

# Prediagnostic Levels of Serum Micronutrients in Relation to Risk of Gastric Cancer in Shanghai, China

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## Abstract

Data on blood levels of specific carotenoids and vitamins in relation to gastric cancer are scarce. Little is known about the relationship between prediagnostic serum levels of carotenoids other than  $\beta$ -carotene and risk of gastric cancer especially in non-Western populations. Prediagnostic serum concentrations of  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein/zeaxanthin, retinol,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and vitamin C were determined on 191 cases and 570 matched controls within a cohort of 18,244 middle-aged or older men in Shanghai, China, with a follow-up of 12 years. High serum levels of  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene were significantly associated with reduced risk of developing gastric cancer (all *P*s for trend  $\leq 0.05$ ); the odds ratios (95% confidence intervals) for the highest versus the lowest quartile of  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene were 0.38 (0.13-1.11), 0.54 (0.32-0.89), and 0.55

(0.30-1.00), respectively. Increased serum level of vitamin C was significantly associated with reduced risk of gastric cancer among men who neither smoked cigarettes over lifetime nor consumed  $\geq 3$  drinks of alcohol per day; the odds ratios (95% confidence intervals) for the second, third, and fourth quartile categories were 0.69 (0.28-1.70), 0.36 (0.14-0.94), and 0.39 (0.15-0.98), respectively, compared with the lowest quartile of vitamin C (*P* for trend = 0.02). There were no statistically significant relationships of serum levels of  $\beta$ -cryptoxanthin, lutein/zeaxanthin, retinol,  $\alpha$ -tocopherol, and  $\gamma$ -tocopherol with gastric cancer risk. The present study implicates that dietary carotenoids, lycopene, and vitamin C are potential chemopreventive agents for gastric cancer in humans. (Cancer Epidemiol Biomarkers Prev 2004;13(11):1772-80)

## Introduction

Despite the continuing decline in both morbidity and mortality of gastric cancer during the past 70 years worldwide (1, 2), this malignancy remains the world's second most common cause of death from cancer and the third most frequent cancer behind cancers of the lung and breast (3). Gastric cancer incidence displays considerable variation worldwide. Gastric cancer is one of the most common cancer in China but is rare in the United States. The average annual age-adjusted incidence rates per 100,000 (world standard) in men and women are 32.3 and 17.6, respectively, in Shanghai, China during 1992 to 1997. The corresponding figures were 6.6 and 2.6 in U.S. Whites (4).

Correa (5) has proposed a detailed hypothetical model of gastric carcinogenesis in which environmental factors contribute to a sequential series of precursor lesions, starting with superficial gastritis and then proceeding through chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally carcinoma. Postulated environmental factors contributing to this sequence include

gastric infection with bacterium *Helicobacter pylori*, high intake of salt, nitrates and related compounds, and low consumption of vitamin C and carotenoids. Ingested nitrate is reduced to nitrite by oral bacteria. Nitrite has a propensity to react with other nitrogen-containing compounds to form nitrosamines, which are potential carcinogens for the stomach in humans. Dietary antioxidants can inhibit the process of nitrosation and are believed to exert protective effects on gastric cancer. Case-control studies consistently found an inverse association between consumption of fresh fruit and vegetables and risk of gastric cancer. Prospective studies, which are less prone to recall bias, showed weak and statistically nonsignificant protective effect of fruit and vegetables on gastric cancer development (6). Possible protective micronutrients include vitamin C (ascorbic acid), vitamin E (mainly  $\alpha$ -tocopherol), and carotenoids (particularly  $\beta$ -carotene). Similarly, retrospective case-control studies showed stronger but limited number of prospective cohort studies showed weaker protective effects of dietary vitamin C and  $\beta$ -carotene on gastric cancer (7, 8).

Given the well-recognized difficulty in the assessment of dietary exposures using an interview instrument, measurements of serum micronutrients are more objective and direct markers of *in vivo* exposure to dietary antioxidants. Data on blood levels of specific carotenoids and vitamins in relation to gastric cancer are scarce. Little is known about the relationship between prediagnostic serum levels of carotenoids other than  $\beta$ -carotene and

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risk of gastric cancer especially in non-Western populations. The present study was designed to investigate the relationships between prospectively collected serum micronutrients and gastric 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.,  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lycopene, and lutein/zeaxanthin), retinol, vitamin C,  $\alpha$ -tocopherol, and  $\gamma$ -tocopherol.

## Materials and Methods

The design of the Shanghai Cohort Study has been described in detail elsewhere (9, 10). Briefly, four small, geographically defined communities over a wide area of the city of Shanghai were selected for a prospective, epidemiologic study of diet and cancer. Complete rosters of all residents in these selected communities for identification of eligible study subjects were obtained from local police stations. The eligibility criteria were men ages between 45 and 64 years and without history of cancer. Between January 1986 and September 1989, 18,244 men (~80% of eligible subjects) participated in the study. Each participant was interviewed in person using a structured questionnaire to obtain demographic information, use of tobacco and alcohol, usual adult diet, and medical history. At completion of the interview, a 10-mL nonfasting blood sample and a single-void (i.e., spot) urine sample were collected from each participant. Blood and urine samples were collected usually between 5 and 9 p.m. and placed in an icebox (~4°C) immediately after collection. Serum was separated within 4 hours after blood draw. Multiple aliquots of serum (1-2 mL each) from each study subject were immediately stored at -70°C and -20°C, respectively. Two aliquots of serum per subject that initially were stored at -20°C were all transferred to -70°C in December 1994. The urine samples were processed after overnight storage at 4°C, and aliquots of the urine samples initially were stored at -20°C and transferred to -70°C in November 2001.

Follow-up of cancer occurrence and death have been conducted through annual in-person re-interviews to all surviving cohort members and routine review of reports from the population-based Shanghai Cancer Registry and from the Shanghai Municipal Vital Statistics Office. Follow-up on the cohort is almost complete; by March 1998 (the cutoff date for the present study), only 207 (1.1%) subjects, including 29 who moved out of Shanghai area, had become lost to follow-up. An additional 248 subjects had moved out of Shanghai area. We were able to follow up all these subjects through mails and/or telephone interviews. The institutional review boards at the University of Southern California and the Shanghai Cancer Institute had approved this study.

By March 1998, 197 cohort participants developed gastric cancer and were eligible for the present study. Diagnoses of 179 (91%) cancers were based on histopathologic evidence. The remaining 18 (9%) cases were diagnosed based on clinical evidence only ( $n = 14$ ) or death certificates only ( $n = 4$ ). For each of the cases, three cancer-free controls matched to index cases by age (within 2 years), month and year of biospecimen collection, and neighborhood of residence at recruitment were randomly chosen among all of the cohort members.

Untreated serum samples that had been continuously frozen at -70°C since their collection were used for measurements of serum micronutrients, including  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein/zeaxanthin, retinol,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and vitamin C. The serum concentrations of all these micronutrients, except vitamin C, were determined by high-performance liquid chromatography using methods described previously (11, 12). The methods used were unable to quantify the lutein and zeaxanthin separately; therefore, these two carotenoids were combined in this report. The serum concentration of vitamin C was determined by a method developed by Roe and Kuether (13) with a modification (14).

Serum samples were arranged in matched sets of four samples, each set containing serum samples from the case and the three matched controls whose disease status was blind to the laboratory personnel. For all 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. Test samples were processed in a dim room to avoid potential change in chemical structures of certain carotenoids by strong light. Six cases and an additional three control subjects not matched to these six cases had missing values on at least one of the serum measurements and were excluded from the study. Thus, the present study included 191 cases and 570 control subjects.

The distributions of all 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 done on logarithmically transformed values, and geometric (as opposed to arithmetic) means and their 95% confidence intervals (95% CI) are presented. The ANOVA method was used to examine the difference in the concentrations of serum micronutrients between men with and men without risk factors for gastric cancer among control subjects (15). We also used the ANOVA method to examine the difference in prediagnostic serum micronutrient concentrations between incident gastric cancer cases and control subjects while maintaining matched case-control sets.

We used conditional logistic regression models to examine associations between serum micronutrient levels and gastric cancer risk (16). The associations were measured by odds ratios (OR) and their corresponding 95% CI and  $P$ s. Study subjects were grouped into quartiles based on the distribution of serum concentrations of micronutrients except for  $\alpha$ -carotene in control subjects (see Appendix 1). In the present study population, 216 (38%) controls and 77 (40%) cases had undetectable level of  $\alpha$ -carotene; thus, study subjects with detectable levels of  $\alpha$ -carotene were classified into tertiles according to the distribution in controls (see Appendix 1). The linear trend tests for exposure-disease associations were based on ordinal values (0-3) for the four exposure categories. We also examined the micronutrient-gastric cancer associations in subgroups stratified by cigarette smoking (ever or never), alcohol consumption (<3 or  $\geq$ 3 drinks per day), positivity of urinary tea polyphenol marker (epigallocatechin), and seropositivity of *H. pylori* antibodies. For the subgroup analyses, matched sets were broken and unconditional logistic regression method was used. The

original matching factors (age, year of blood biospecimen collection, and neighborhood of residence at recruitment) were included in the unconditional logistic regression models as covariates.

The presence of *H. pylori* antibodies in serum is an independent risk factor (17) and detectable level of urinary epigallocatechin is a protective factor for gastric cancer in the study population (18). Cigarette smoking and heavy alcohol consumption ( $\geq 3$  drinks per day) were significantly associated with increased risk of gastric cancer risk. These potential confounders were included in the multivariate logistic regression models when we examined the independent effects of micronutrients on gastric cancer risk.

Statistical computing was conducted using the SAS version 8.0 (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 (SD) ages of cases at cancer diagnosis and control subjects at the time of cancer diagnosis of index cases were 63.4 (5.6) and 63.4 (5.5) years, respectively. The average time interval between blood sample collection and diagnosis of gastric cancer among cases was 5.1 (3.0) years (ranging from 1 month to 12 years).

At recruitment, 69% of cases and 59% of controls reported having smoked  $\geq 1$  cigarettes per day for at least 6 months. Ever smokers had a 50% excess risk (OR, 1.53; 95% CI, 1.07-2.19) of gastric cancer compared with never smokers. Among cases, 19% consumed  $\geq 3$  drinks of alcohol per day (heavy drinkers) and 31% consumed  $< 3$  drinks per day (light drinkers). The percentages of heavy and light drinkers in controls were 12% and 31%, respectively. Heavy drinkers had an OR (95% CI) of 1.63 (1.02-2.60) for gastric cancer compared with nondrinkers after adjustment for cigarette smoking.

Table 1 shows geometric means of serum micronutrients among control subjects only stratified by four identified risk (or protective) factors for gastric cancer. Cigarette smoking and alcohol drinking were associated with reduced levels of individual carotenoids and

vitamin C. After adjustment for alcohol intake, smokers still had significantly lower levels of individual carotenoids and vitamin C. On the other hand, adjustment for cigarette smoking reduced the difference in certain micronutrients between alcohol drinkers and nondrinkers, but alcohol consumers still showed statistically significantly lower levels of  $\beta$ -carotene, lycopene, and vitamin C and higher level of retinol than nondrinkers. Subjects with detectable levels of urinary epigallocatechin had significantly higher level of  $\beta$ -cryptoxanthin and lower level of  $\alpha$ -carotene than those with undetectable urinary epigallocatechin. Subjects with serum antibodies to *H. pylori* had significantly lower levels of retinol than those without the antibodies. No significant differences in the concentrations of serum tocopherols were seen according to the status of cigarette smoking, alcohol drinking, positivity of urinary epigallocatechin, and seropositivity of *H. pylori* antibodies.

The correlation coefficients between individual carotenoids ranged from 0.23 to 0.53 (Table 2). Vitamin C was correlated with all specific carotenoids; the highest correlation coefficient was for  $\beta$ -carotene (0.37) and  $\beta$ -cryptoxanthin (0.31). Retinol was weakly correlated with carotenoids and vitamin C but highly correlated with  $\alpha$ -tocopherol.

Table 3 shows geometric means of serum micronutrients in gastric cancer cases and control subjects. Compared with controls, cases had statistically significantly lower prediagnostic serum levels of  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene. Overall, there were no significant differences in prediagnostic serum concentrations of  $\beta$ -cryptoxanthin, lutein/zeaxanthin, retinol,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and vitamin C between cases and controls.

Table 4 shows the relative risk of gastric cancer in relation to quartile levels of serum micronutrients in total study subjects. Before adjustment for potential confounders, increased levels of  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene were significantly associated with reduced gastric cancer risk (all *P*s for trend  $\leq 0.05$ ). After adjustment for cigarette smoking, heavy alcohol consumption, positivity of urinary epigallocatechin, and seropositivity of *H. pylori* antibodies, the inverse association between  $\beta$ -carotene and gastric cancer risk remained statistically significant (*P* for trend = 0.05).

**Table 1. Geometric mean concentrations of serum micronutrients by selected risk factors for gastric cancer among control subjects only, Shanghai Cohort Study, 1986-1998**

	Cigarette smoking			Alcohol drinking			Urinary epigallocatechin status*			<i>H. pylori</i> status†		
	No	Yes	<i>P</i>	No	Yes	<i>P</i>	Negative	Positive	<i>P</i>	Negative	Positive	<i>P</i>
Subjects ( <i>n</i> )	234	336	—	326	244	—	104	461	—	98	448	—
$\alpha$ -Carotene ( $\mu\text{g/dL}$ )	0.79	0.55	$<0.001$	0.70	0.58	0.07	0.83	0.61	0.02	0.73	0.65	0.42
$\beta$ -Carotene ( $\mu\text{g/dL}$ )	10.5	8.4	$<0.001$	9.9	8.3	0.003	8.2	9.4	0.08	9.9	9.0	0.24
$\beta$ -Cryptoxanthin ( $\mu\text{g/dL}$ )	3.3	2.6	$<0.001$	3.0	2.6	0.04	2.3	3.0	0.004	2.5	3.0	0.07
Lycopene ( $\mu\text{g/dL}$ )	3.0	2.2	$<0.001$	2.8	2.1	0.003	2.5	2.5	0.94	2.9	2.5	0.14
Lutein/zeaxanthin ( $\mu\text{g/dL}$ )	30.7	28.6	0.04	29.3	29.6	0.77	28.6	29.6	0.42	29.6	29.5	0.93
Retinol ( $\mu\text{g/dL}$ )	46.8	47.1	0.79	45.0	49.7	$<0.001$	47.4	46.9	0.73	50.2	46.4	0.01
Vitamin C (mg/L)	7.0	5.3	$<0.001$	6.4	5.3	$<0.001$	5.6	6.0	0.29	6.1	5.9	0.75
$\alpha$ -Tocopherol (mg/L)	8.7	8.5	0.43	8.6	8.5	0.87	8.2	8.7	0.12	8.3	8.6	0.30
$\gamma$ -Tocopherol (mg/L)	1.6	1.6	0.53	1.6	1.6	0.55	1.5	1.6	0.12	1.6	1.6	0.90

\*Detectable levels of urinary epigallocatechin. Five subjects with missing epigallocatechin status were excluded from these data analyses.

†Seropositivity of *H. pylori* antibodies. Twenty-four subjects with missing *H. pylori* status were excluded from these data analyses.

**Table 2. Pearson correlation coefficients between logarithmically transformed values of serum micronutrient concentrations among control subjects only, Shanghai Cohort Study, 1986-1998**

	$\alpha$ -Carotene	$\beta$ -Carotene	$\beta$ -Cryptoxanthin	Lycopene	Lutein/zeaxanthin	Retinol	Vitamin C	$\alpha$ -Tocopherol
$\beta$ -Carotene	0.44*	—	—	—	—	—	—	—
$\beta$ -Cryptoxanthin	0.23*	0.51*	—	—	—	—	—	—
Lycopene	0.50*	0.36*	0.25*	—	—	—	—	—
Lutein/zeaxanthin	0.24*	0.53*	0.29*	0.19*	—	—	—	—
Retinol	0.09†	0.18*	0.08†	0.12*	0.23*	—	—	—
Vitamin C	0.13*	0.37*	0.31*	0.17*	0.19*	0.06	—	—
$\alpha$ -Tocopherol	-0.03	0.13*	0.23*	0.12*	0.23*	0.39*	0.15*	—
$\gamma$ -Tocopherol	-0.04	0.02	-0.001	0.04	0.21*	0.16*	-0.06	0.28*

\* $P < 0.01$ .† $P < 0.05$ .

The associations of other micronutrients including  $\beta$ -cryptoxanthin, lutein/zeaxanthin, retinol, vitamin C,  $\alpha$ -tocopherol, and  $\gamma$ -tocopherol with gastric cancer risk were all statistically nonsignificant (all  $P$ s for trend  $> 0.05$ ) before as well as after adjustment for potential confounders.

Table 5 shows the relative risk of gastric cancer in relation to quartile levels of serum micronutrients stratified by the joint status of cigarette smoking and alcohol consumption. Fifty-six gastric cancer patients and 227 control subjects neither smoked cigarettes over lifetime nor consumed  $\geq 3$  drinks of alcohol per day. Among them, increased levels of  $\beta$ -carotene and vitamin C were associated with significantly reduced risk of gastric cancer. Multivariate-adjusted ORs (95% CI) for gastric cancer associated with the highest quartile of  $\beta$ -carotene and vitamin C were 0.31 (0.13-0.75) and 0.39 (0.15-0.98), respectively, compared with the lowest quartile of respective micronutrients (both  $P$ s for trend  $< 0.05$ ). A reduced risk of gastric cancer also was observed for increased levels of  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, and retinol although the inverse associations were not statistically significant. No associations between tocopherols and gastric cancer risk was observed among nonsmokers and nondrinkers or light drinkers. Among men who either smoked cigarettes regularly or consumed at least 3 drinks of alcohol per day (135 cases and 343 controls), the micronutrient-gastric cancer associations all were statistically nonsignificant (all  $P$ s for trend  $\geq 0.39$ ; Table 5).

We also examined the associations between serum levels of micronutrients and gastric cancer risk stratified by positivity of serum antibodies to *H. pylori* and urinary

epigallocatechin. There were small numbers of subjects with negative *H. pylori* antibodies (20 cases and 98 controls) or negative urinary epigallocatechin (38 cases and 104 controls). We did not detect any significant modifying effects of *H. pylori* infection and urinary epigallocatechin on the micronutrient-gastric cancer associations (data not shown).

Serum vitamin C was correlated with  $\beta$ -carotene (Pearson correlation coefficient = 0.31). After adjustment for vitamin C, the multivariate-adjusted OR (95% CI) for gastric cancer in the second, third, and fourth quartile of  $\beta$ -carotene were 0.77 (0.33-1.80), 0.61 (0.26-1.42), and 0.39 (0.15-1.00), respectively, compared with the lowest quartile of  $\beta$ -carotene among those who neither smoked cigarettes nor consumed  $\geq 3$  drinks of alcohol ( $P$  for trend = 0.04). On the other hand, the corresponding figures for vitamin C was 0.75 (0.30-1.92), 0.45 (0.17-1.19), and 0.54 (0.20-1.45;  $P$  for trend = 0.14) after adjustment for  $\beta$ -carotene.

Incident gastric cancer cases diagnosed within a short period after entry into the study might have changed their dietary habits already (because of disease symptoms) at the time of blood collection. Therefore, we repeated all of the analyses after excluding cases ( $n = 32$ ) diagnosed during the first 2 years of follow-up and their matched controls. Results were similar to those based on the entire data set. Among total study subjects, the multivariate-adjusted OR (95% CI) for gastric cancer associated with the highest quartile of  $\beta$ -carotene and lycopene were 0.62 (0.35-1.11) and 0.55 (0.28-1.06), respectively, relative to the lowest quartile of respective micronutrients ( $P$  for trend = 0.08 for  $\beta$ -carotene and 0.09 for lycopene). Among men who neither smoked

**Table 3. Geometric mean concentrations (95% CI) of prediagnostic serum micronutrients in gastric cancer patients and control subjects, Shanghai Cohort Study, 1986-1998**

Micronutrients	Cancer patients ( $n = 191$ )	Control subjects ( $n = 570$ )	$P^*$
$\alpha$ -Carotene ( $\mu\text{g/dL}$ )	0.54 (0.47-0.60)	0.64 (0.60-0.68)	0.006
$\beta$ -Carotene ( $\mu\text{g/dL}$ )	7.9 (7.2-8.7)	9.1 (8.7-9.6)	0.01
$\beta$ -Cryptoxanthin ( $\mu\text{g/dL}$ )	2.7 (2.4-2.9)	2.8 (2.7-3.0)	0.30
Lycopene ( $\mu\text{g/dL}$ )	2.2 (1.9-2.4)	2.5 (2.3-2.7)	0.03
Lutein/zeaxanthin ( $\mu\text{g/dL}$ )	29.9 (28.4-31.5)	29.4 (28.5-30.3)	0.57
Retinol ( $\mu\text{g/dL}$ )	46.5 (44.7-48.3)	47.0 (45.9-48.0)	0.65
Vitamin C (mg/L)	5.5 (5.0-6.0)	5.9 (5.7-6.2)	0.12
$\alpha$ -Tocopherol (mg/L)	8.6 (8.2-9.0)	8.6 (8.3-8.8)	0.77
$\gamma$ -Tocopherol (mg/L)	1.7 (1.6-1.8)	1.6 (1.5-1.7)	0.18

\*Derived from ANOVA, which retained a matched set consisting of three control subjects individually matched to the index case by age, month and year of biospecimen collection, and neighborhood of residence at recruitment.

**Table 4. Serum micronutrient levels in relation to risk of gastric cancer, Shanghai Cohort Study, 1986-1998**

Micronutrients	Quartile*				P for trend
	First	Second	Third	Fourth	
$\alpha$ -Carotene					
OR (95% CI) <sup>†</sup>	1.00	0.63 (0.22-1.83)	0.54 (0.19-1.55)	0.38 (0.13-1.11)	0.04
Adjusted OR (95% CI) <sup>‡</sup>	1.00	0.55 (0.19-1.59)	0.52 (0.18-1.50)	0.39 (0.13-1.14)	0.09
$\beta$ -Carotene					
OR (95% CI) <sup>†</sup>	1.00	0.74 (0.47-1.16)	0.58 (0.36-0.95)	0.54 (0.32-0.89)	0.01
Adjusted OR (95% CI) <sup>‡</sup>	1.00	0.81 (0.51-1.29)	0.64 (0.40-1.05)	0.62 (0.37-1.06)	0.05
$\beta$ -Cryptoxanthin					
OR (95% CI) <sup>†</sup>	1.00	0.84 (0.51-1.39)	0.70 (0.41-1.21)	0.80 (0.47-1.38)	0.41
Adjusted OR (95% CI) <sup>‡</sup>	1.00	0.90 (0.53-1.52)	0.74 (0.43-1.30)	0.90 (0.51-1.58)	0.66
Lycopene					
OR (95% CI) <sup>†</sup>	1.00	0.85 (0.51-1.41)	0.73 (0.43-1.25)	0.55 (0.30-1.00)	0.05
Adjusted OR (95% CI) <sup>‡</sup>	1.00	0.89 (0.53-1.49)	0.80 (0.46-1.37)	0.63 (0.34-1.15)	0.13
Lutein/zeaxanthin					
OR (95% CI) <sup>†</sup>	1.00	1.19 (0.75-1.89)	0.96 (0.59-1.58)	1.10 (0.67-1.81)	0.91
Adjusted OR (95% CI) <sup>‡</sup>	1.00	1.20 (0.75-1.92)	0.96 (0.57-1.60)	1.12 (0.67-1.86)	0.88
Retinol					
OR (95% CI) <sup>†</sup>	1.00	0.97 (0.62-1.52)	0.85 (0.53-1.36)	0.88 (0.54-1.44)	0.51
Adjusted OR (95% CI) <sup>‡</sup>	1.00	0.97 (0.62-1.53)	0.83 (0.51-1.34)	0.86 (0.52-1.43)	0.45
Vitamin C					
OR (95% CI) <sup>†</sup>	1.00	1.02 (0.65-1.58)	0.82 (0.50-1.33)	0.72 (0.44-1.19)	0.14
Adjusted OR (95% CI) <sup>‡</sup>	1.00	1.19 (0.76-1.88)	0.97 (0.58-1.60)	0.92 (0.55-1.55)	0.57
$\alpha$ -Tocopherol					
OR (95% CI) <sup>†</sup>	1.00	0.94 (0.58-1.52)	1.11 (0.68-1.83)	1.26 (0.76-2.09)	0.28
Adjusted OR (95% CI) <sup>‡</sup>	1.00	0.96 (0.59-1.58)	1.10 (0.66-1.81)	1.23 (0.74-2.06)	0.36
$\gamma$ -Tocopherol					
OR (95% CI) <sup>†</sup>	1.00	1.11 (0.69-1.77)	1.01 (0.62-1.65)	1.18 (0.70-1.97)	0.65
Adjusted OR (95% CI) <sup>‡</sup>	1.00	1.14 (0.70-1.83)	1.10 (0.67-1.81)	1.19 (0.70-2.00)	0.57

\*See Appendix 1 for quartile cut points.

<sup>†</sup>ORs were derived from conditional logistic regression models that retained a matched set consisting of three control subjects individually matched to the index case by age, month and year of biospecimen collection, and neighborhood of residence at recruitment.

<sup>‡</sup>Further adjusted for cigarette smoking (never or ever), alcohol consumption (<3 or  $\geq$ 3 drinks per day), positivity of urinary epigallocatechin (no or yes), and seropositivity of *H. pylori* antibodies (no or yes).

cigarettes over lifetime nor consumed  $\geq$ 3 drinks of alcohol per day, multivariate-adjusted OR (95% CI) for gastric cancer in the second, third, and fourth quartiles of  $\beta$ -carotene were 0.65 (0.25-1.71), 0.44 (0.17-1.14), and 0.28 (0.10-0.75), respectively, compared with the lowest quartile (*P* for trend = 0.008). The corresponding figures for vitamin C were 0.67 (0.24-1.92), 0.40 (0.14-1.17), and 0.42 (0.15-1.19; *P* for trend = 0.068).

Of the 191 gastric cancer patients, 44 (23%) had cardia cancer, 117 (61%) had noncardia cancer, and 30 (16%) had unknown subsite. Risk estimates by subsite were relatively unstable because of small sample sizes on stratification. Nonetheless, we examined the associations between micronutrients and cardia/noncardia cancer risk and did not detect any meaningful variations in disease risk by subsite (data not shown).

## Discussion

To our knowledge, the present study is the first to examine the associations between prediagnostic serum levels of specific carotenoids other than  $\beta$ -carotene and risk of gastric cancer. A statistically significant inverse association between prediagnostic serum level of  $\beta$ -carotene and gastric cancer risk was observed even after adjustment for *H. pylori* infection, cigarette smoking, heavy alcohol consumption, and ingestion of tea polyphenols.  $\beta$ -Carotene has shown to reduce carcino-

gen-induced gastric tumor in experimental rodents by >50% (19, 20). A decreased level of serum  $\beta$ -carotene was observed in patients with more advanced precancerous gastric lesions (intestinal metaplasia and/or dysplasia) than those with less advanced lesions (superficial gastritis and/or chronic atrophic gastritis) in a high-risk population in northern China (21). Four cohort studies examined the relationship between blood total or  $\beta$ -carotene and gastric cancer risk (22-25). Three of the four studies reported a lower baseline blood level of  $\beta$ -carotene in persons developing gastric cancer than those who remained free of cancer during follow-up (22, 24, 25), but only one of the three studies showed a statistically significant difference in  $\beta$ -carotene concentration between cases and controls (25). The other study did not find an inverse association between serum  $\beta$ -carotene and gastric cancer risk (23). All four studies were conducted in low-risk Western populations that had high serum levels of  $\beta$ -carotene and low incidence rate of gastric cancer. Furthermore, all those studies had a small sample sizes (the number of gastric cancer of all four studies combined is 187, fewer than that of the present study) and did not account for all potential confounding factors, particularly *H. pylori* infection and exposure to other antioxidants such as tea polyphenols. Intervention trials of  $\beta$ -carotene on the protection against the development of gastric dysplasia and cancer showed different results in high-risk versus low-risk populations. Supplementation with 20 mg/d of  $\beta$ -carotene for 5 years

**Table 5. Adjusted\* ORs (95% CI) for gastric cancer associated with serum micronutrient levels stratified by the status of cigarette smoking and alcohol consumption, Shanghai Cohort Study, 1986-1998**

Micronutrients	Quartile <sup>†</sup>				P for trend
	First	Second	Third	Fourth	
$\alpha$ -Carotene					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	0.49 (0.18-1.39)	1.19 (0.50-2.84)	0.43 (0.15-1.22)	0.35
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	1.24 (0.60-2.31)	0.84 (0.42-1.67)	0.98 (0.46-2.09)	0.62
$\beta$ -Carotene					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	0.68 (0.30-1.55)	0.53 (0.24-1.21)	0.31 (0.13-0.75)	0.008
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	0.93 (0.54-1.59)	0.77 (0.43-1.38)	0.99 (0.55-1.76)	0.76
$\beta$ -Cryptoxanthin					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	0.58 (0.23-1.47)	0.56 (0.22-1.42)	0.46 (0.18-1.14)	0.13
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	1.16 (0.65-2.04)	0.87 (0.47-1.62)	1.35 (0.73-2.50)	0.54
Lycopene					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	0.66 (0.27-1.59)	0.46 (0.18-1.14)	0.59 (0.23-1.51)	0.22
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	1.27 (0.72-2.26)	1.34 (0.74-2.42)	0.86 (0.44-1.66)	0.82
Lutein/zeaxanthin					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	1.14 (0.49-2.66)	0.67 (0.27-1.62)	0.85 (0.37-1.93)	0.45
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	1.31 (0.75-2.31)	1.21 (0.68-2.17)	1.34 (0.75-2.41)	0.39
Retinol					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	1.06 (0.50-2.27)	0.80 (0.34-1.84)	0.47 (0.19-1.18)	0.09
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	0.94 (0.53-1.68)	0.80 (0.44-1.45)	1.02 (0.57-1.82)	0.94
Vitamin C					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	0.69 (0.28-1.70)	0.36 (0.14-0.94)	0.39 (0.15-0.98)	0.02
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	1.31 (0.76-2.24)	1.26 (0.71-2.24)	1.22 (0.66-2.27)	0.50
$\alpha$ -Tocopherol					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	1.15 (0.47-2.83)	1.48 (0.60-3.64)	1.36 (0.57-3.24)	0.42
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	0.82 (0.45-1.49)	0.87 (0.49-1.55)	1.07 (0.60-1.89)	0.77
$\gamma$ -Tocopherol					
Nonsmokers and <3 drinks per day <sup>‡</sup>	1.00	1.39 (0.53-3.63)	1.59 (0.66-3.84)	1.26 (0.50-3.20)	0.60
Ever smokers or $\geq$ 3 drinks per day <sup>§</sup>	1.00	1.00 (0.57-1.76)	0.87 (0.47-1.59)	1.18 (0.67-2.06)	0.67

\*All ORs were adjusted for age, year of biospecimen collection, neighborhood of residence at recruitment, batch of assays for serum micronutrients, positivity of urinary epigallocatechin (no or yes), and seropositivity of *H. pylori* antibodies (no or yes).

<sup>†</sup>See Appendix 1 for quartile cut points.

<sup>‡</sup>Including 56 gastric cancer patients and 227 control subjects who neither smoked cigarettes over lifetime nor consumed  $\geq$ 3 drinks of alcohol per day.

<sup>§</sup>Including 135 gastric cancer patients and 343 control subjects who either smoked cigarettes or consumed at least 3 drinks of alcohol per day. ORs were further adjusted for cigarette smoking (never or ever) and alcohol consumption (<3 or  $\geq$ 3 drinks per day).

had no effect on the development of gastric dysplasia or carcinoma among Finnish men, a low-risk population for gastric cancer (26). On the other hand, supplementation with 30 mg/d of  $\beta$ -carotene for 6 years resulted in a statistically significant increase in the rate of regression from more advanced lesions (intestinal metaplasia) to less advanced lesions (nonmetaplastic atrophy) as well as a decrease in the rate of progression from less advanced lesions (intestinal metaplasia) to more advanced gastric lesions (dysplasia or carcinoma) in Colombia, a high-risk area for gastric cancer (27). Similarly, supplementation with 50 mg of  $\beta$ -carotene on every other day to male physicians in the United States for 12 years or 20 mg/d to adult men in Finland showed no effect on prevention of gastric cancer incidence (28, 29). On the other hand, Chinese in Linxian, China, one of the areas with the world's highest incidence rates of esophageal and gastric cancers, given 15 mg/d of  $\beta$ -carotene for 5 years experienced a statistically significant 21% reduction in risk of developing gastric cancer, although the protective effect was unable to attribute to  $\beta$ -carotene alone due to the treatment agent combined with selenium and  $\alpha$ -tocopherol (30). Those data along with ours suggest that  $\beta$ -carotene exerts protective effect on gastric cancer in high-risk populations in which a low serum level of  $\beta$ -carotene is present. The baseline serum concentration of  $\beta$ -carotene was 9.1  $\mu$ g/dL in the present study

population and 6.9  $\mu$ g/dL in participants of the Linxian trial discussed above, less than half of people in the United States and Europe (25, 28, 31, 32). It is conceivable that the protection of  $\beta$ -carotene against gastric cancer exists in populations with insufficient dietary  $\beta$ -carotene.

The present study showed an inverse association between serum level of lycopene and gastric cancer risk. The inverse lycopene-gastric cancer association strengthened after excluding cases of gastric cancer diagnosed within 2 years of follow-up. Lycopene seems to be the most efficient quencher of singlet oxygen and free radicals among the common carotenoids *in vitro* (33, 34). Experimental study showed that administration of lycopene completely inhibited the development of carcinogen-induced gastric cancer in rats and that the anticarcinogenic effect might be through its antioxidative property (35). Tomato and tomato-based products are the predominant sources of lycopene. Numerous case-control studies have consistently shown an inverse association between tomato intake and risk of gastric cancer across various populations with a wide range of incidence rates (36). Our results along with findings by others support a protective role of lycopene in risk of gastric cancer.

Ascorbic acid (a reduced form of vitamin C) has shown to inhibit endogenous nitrosation in humans (37); endogenously formed nitrosamines are postulated

carcinogens for the stomach in humans (5). Clinical studies have shown a stepwise decrease in the ascorbic acid concentration in gastric juice of persons with the histologic changes from normal to chronic gastritis, atrophy, and intestinal metaplasia (38). Similarly, serum level of ascorbic acid was significantly lower in patients with advanced gastric lesions (intestinal metaplasia) than less advanced gastric lesions (superficial gastritis or chronic atrophic gastritis; ref. 21). A statistically significant inverse association between baseline serum vitamin C level and risk of progression from gastric intestinal metaplasia to dysplasia and/or carcinoma or from dysplasia to carcinoma was observed in a high-risk population of northern China after >4 years of follow-up (39). Case-control studies revealed that gastric juice concentration of ascorbic acid were statistically significant lower in patients with advanced gastric lesions (atrophic gastritis, intestinal metaplasia, dysplasia, and carcinoma) than those with normal gastric mucosa (40, 41). A prospective cohort study observed a statistically significant inverse association between dietary intake of vitamin C and gastric cancer in the Netherlands after >6 years of follow-up (8). There has been only one prospective study examining the association between serum vitamin C and risk of gastric cancer. The study was conducted in a cohort of ~3,000 men in Switzerland and included only 28 gastric cancer patients after 17 years of follow-up. Gastric cancer patients had a statistically nonsignificant lower level of vitamin C than cohort participants who remained free of cancer (25). Similarly, the present study showed a statistically nonsignificant inverse association between serum vitamin C level and gastric cancer risk in total subjects. However, a statistically significant reduced risk of gastric cancer associated with increased level of vitamin C was seen among men who neither smoked cigarettes over lifetime nor consumed  $\geq 3$  drinks of alcohol per day. Cigarette smokers are exposed to high levels of free radicals in cigarette smoke. High consumption of alcohol can lead to increased generation of free radicals and oxidative stress (42). The present study showed that smokers and drinkers had significantly lower serum levels of carotenoids and vitamin C, reflecting their lower ingestion of these micronutrients (5-10% lower;  $P = 0.03-0.12$  for the differences) and greater exhaustion of *in vivo* antioxidants by free radicals than nonsmokers and nondrinkers, respectively. It is conceivable that smokers and heavy alcohol drinkers may require an increased amount of antioxidants such as vitamin C to protect themselves from oxidative damage to DNA and other cellular structures by free radicals related to smoking and drinking. This could be one of the explanations that the present study did not find a protective effect of vitamin C on gastric cancer in cigarette smokers and heavy alcohol drinkers, especially in a population generally possessing a relatively low serum level of vitamin C (5.9 mg/L) compared with their counterparts in the United States (12.3 mg/L; ref. 43). Alternatively, serum vitamin C might merely be a surrogate of fruit and vegetable intake. In fact, the present study also showed a reduced risk of gastric cancer in subjects with high levels of  $\beta$ -carotene and lycopene that are abundant in fruit and vegetables rich in vitamin C. It is possible that the observed association between vitamin C and gastric cancer was a marker of as-yet unidentified chemopreventive agent in fruit and vegetables.

Limited number of epidemiologic studies have examined the associations between dietary intakes of  $\beta$ -cryptoxanthin and lutein/zeaxanthin and gastric cancer. An ecologic study did not find a correlation between serum levels of lutein and zeaxanthin and gastric cancer mortality across different regions with various incidence rates of gastric cancer in Japan (44). Two case-control and one cohort studies have examined the relationships between dietary intakes of  $\beta$ -cryptoxanthin and/or lutein/zeaxanthin and gastric cancer. None of them found a statistically significant association (8, 45, 46). There have been no studies examining the associations between prediagnostic serum levels of  $\beta$ -cryptoxanthin and lutein/zeaxanthin and risk of gastric cancer. We reported previously a statistically significant inverse association between serum levels of  $\beta$ -cryptoxanthin and lutein/zeaxanthin and risk of lung cancer in our study population (47). This is the first study to examine the associations between these two carotenoids and gastric cancer. Although an elevated level of prediagnostic serum  $\beta$ -cryptoxanthin was associated with a reduced risk of gastric cancer, particularly among men who were neither smokers nor heavy drinkers, the inverse association was not statistically significant. We did not observe an inverse association between serum level of lutein/zeaxanthin and gastric cancer risk in our study population. Limited data accumulated thus far do not support a strong protection of dietary  $\beta$ -cryptoxanthin and lutein/zeaxanthin against the development of gastric cancer.

Several studies have examined the association between prediagnostic serum levels of retinol and gastric cancer risk (23-25, 48). Consistent with the findings of the present study, none of those previous studies found a statistically significant protective or risk effect of retinol on the development of gastric cancer.

Several studies also examined the association between prediagnostic serum levels of vitamin E (mainly  $\alpha$ -tocopherol) and gastric cancer (24, 25, 49, 50). In general, those studies showed a null or weak inverse association. In a recent report, on the other hand, a statistically significant positive association between baseline serum level of  $\alpha$ -tocopherol and gastric cancer was observed in participants of the intervention trial in Linxian, China. The study did not observe a significant relationship between  $\gamma$ -tocopherol and gastric cancer risk (51). Data from an intervention trial did not show a preventive effect of  $\alpha$ -tocopherol on the development of gastric cancer in Finnish smokers (32). Consistent with most of previous studies, our study did not show a protective or risk effect of tocopherols on gastric cancer in this high-risk population.

The strengths of the present study include (a) the prospective study design and the availability of prediagnostic serum specimens, minimizing the possible influence of disease symptoms on dietary intake of various micronutrients and other lifestyle factors; (b) the measurements of multiple serum micronutrients, allowing for simultaneous evaluations of the relationships between measured micronutrients and gastric cancer risk; (c) the comprehensive measurements of potential confounders, minimizing the possible confounding effects on the micronutrient-disease associations; (d) the size of the cohort (>18,000 men) with up to 12 years of follow-up, providing relatively high statistical power to detect

**Appendix 1: Quartile cut-points of serum concentrations of various micronutrients, Shanghai Cohort Study, 1986-1998**

Micronutrient	Quartile			
	First	Second	Third	Fourth
α-Carotene (μg/dL)*	ND (77/216) <sup>†</sup>	<0.8 (46/123)	0.9-1.4 (38/112)	>1.4 (30/119)
β-Carotene (μg/dL)	<6.0 (64/143)	6.0-9.4 (50/144)	9.5-14.8 (39/140)	>14.8 (38/143)
β-Cryptoxanthin (μg/dL)	<1.8 (53/139)	1.8-2.9 (48/142)	3.0-4.3 (42/145)	>4.3 (48/144)
Lycopene (μg/dL)	<1.2 (57/148)	1.2-2.5 (51/140)	2.6-4.6 (45/139)	>4.6 (38/143)
Lutein/zeaxanthin (μg/dL)	<23.0 (46/146)	23.0-30.1 (53/140)	30.2-38.3 (43/142)	>38.3 (49/142)
Retinol (μg/dL)	<38.9 (52/144)	38.9-47.6 (50/143)	47.7-57.0 (43/140)	>57.0 (46/143)
Vitamin C (mg/L)	<3.4 (54/145)	3.4-5.3 (55/143)	5.4-8.4 (43/140)	>8.4 (39/142)
α-Tocopherol (mg/L)	<7.0 (45/141)	7.0-8.3 (42/142)	8.4-10.5 (49/143)	>10.5 (55/144)
γ-Tocopherol (mg/L)	<1.1 (44/140)	1.1-1.5 (50/144)	1.6-2.1 (45/142)	>2.1 (42/144)

\*ND, nondetectable; the cut points were the first, second, and third tertiles among all controls with detectable serum level of α-carotene.

<sup>†</sup>Number of cases/number of controls in parentheses.

moderate effects of micronutrients on gastric cancer occurrence; (e) almost complete follow-up [only 207 (1.1%) subjects were lost to follow-up by the cutoff date of the present study], minimizing the possibility of selection bias; and (f) the relatively low dietary intake levels of certain micronutrients in the study population, providing an opportunity to examine these associations with gastric cancer in the lower ranges of exposure levels. The chief limitation of the present study is the potential degradation of vitamin C in nonacidified serum with long-term storage. However, the comparison of vitamin C levels between cases and their individually matched controls was valid because the time interval between sample collection and testing was one of the matching variable, with no more than 2 months of difference between the index case and any of his matched control subjects. If the degradation of vitamin C indeed existed and such degradation was greater in sera with high than those with low level of vitamin C, this would lead to an underestimate of effect of vitamin C on gastric cancer risk.

In summary, the present study showed statistically significant inverse associations between prediagnostic serum levels of carotenes and lycopene and gastric cancer development. The inverse carotene-gastric cancer association was independent of *H. pylori* infection, cigarette smoking, heavy alcohol consumption, and exposure to tea polyphenol. Our data also implicated a protective role of vitamin C in the development of gastric cancer especially among men who were neither smokers nor heavy drinkers. The present study gives additional credence to prior experimental and epidemiologic data in support of the hypothesis that dietary carotenes, lycopene, and vitamin C are potential chemopreventive agents for gastric cancer in humans.

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