.82)	0.002
All subjects: adjusted OR (95% CI)[c]	1.00	0.86 (0.52–1.42)	0.68 (0.40–1.18)	0.74 (0.42–1.30)	0.20
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	0.96 (0.58–1.61)	0.86 (0.50–1.47)	0.81 (0.45–1.44)	0.41
β-Cryptoxanthin					
All subjects: OR (95% CI)[b]	1.00	0.62 (0.39–1.01)	0.28 (0.16–0.52)	0.38 (0.21–0.69)	<0.001
All subjects: adjusted OR (95% CI)[c]	1.00	0.72 (0.41–1.26)	0.42 (0.21–0.84)	0.45 (0.22–0.92)	0.02
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	0.68 (0.39–1.18)	0.42 (0.22–0.83)	0.48 (0.24–0.94)	0.02
Lycopene					
All subjects: OR (95% CI)[b]	1.00	0.77 (0.50–1.19)	0.57 (0.35–0.93)	0.46 (0.27–0.79)	0.003
All subjects: adjusted OR (95% CI)[c]	1.00	0.85 (0.51–1.42)	0.84 (0.48–1.46)	0.59 (0.31–1.14)	0.15
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	0.86 (0.51–1.46)	0.78 (0.44–1.37)	0.80 (0.43–1.49)	0.40
Lutein/zeaxanthin					
All subjects: OR (95% CI)[b]	1.00	1.13 (0.74–1.73)	0.75 (0.47–1.20)	0.69 (0.43–1.12)	0.047
All subjects: adjusted OR (95% CI)[c]	1.00	1.58 (0.95–2.63)	1.05 (0.60–1.84)	0.97 (0.55–1.71)	0.53
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	1.34 (0.80–2.25)	0.81 (0.47–1.40)	0.75 (0.43–1.32)	0.15
Retinol[e]					
All subjects: OR (95% CI)[b]	1.00	0.61 (0.40–0.95)	0.70 (0.45–1.09)	0.69 (0.44–1.09)	0.14
All subjects: adjusted OR (95% CI)[c]	1.00	0.51 (0.29–0.88)	0.66 (0.39–1.12)	0.65 (0.37–1.09)	0.18
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	0.59 (0.33–1.03)	0.65 (0.38–1.12)	0.60 (0.35–1.05)	0.09
Total tocopherols					
All subjects: OR (95% CI)[b]	1.00	0.86 (0.55–1.35)	0.86 (0.55–1.35)	0.84 (0.53–1.34)	0.48
All subjects: adjusted OR (95% CI)[c]	1.00	0.81 (0.49–1.36)	0.90 (0.52–1.55)	0.83 (0.49–1.43)	0.61
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	0.82 (0.48–1.40)	0.85 (0.49–1.49)	0.82 (0.46–1.44)	0.53
α-Tocopherol					
All subjects: OR (95% CI)[b]	1.00	1.10 (0.70–1.72)	0.97 (0.61–1.54)	0.97 (0.60–1.55)	0.75
All subjects: adjusted OR (95% CI)[c]	1.00	0.85 (0.49–1.47)	0.91 (0.52–1.61)	0.84 (0.47–1.49)	0.65
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	0.90 (0.52–1.55)	0.91 (0.52–1.59)	0.84 (0.47–1.51)	0.60
γ-Tocopherol					
All subjects: OR (95% CI)[b]	1.00	1.02 (0.65–1.60)	1.04 (0.67–1.64)	0.79 (0.49–1.27)	0.38
All subjects: adjusted OR (95% CI)[c]	1.00	1.04 (0.61–1.75)	1.23 (0.72–2.10)	0.88 (0.50–1.54)	0.83
Ever-smokers only: adjusted OR (95% CI)[d]	1.00	1.16 (0.68–1.98)	1.23 (0.71–2.14)	0.90 (0.51–1.57)	0.79

[a] See Appendix A for quartile cut-points.
[b] Based on conditional logistic regression method. A matched set consisted of three control subjects individually matched to the index case by age, mo and yr of blood sample collection, and neighborhood of residence at recruitment.
[c] Further adjusted for age at starting to smoke, average no. of cigarettes smoked/day, and smoking status at the time of blood draw (nonsmoker, smoker).
[d] Based on conditional logistic regression method. A matched set consisted of all cases and controls with serum measurement from the same batch. Further adjusted for matching factors (age, mo and yr of blood sample collection, and neighborhood of residence at recruitment), age at starting to smoke, average no. of cigarettes smoked/day, and smoking status at the time of blood draw (nonsmoker, smoker).
[e] Compared with the lowest quartile, the smoking-unadjusted OR for the 2nd–4th quartiles of retinol values combined was 0.67 (95% CI = 0.47–0.94); the smoking-adjusted OR was 0.60 (95% CI = 0.39–0.92) in all subjects and 0.62 (95% CI = 0.40–0.96) in ever-smokers only.

retinol-lung cancer association (5, 6, 21–24, 39). All were conducted in well-nourished, Western populations of which the average level of circulating retinol ranges from 60 μg/dl to 82 μg/dl (the mean level was 47 μg/dl in these Shanghai men). None of the six studies observed a difference in levels of blood retinol between cases and controls. The present investigation, which shows no additional protective effect of retinol beyond a level of 40 μg/dl, may explain why these previous studies, all conducted in Occidental populations (with <5% of population values <40 μg/dl), have yielded null results.

A number of studies have examined the association between serum levels of α-tocopherol and lung cancer. In general, null or weak inverse associations have been observed. Of nine prospective studies evaluating serum levels of α-tocopherol (5, 6, 8, 9, 22, 27–29, 36), only one showed a statistically significant inverse association (6). Recent data from the Alpha-Tocopherol Beta-Carotene Study showed baseline serum α-tocopherol concentrations to be inversely associated with risk of lung cancer among 29,133 Finnish smokers (P = 0.09; Ref. 27). Consistent with most of the previous studies, the present study did not observe any protective effects of α- and γ-tocopherols separately or in combination on lung cancer development. The mean serum level of α-tocopherol (8.2 mg/liter) in this Chinese population is in the low range of comparable values in Western countries (8.0–11.6 mg/liter; Ref. 9, 27, 28).

The strengths of the present study include: (*a*) the availability of prediagnostic serum specimens and the exclusion of

Table 4 Serum micronutrient levels in relation to risk of lung cancer by smoking status, Shanghai Cohort Study, 1986–1998

Micronutrients	Level[a]	Never-smokers		Ever-smokers	
		Ca/Co[b]	OR (95% CI)[c]	Ca/Co[b]	OR (95% CI)[c]
Total carotenoids	Low	10/140	1.00	122/172	1.00
	High	10/147	1.09 (0.42–2.76)	67/163	0.63 (0.42–0.94)
α-Carotene	Low	10/144	1.00	130/204	1.00
	High	10/143	0.77 (0.30–2.01)	59/131	0.83 (0.54–1.28)
β-Carotene	Low	12/135	1.00	115/176	1.00
	High	8/152	0.69 (0.26–1.79)	74/159	0.85 (0.57–1.27)
β-Cryptoxanthin	Low	11/133	1.00	120/182	1.00
	High	9/154	0.90 (0.31–2.60)	69/153	0.58 (0.36–0.93)
Lycopene	Low	10/132	1.00	116/184	1.00
	High	10/155	0.65 (0.23–1.83)	73/151	0.85 (0.55–1.30)
Lutein/zeaxanthin	Low	8/148	1.00	116/164	1.00
	High	12/139	1.82 (0.71–4.65)	73/171	0.68 (0.46–1.00)
Retinol	Low[d]	6/74	1.00	63/82	1.00
	High[d]	14/213	0.75 (0.27–2.07)	126/253	0.62 (0.40–0.96)
Total tocopherols	Low	10/140	1.00	99/172	1.00
	High	10/147	0.90 (0.36–2.25)	90/163	0.93 (0.62–1.38)
α-Tocopherol	Low	10/148	1.00	99/165	1.00
	High	10/139	0.94 (0.36–2.42)	90/170	0.93 (0.63–1.39)
γ-Tocopherol	Low	8/139	1.00	102/174	1.00
	High	12/148	1.42 (0.56–3.61)	87/161	0.98 (0.66–1.45)

[a] See Appendix A for high/low cut-points; low, less than or equal to median value; high, greater than median value.
[b] No. of cases/ no. of controls.
[c] Based on conditional logistic regression method. A matched set consisted of all cases and controls with serum measurement from the same batch. For never-smokers, adjusted for matching factors (age, mo and yr of blood sample collection, and neighborhood of residence at recruitment); for ever-smokers, further adjusted for age at starting to smoke, average number of cigarettes smoked/day, and smoking status at the time of blood draw (nonsmoker, smoker).
[d] For retinol: low = 1st quartile (<39.61 μg/dl); high = 2nd–4th quartiles (≥39.61 μg/dl).

cases occurring within 2 years of recruitment, minimizing the possible influence of disease symptoms on dietary intake of various micronutrients; (*b*) the size of the cohort (>18,000 men) with ≤12 years of follow-up providing relatively high statistical power to detect moderate effects of exposure on disease occurrence; (*c*) almost complete follow-up [only 207 (1.1%) subjects were lost to follow-up] minimizing the possibility of selection bias; and (*d*) the relatively low dietary intake levels of certain micronutrients in the study population providing an opportunity to examine these associations with lung cancer in the lower ranges of exposure levels.

In summary, our study demonstrates a statistically significant inverse association between prediagnostic serum concentration of β-cryptoxanthin and lung cancer development, independent of smoking, among middle-aged or older men in Shanghai, China. Serum levels of other specific carotenoids including α-carotene, β-carotene, lycopene, and lutein/zeaxanthin also exhibited inverse associations with lung cancer risk, although none remained statistically significant after adjustment for smoking. A statistically significant reduction in lung cancer risk was noted in smokers with above *versus* below median level of serum total carotenoids. Low levels of serum retinol (with a threshold effect) were associated with increased lung cancer risk in our study population, of which the mean serum retinol level is 50–70% that of Western populations. Serum levels of α- and γ-tocopherol were unrelated to lung cancer development in this population.

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Appendix A

Appendix A Quartile cut-points of serum concentrations of various micronutrients

Micronutrient	Quartile			
	1st	2nd	3rd	4th
Total carotenoids (μg/dl)	<40.48	40.48–51.30	51.31–66.56	≥66.57
α-Carotene (μg/dl)	<0.71	0.71–1.10	1.11–1.60	≥1.61
β-Carotene (μg/dl)	<7.10	7.10–11.00	11.01–16.20	≥16.21
β-Cryptoxanthin (μg/dl)	<1.81	1.81–3.00	3.01–4.53	≥4.54
Lycopene (μg/dl)	<1.61	1.61–2.60	2.61–4.30	≥4.31
Lutein/zeaxanthin (μg/dl)	<24.27	24.27–32.10	32.11–40.63	≥40.64
Retinol (μg/dl)	<39.61	39.61–47.10	47.11–56.58	≥56.58
Total tocopherols (mg/liter)	<8.14	8.14–9.57	9.58–11.77	≥11.78
α-Tocopherol (mg/liter)	<6.71	6.71–7.98	7.99–10.00	≥10.01
γ-Tocopherol (mg/liter)	<1.17	1.17–1.53	1.54–2.10	≥2.11

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