The Relation of Serum Levels of Antioxidant Vitamins C and E, Retinol and Carotenoids with Pulmonary Function in the General Population

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Reduced pulmonary function is an important predictor of mortality in the general population, and antioxidant vitamins are thought to positively influence pulmonary function. Vitamin C, vitamin E, retinol, and carotenoids are powerful antioxidants but information about the joint relation of serum levels of these antioxidants to pulmonary function is limited. We analyzed the association of FEV_1 and FVC with serum vitamins C and E, retinol, and carotenoids (β-cryptoxanthin, lutein/zeaxanthin, β-carotene, and lycopene) in a cross-sectional study. The analysis was carried out in a sample of 1,616 randomly selected residents of Western New York, USA, age 35 to 79 yr and free of respiratory disease. Lung function was adjusted for height, age, sex, and race and expressed as percentage of predicted normal FEV_1 (FEV_1%) and FVC (FVC%). Participants in the lowest quartile of each of the serum antioxidants had consistently lower FEV_1% and FVC% than those in higher quartiles. Multiple linear regression analysis revealed significant associations of vitamin C, vitamin E, β-cryptoxanthin, lutein/zeaxanthin, β-carotene, and retinol with FEV_1% when these variables were investigated individually after adjustment for other covariates (smoking status, pack-years of smoking, weight, eosinophil count, and education). When all of these antioxidant vitamins were analyzed simultaneously in a multivariate regression model, the strongest association was seen with vitamin E and β-cryptoxanthin. Only retinol showed an independent effect on FEV_1% after controlling for vitamin E and β-cryptoxanthin. As for FEV_1%, vitamin E and β-cryptoxanthin were most strongly related to FVC% when all variables were considered in the multivariate regression model. The differences in FEV_1 associated with a reduction of one standard deviation of serum vitamin E or β-cryptoxanthin were equivalent to the negative influence of approximately 1 to 2 yr of aging. Our findings support the hypothesis that antioxidant vitamins may play a role in respiratory health and that vitamin E and β-cryptoxanthin appear to be stronger correlates of lung function than other antioxidant vitamins.

Reduced pulmonary function is an important predictor of mortality in the general population (1, 2). The factors that influence pulmonary function, however, have not been completely understood, and oxidant exposure has been suggested to be a potential mechanism linking reduced pulmonary function to mortality (3, 4). The role of the balance between the oxidative burden and the body antioxidant potential in the pathogenesis of airway obstruction has been recently the focus of investigation (5, 6).

Vitamin C and vitamin E are powerful antioxidants found in the lung where they protect against oxidative damage (7). Although vitamin E is predominantly membrane bound, there is a close interaction between vitamins C and E (8), because vitamin C not only functions directly as an antioxidant, but it also recycles the antioxidant capacity of oxidized vitamin E (9). Retinol (vitamin A) and carotenoids, because of their anti-inflammatory and antioxidant activity (10, 11), have been investigated in pulmonary disease for many years (12–15). These compounds have been thought to protect against development of lung cancer and other respiratory illnesses (12, 16).

There are several studies that have analyzed the association between dietary intake of these antioxidant vitamins and respiratory function (17). A large number of studies have found positive associations between pulmonary function and intake of vitamin C or foods high in vitamin C content (i.e., fresh fruit and vegetable) (18–22). Fewer studies have analyzed the relation of dietary vitamin E intake with pulmonary function and have reported contradictory findings (19, 20).

Two large prevention trials in high-risk populations found an increased lung cancer risk in the arm receiving large doses of β-carotene supplementation (23, 24). In contrast to these discouraging results, serum β-carotene at baseline was positively associated with lung function in one of these trials (25) and in a cross-sectional study β-carotene intake was positively associated with pulmonary function (19). Several reasons for this discrepancy have been hypothesized (26), and it is not clear whether the observed association between β-carotene intake and pulmonary function is, in fact, related to β-carotene itself or whether other carotenoids and nutrients correlated with β-carotene intake may be involved. To date, more than 600 carotenoids have been described and many of them (e.g., β-cryptoxanthin, lutein/zeaxanthin, β-carotene, and lycopene) are known to have strong antioxidant activity (11). If carotenoids other than β-carotene, not yet well studied, would play a role in antioxidant defense mechanisms, differences between associations of β-carotene supplementation and dietary intake with lung health could, at least in part, be explained.

Studies focusing on dietary intake leave uncertainty as to the real nature of the association between these antioxidants and lung function because intake of food items rich in antioxidant vitamins is associated with other life-style habits that may influence lung function (27). Therefore, the measurement of antioxidant vitamin serum levels has been advocated to analyze this association (13, 15, 28). Only few studies have investigated blood levels of vitamins C and E in relation to lung function; they found inconsistent results. These studies, for the most part, have focused on small or selected samples (14, 21, 22, 29,

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30). There is still considerable uncertainty about the association of serum antioxidant vitamins C and E with lung function. Moreover, the interrelation of serum vitamin C with vitamin E and their association with pulmonary function have not been investigated contemporaneously in epidemiologic studies.

Few epidemiologic studies have considered serum carotenoids in relation to lung function (14, 25, 29, 31), and only in one study, conducted in a sample of Dutch elderly, were several antioxidant carotenoids measured (30). In this latter report α-carotene, β-carotene, and lycopene had the strongest relation to lung function, but α-carotene and lycopene serum levels were lower than in the United States.

No study has simultaneously investigated the relation of vitamin C, vitamin E, and carotenoids in serum. The present study had the following aims: to analyze the association of FVC and FEV_1 with vitamin C and E serum levels in a large random sample of the general population and to describe the relation of serum vitamin C, vitamin E, retinol, and several potent antioxidant carotenoids (β-cryptoxanthin, lutein/zeaxanthin, β-carotene, and lycopene) with pulmonary function (FEV_1 and FVC).

METHODS

The present study focuses on a general population sample from Erie and Niagara Counties, New York, recruited as part of an ongoing series of population-based case-control studies. Residents of Erie and Niagara counties age 35 to 64 yr were randomly selected from the New York State Department of Motor Vehicles records. Participants age 65 to 79 yr were randomly selected from the rolls of the Health Care Finance Association.

We assigned a computer-generated random number to each person on the complete lists of all potential participants supplied by the New York State Department of Motor Vehicles and the Health Care Finance Association. The potential participants were then sorted with ascending numbers according to their randomly assigned number and contacted in sequential fashion. Introductory letters were mailed to potential interviewees before to interviewers' telephone calls. Up to 12 callbacks were made. Of the first 4,946 eligible subjects we contacted, 2,409 (48.7%) refused to participate; we were able to interview 2,537 (51.3%; 1,322 female, 1,215 male).

In the presented analysis, participants were excluded for the following reasons: race other than white or African-American (n = 33, 1.3%); missing information on height, weight, smoking status, or education (n = 185, 6.2%); missing blood determination of vitamin C, fat soluble vitamins, or triglycerides (n = 94, 3.7%, including participants who refused to give blood samples); missing pulmonary function tests as a result of randomly occurring absence of the responsible personnel or home-based interviews (n = 257, 10.1%). Pulmonary function tests were unacceptable or not reproducible in 109 (5.5%) of the remaining 1,968 participants. We further excluded 240 participants who reported a diagnosis of asthma or chronic obstructive pulmonary disease (COPD) (including chronic bronchitis and emphysema) and three with a history of lung fibrosis.

The excluded participants for whom information was available were similar to included participants in age, height, weight, triglyceride levels, serum vitamin E, retinol, and β-carotene and the distribution of sex and race categories. Serum vitamin C levels (1.14 versus 1.26 mg/dl, $p < 0.05$) and carotenoid levels other than β-carotene were slightly lower in the excluded participants (β-cryptoxanthin: 0.089 versus 0.098 μg/dl; lutein/zeaxanthin: 0.138 versus 0.150 mg/dl; lycopene: 0.432 versus 0.480 μg/dl, $p < 0.05$ for all of these serum antioxidants). Excluded participants were more likely to be current smokers (19.2% versus 13.7%, $p < 0.05$). Approximately 26.4% of the excluded participants were excluded because of an underlying history of asthma or COPD and had lower pulmonary function levels than the included participants (FEV_1 2.69 versus 2.95 L and FVC 3.62 versus 3.83 L, both $p < 0.05$).

Interview

The examination included both an in-person interview that addressed a number of life-style habits, including questions on the duration and intensity of lifetime cigarette smoking, and a self-administered questionnaire. The questionnaire included questions on education, medical history, and vitamin supplement use. Before the interview all answers in the questionnaire were reviewed by trained personnel and participants were asked to clarify any uncertain aspects.

Pulmonary Function Tests

Spirometry was performed between 6:30 and 9:30 AM by trained personnel according to the American Thoracic Society (ATS) guidelines on the Standardization of Spirometry, 1994 update (32), using a Vitalograph Compact for the first 562 participants and a 2170 Spirometer for all other participants (both from Vitalograph Medical Instruments, Lenexa, Kansas). The spirometers were calibrated daily with a 3-L syringe before testing. The procedures and test maneuver were explained in detail to the participant. Two to three slow practice maneuvers were performed. Then at least three technically acceptable and reproducible FVC maneuvers were performed (4). All pulmonary function results were reported as BTPS volumes. Following the current standard, reproducibility criteria were met if the difference between the two best FVC and the two best FEV_1 maneuvers was ⩽ 200 ml. A maximum of eight test maneuvers were performed (32). The current ATS guidelines recommend that FEV_1 is backextrapolated to determine the start of the test (32); however, older devices such as the Vitalograph Compact used do not report backextrapolated results. Therefore, we made adjustments for differences in FEV_1 between the two spirometers based on the mean backextrapolated volume in percentage of the FVC obtained with the Vitalograph 2170 device. The backextrapolated volume was 3.13% (SD 1.3%) of the FVC and adjustment for this factor was made. Our estimates are lower than the 4.35% (SD 2.71%) reported by Knudson and coworkers (33), indicating that the test effort was uniformly good and that the backextrapolated volume was small. We also performed a sequence of tests to quantify the difference in FEV_1 between the two spirometers caused by backextrapolation under controlled laboratory conditions. In a series of 34 blows with a calibrated 3-L syringe, the FEV_1 was 3.36% (SD 2.55%) lower using the Vitalograph Compact device in series with the 2170 model. This difference was not significantly different from the factor (3.13%) we used to adjust for backextrapolation.

FEV_1 and FVC prediction equations were calculated for men and women separately with values obtained from the included participants who were lifelong nonsmokers. The following equations were obtained for men:

$$\text{predicted } FEV_1 = -0.550 - 0.0359 \times \text{age (years)} + 3.603 \times \text{height (m)} - 0.478 \times \text{race}$$

$$\text{predicted FVC} = -2.229 - 0.0382 \times \text{age (years)} + 5.210 \times \text{height (m)} - 0.739 \times \text{race}$$

and for women:

$$\text{predicted } FEV_1 = -0.184 - 0.0298 \times \text{age (years)} + 2.829 \times \text{height (m)} - 0.309 \times \text{race}$$

$$\text{predicted FVC} = -1.413 - 0.0318 \times \text{age (years)} + 4.104 \times \text{height (m)} - 0.514 \times \text{race}$$

A dummy variable was used for race (white = 0, African-American = 1). These coefficients were derived from pulmonary function records of 267 men and 427 women who never smoked and did not report a history of chronic lung disease. The age and height coefficients and the intercept are in agreement with other published prediction equations (34). Inclusion of nonlinear prediction terms for height or age did not significantly improve prediction of FEV_1 or FVC. FEV_1 in percentage of the predicted values (FEV_1%) and FVC in percentage of the predicted values (FVC%) were calculated for all participants.

Blood Determinations

Blood samples were obtained between 7:30 and 9:30 A.M. after a fasting of 8 to 12 h. Vitamin measurements were made in the laboratory of the Department of Clinical Laboratory Sciences, School of Health Related Sciences, University at Buffalo, New York. Fat-soluble vitamins were measured in serum by high-pressure liquid chromatography (HPLC) on a Shimadzu LC-7A device with SPD-M6A photodiode array (Shimadzu Scientific Instruments, Inc., Braintree, MA) and expressed as μg/ml (35). Quality control of the HPLC determination of fat-soluble vitamins (α-tocopherol) was maintained through participation in the Micronutrients Measurement Quality Assurance

TABLE 1. CHARACTERISTICS OF PARTICIPANTS BY SEX*

Variable	Men (*n* = 777)	Women (*n* = 839)
Age, yr†	60.6 ± 10.9	59.3 ± 10.4
Height, m‡	1.75 ± 0.07	1.62 ± 0.06
Weight, kg‡	87.2 ± 14.6	72.9 ± 16.1
FVC, L‡	4.45 ± 0.96	3.26 ± 0.68
FEV_1, L‡	3.35 ± 0.80	2.50 ± 0.55
FVC%	97.8 ± 15.7	98.9 ± 14.7
FEV_1%‡	95.3 ± 17.2	97.7 ± 15.5
Plasma vitamin C, mg/dl‡	1.19 ± 0.58	1.33 ± 0.64
Plasma vitamin E, μg/ml‡	13.91 ± 6.28	15.00 ± 7.00
Plasma β-cryptoxanthin, μg/ml‡	0.093 ± 0.066	0.103 ± 0.073
Plasma lutein/zeaxanthin, μg/ml	0.149 ± 0.066	0.152 ± 0.071
Plasma β-carotene, μg/ml‡	0.171 ± 0.170	0.216 ± 0.189
Plasma lycopene, μg/ml	0.481 ± 0.234	0.478 ± 0.226
Plasma retinol, μg/ml‡	0.587 ± 0.142	0.548 ± 0.152
Eosinophil count, cells/μl†	3.4 ± 1.9	3.0 ± 1.8
Plasma triglyceride, mg/dl	148.7 ± 93.9	143.2 ± 93.0
African-American, %	6.8%	6.9%
Never-smoker,‡	33.9%	50.9%
Current smoker, %	12.2%	15.0%
Ex-smoker, %‡	53.9%	34.1%
Pack-years of smoking‡	19.6 ± 26.0	10.3 ± 18.5

* Values are mean ± SD.
† $p < 0.05$.
‡ $p < 0.01$ for difference between sex groups using Student's *t* test for continuous variables or chi-square for categorical variables.

Program, Round Robin Proficiency Testing from the National Institute of Standards and Technology (NIST Gaithersburg, MD). Twelve blind samples were analyzed and submitted yearly for the entire study period. Scores consisted of 1 = excellent (all values reported within ± 1 SD of NIST assigned value), 2 = acceptable (all values reported within ± 2 SD of NIST assigned value), 3 = marginal (all values reported within ± 3 SD of NIST assigned value), and 4 = poor (any value reported > or < 3 SD of NIST assigned value). Our laboratory consistently achieved grades of 1 and rarely 2 for all test specimens. Total vitamin C (ascorbic acid) was determined after stabilization with meta-phosphoric acid by the dinitrophenylhydrazine method and expressed as mg/dl (36). Triglycerides and cholesterol concentrations were determined using a commercial kit and expressed as mg/dl (Roche Diagnostic Systems, Inc., Indianapolis, IN). All samples were processed within 30 min after the blood was obtained, frozen at −80° C, and analyzed in batches. The intra-assay variability (coefficient of variation) was 8.5% and 2.5% for vitamin C and vitamin E, respectively. The corresponding interassay variability was 14.3% and 3.5% for vitamins C and E, respectively. For all carotenoids and retinol the intraassay and interassay variability (coefficient of variation) ranged from 3.45 to 4.79% and 4.45 to 5.45%, respectively. An automated differential cell blood count (including eosinophil counts) was determined at the laboratory of the Kaleida/Millard Fillmore Hospital Center for Laboratory Medicine in Buffalo, New York, using a Coulter Counter (Beckman Coulter, Inc., Fullerton, CA).

Anthropometry

All anthropometric measurements were taken according to a standardized protocol: body weight was measured with participants wearing light clothes and no shoes, using a balance beam scale (Detecto, Inc., Webb City, MO); height was measured without shoes using standardized scales (Perspective Enterprises, Kalamazoo, MI).

Statistical Analysis

Never-smokers were defined as those who had smoked less than 100 cigarettes during their entire lifetime. Pack-years of smoking exposure was derived from total years of smoking multiplied by the number of cigarettes per day divided by 20. Mean values and standard deviation (SD) for all relevant variables were calculated. The distributions of the continuous variables were examined to determine if they were normally distributed. All of the analyzed dependent variables showed normal distribution. Continuous independent variables were not significantly skewed or their transformation did not significantly alter the results of the analyses.

Bivariate analysis was performed to examine interrelations among variables with simple Pearson's and partial correlation coefficients (r). Multiple linear regression analysis was performed to analyze the relation between antioxidant blood levels and FEV_1 or FVC. Because of their proposed physiologic interaction, we first analyzed the association of vitamins C and E with pulmonary function. In this analysis, the dependent variables were FEV_1% and FVC% and the independent variables were vitamin C, vitamin E [adjusted for triglyceride levels expressed as μg vitamin E/mg triglyceride per dl serum (27)], and other covariates. Because little is known about vitamin A and carotenoids other than β-carotene, we then investigated the antioxidants retinol, β-cryptoxanthin, lutein/zeaxanthin, β-carotene, and lycopene as independent variables separately from vitamins C and E. Finally, we included all serum antioxidant vitamins as independent variables in the regression model. For better comparison among the various antioxidant vitamins in the regression models, the vitamin variables were expressed as a serum level change of 1 SD to account for the differences between the vitamins in absolute levels and to investigate the relative contribution of each antioxidant vitamin. FEV_1% and FVC% were used because this allowed analysis of both men and women together. The following covariates were considered and included in the analysis of FEV_1% and FVC%: weight (kg), blood eosinophil count (cells/μl), education (less than 12 yr of school, high school diploma, vocational school, some college, associate degree, bachelors degree, graduate degree), smoking status (never, former, current), and cumulative tobacco smoke exposure (pack-years of smoking). Education was used as an indicator of socioeconomic status in this analysis.

Twenty-four percent of the study population reported using supplemental vitamins regularly (≥ 1 supplement daily on average during the past year). Adjustment for supplement vitamin use (yes/no) did not affect the magnitude or the significance levels of the regression coefficients for the antioxidant vitamin variables. No significant change in the regression coefficients was seen when vitamin E was analyzed without adjustment for serum triglycerides and, therefore, only the adjusted values are presented (27). We also did not observe significant changes in the regression coefficients if we adjusted carotenoid levels for serum cholesterol (divided by cholesterol) or inclusion of cholesterol in the regression model. Interaction was investigated by

TABLE 2. CORRELATION* BETWEEN SERUM RETINOL AND CAROTENOIDS

	Vitamin C	Vitamin E	β-cryptoxanthin	Lutein/ zeaxanthin	β-carotene	Lycopene	Retinol
Vitamin C	x	0.21‡	0.20‡	0.13‡	0.13‡	0.01	0.09‡
Vitamin E		x	0.21‡	0.19‡	0.13‡	0.06†	−0.07†
β-cryptoxanthin			x	0.42‡	0.37‡	0.27‡	0.10‡
Lutein/zeaxanthin				x	0.21‡	0.31‡	0.13‡
β-carotene					x	0.20‡	0.08‡
Lycopene						x	0.09‡
Retinol							x

* Partial correlation coefficient adjusted for age, sex, race, and weight, vitamin E adjusted for triglyceride.
† $p < 0.05$.
‡ $p < 0.001$.

TABLE 3a. FEV_1% (MEAN ± SD*) BY QUARTILES OF ANTIOXIDANT VITAMINS

	Quartile Mean* (± SD)				
	I	II	III	IV	p for Linear Trend
Vitamin C	93.7 ± 16.9	96.9 ± 16.1	96.3 ± 16.5	99.1 ± 15.6	< 0.001
Vitamin E	94.3 ± 16.7	95.7 ± 16.3	97.4 ± 16.1	98.7 ± 16.1	< 0.001
β-cryptoxanthin	92.1 ± 17.9	97.9 ± 14.4	96.2 ± 15.6	99.6 ± 16.4	< 0.001
Lutein/zeaxanthin	93.5 ± 16.2	97.5 ± 16.0	97.5 ± 16.9	97.4 ± 15.9	0.001
β-carotene	94.9 ± 16.5	95.1 ± 15.4	96.7 ± 16.5	99.3 ± 16.6	< 0.001
Lycopene	95.4 ± 16.8	96.5 ± 16.4	96.8 ± 16.9	97.3 ± 15.2	0.096
Retinol	95.2 ± 17.5	95.1 ± 16.5	98.1 ± 15.3	97.6 ± 15.9	0.006

* Quartile mean concentration for vitamin C: 0.545, 1.053, 1.395, and 2.042 mg/dl; for vitamin E: 0.055, 0.089, 0.129, 0.222 μg vitamin E/mg triglyceride (per dl serum); β-cryptoxanthin: 0.033, 0.066, 0.102, and 0.191 μg/dl; for lutein/zeaxanthin: 0.078, 0.122, 0.159, and 0.240 μg/dl; for β-carotene: 0.055, 0.113, 0.183, 0.419 μg/dl; for lycopene: 0.222, 0.382, 0.528, and 0.787 μg/dl; for retinol 0.397, 0.511, 0.597, and 0.759 μg/dl.

including interaction terms of the antioxidant vitamins serum levels multiplied by smoking status and other covariates. Interaction terms were determined significant if the level of significance was $p < 0.1$, but no statistically significant interaction was observed (data not shown).

Differences in FEV_1% and FVC% levels between quartiles of vitamin C and vitamin E adjusted for serum triglyceride were analyzed using general linear models with adjustment for unbalanced design to account for unequal cell sizes. Linear trends were calculated for the analysis in quartiles. Student's *t* test and chi-square test were used to compare characteristics of included and excluded participants or men and women. Statistical significance was considered if p values were less than 0.05 (two-tailed). The Statistical Package for Social Sciences was used for the analyses (37).

RESULTS

Demographic Characteristics, Spirometry, and Blood Results

The baseline characteristics of the 1,616 participants are shown in Table 1 by sex. On average men were older, taller, heavier, and more likely to be ex-smokers than women. As expected, absolute FEV_1 and FVC were higher in men, but FEV_1% was lower in men than in women. Women showed higher serum concentrations of vitamins C, vitamin E, β-carotene, and β-cryptoxanthin than men and men had higher eosinophil counts.

Table 2 shows partial correlation coefficients among the antioxidant vitamins after adjustment for sex, age, race, and weight. Although, for the most part, these correlations were statistically significant, many correlations were small. Vitamins C and E were weakly correlated with each other and with the carotenoids β-cryptoxanthin, lutein/zeaxanthin, and β-carotene. The correlation coefficients of vitamins C and E with retinol and lycopene were less than 0.1. Moderately strong correlations were observed among the various carotenoids. β-cryptoxanthin was most strongly correlated with lutein/zeaxanthin, followed by β-carotene and lycopene. Lutein/zeaxanthin was more strongly correlated with lycopene than with β-carotene, and the weakest correlation among the carotenoids was seen between β-carotene and lycopene. There was a weak correlation of similar magnitude between retinol serum levels and all carotenoids.

Analysis of FEV_1 by Quartiles of Vitamin Blood Levels

Tables 3a and 3b display the average levels of FEV_1% and FVC% by quartiles of antioxidant vitamins. For both FEV_1% and FVC%, participants with the lowest serum levels of antioxidant vitamins consistently exhibited the lowest value. The increase of FEV_1% and FVC% across quartiles of the antioxidant vitamins was highly significant except for lycopene and retinol.

One aim of this study was to investigate the interrelation between serum vitamins C and E. Figures 1 and 2 depict mean pulmonary function levels cross-classified by quartiles of vitamins C and E after adjustment for pack-years of smoking, weight, and education. FEV_1% was also adjusted for smoking status and eosinophil count because these variables were significantly related in the bivariate analysis. For each vitamin there was evidence of an increase in FEV_1% with increasing level of the other vitamin (Figure 1), but the linear trends were not statistically significant across any of the quartiles ($p > 0.05$). For FVC% (Figure 2), the trends for vitamins C across quartiles of vitamin E were not statistically significant whereas those for vitamin E were significant across the first and the third quartiles of vitamin C (p for both < 0.008).

Multiple Linear Regression Analysis

Vitamins C and E. To investigate the relation of vitamins C and E with lung function, regression analyses were performed with FEV_1% and FVC% as dependent variables and serum antioxidant levels of vitamins C and E and other covariates as

TABLE 3b. FVC% (MEAN ± SD*) BY QUARTILES OF ANTIOXIDANT VITAMINS

	Quartile Mean* (± SD)				
	I	II	III	IV	p for Linear Trend
Vitamin C	96.6 ± 16.0	98.2 ± 14.9	98.2 ± 15.3	100.6 ± 14.5	< 0.001
Vitamin E	95.0 ± 14.7	97.5 ± 14.4	100.1 ± 15.7	101.0 ± 15.4	< 0.001
β-cryptoxanthin	94.9 ± 16.0	99.4 ± 14.5	98.0 ± 14.2	101.3 ± 15.5	< 0.001
Lutein/zeaxanthin	95.3 ± 14.8	98.7 ± 14.5	99.3 ± 16.2	100.3 ± 14.9	< 0.001
β-carotene	96.1 ± 15.1	97.2 ± 14.9	98.6 ± 14.7	101.7 ± 15.6	< 0.001
Lycopene	97.4 ± 15.5	98.6 ± 1.52	98.7 ± 15.7	99.0 ± 14.5	0.166
Retinol	97.4 ± 15.6	97.4 ± 15.0	99.4 ± 15.0	99.42 ± 15.3	0.016

* Quartile mean concentrations are same as Table 3a (*see* note).

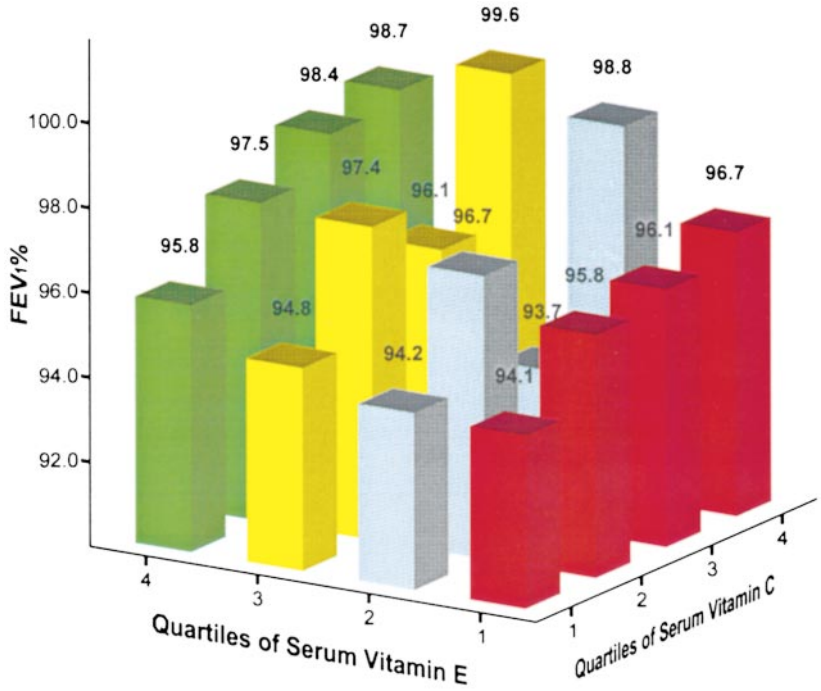

Figure 1. Depicted is the mean FEV_1% after cross-classification by quartiles of vitamin C and E after adjustment for smoking status, pack-years of smoking, eosinophil count, education, and weight. For example, the blue columns show the FEV_1% of participants in the highest quartile of vitamin E by quartiles of vitamin C. There is a trend toward increasing FEV_1% within each quartile of vitamin E with increasing levels of vitamin C and vice versa, but these trends were not statistically significant.

independent variables. Tables 4a and 4b show the regression coefficients and their standard error (SE) for the antioxidant vitamins when they were included in the model individually (first column, vitamin C; second column, adjusted vitamin E) and when both variables were included simultaneously (third column).

For FEV_1% (Table 4a), as expected, lifetime exposure to tobacco smoke (pack-years of smoking), current smoking status, weight, education, and eosinophil count were all significant correlates in all three models. Both vitamins C and E were significantly associated with FEV_1% when they were included individually, but only vitamin E remained significantly associated when both vitamins were included in the model.

When FVC% was the dependent variable, pack-years of smoking, weight, and education were significant correlates in all three models (Table 4b). Of the two antioxidant vitamins, only vitamin E showed a significant association with FVC%.

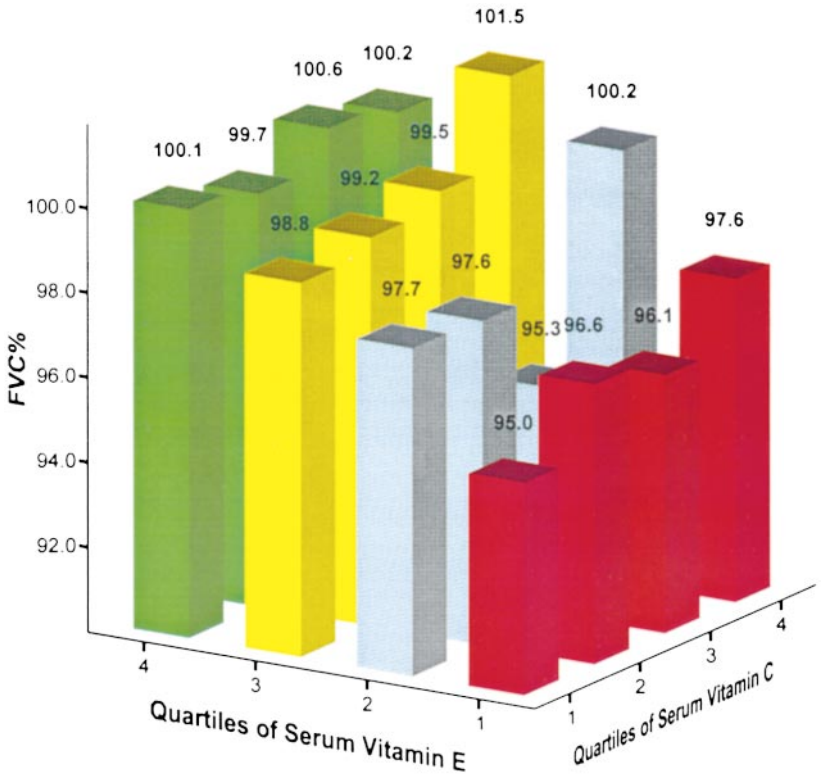

Figure 2. Shown is the mean FVC% after cross-classification by quartiles of vitamin C and E after adjustment for pack-years of smoking, education, and weight. Participants in the lowest quartile of each vitamin had the lowest pulmonary function tests compared with those who had high levels of at least one antioxidant vitamin. The linear trends for vitamins C across quartiles of vitamin E were not statistically significant whereas those for vitamin E were significant across the first and the third quartiles of vitamin C (p for both < 0.008).

TABLE 4a. MULTIPLE LINEAR REGRESSION COEFFICIENTS AND p VALUES FOR REGRESSION OF FEV_1% AGAINST VITAMIN C AND VITAMIN E

	Dependent Variable: FEV_1%					
	Model with Vitamin C		Model with Vitamin E		Model with Vitamins C and E	
Variable	β	SE	β	SE	β	SE
Smoking status (never, former, current)	−2.39*	(0.68)	−2.38†	(0.68)	−2.35†	(0.68)
Pack–years of smoking	−0.168†	(0.021)	−0.167*	(0.021)	−0.167*	(0.021)
Weight, kg	−0.059†	(0.023)	−0.049‡	(0.023)	−0.046‡	(0.024)
Education, degree	0.442‡	(0.138)	0.445†	(0.137)	0.437†	(0.138)
Eosinophil count, cells/μl	−0.496‡	(0.205)	−0.473‡	(0.204)	−0.494‡	(0.205)
Vitamin C (SD)	0.772‡	(0.388)	—	—	0.578	(0.396)
Vitamin E (SD)	—	—	1.120*	(0.399)	1.002*	(0.407)

* $p < 0.001$.
† $p < 0.01$.
‡ $p < 0.05$.

The association of vitamin E with FVC% was unchanged when both vitamins were included in the model.

Retinol and carotenoids. Tables 5a and 5b summarize the results of multiple linear regression analysis when retinol and carotenoids were added to the baseline model individually (left column) and for the model in which all variables were included simultaneously (right column).

We observed a statistically significant association of β-cryptoxanthin, lutein/zeaxanthin, β-carotene, and retinol with FEV_1% when these variables were entered individually and separately into the regression models (Table 5a, antioxidant vitamins entered individually). The strongest association was seen with β-cryptoxanthin. None of the other individual carotenoids remained statistically significant when β-cryptoxanthin was added to the model, whereas the coefficient for retinol showed a significant independent effect. Finally, inclusion of all carotenoids and retinol simultaneously led to a small decrease in the coefficient for β-cryptoxanthin that remained the only and independently significant correlate of FEV_1% among the carotenoids, whereas the coefficient for retinol was only slightly reduced.

FVC% was significantly correlated with β-cryptoxanthin, β-carotene, and lutein/zeaxanthin when the antioxidant variables were investigated individually and separately (Table 5b). Among these antioxidants, as for FEV_1%, β-cryptoxanthin was most strongly related to FVC% and the regression coefficient for lutein/zeaxanthin was similar in magnitude. After inclusion of β-cryptoxanthin in the model, only lutein/zeaxanthin showed an independent association with FVC%. Addition of all carotenoids and retinol simultaneously to the baseline model showed that only the regression coefficients for β-cryptoxanthin and lutein/zeaxanthin reached statistical significance.

Combined effect of vitamin C, vitamin E, retinol, and carotenoids. In the final model we examined the association of all carotenoids, retinol, and, in addition, vitamins C and E with FEV_1% and FVC%. Table 6 shows the results of multiple linear regression after inclusion of these variables in a regression model adjusted for other covariates. The correlation of FEV_1% with β-cryptoxanthin was stronger than with vitamin E, and the regression coefficients were little changed when both variables were included in the regression models. Retinol maintained an independent association with FEV_1% even after controlling for β-cryptoxanthin and vitamin E. For FVC%, a stronger association with vitamin E than with β-cryptoxanthin was observed, but both antioxidant vitamins were significantly related to FVC%. The correlation of lutein/zeaxanthin was only borderline statistically significant when vitamin E and β-cryptoxanthin were included in the regression model. No other serum antioxidant was significantly related to FEV_1% or FVC in these models.

DISCUSSION

The results of this population-based study support the hypothesis that serum antioxidant vitamins positively influence pulmonary function. Vitamin C, vitamin E, β-cryptoxanthin, lutein/zeaxanthin, β-carotene, and retinol were positively related to FEV_1, and β-cryptoxanthin, lutein/zeaxanthin, and β-carotene were positively related to FVC when considered individually. However, when considered together, β-cryptoxanthin and vitamin E were the serum parameters with the strongest relation to both FEV_1 and FVC. In addition to β-cryptoxanthin and vitamin E, only retinol and lutein/zeaxanthin had independent effects on FEV_1% (retinol) and FVC% (lutein/zeaxanthin).

TABLE 4b. MULTIPLE LINEAR REGRESSION COEFFICIENTS AND p VALUES FOR REGRESSION OF FEV_1% AGAINST VITAMIN C AND VITAMIN E

	Dependent Variable: FVC%					
	Model with Vitamin C		Model with Vitamin E		Model with Vitamins C and E	
Variable	β	SE	β	SE	β	SE
Smoking status (never, former, current)	−1.07	(0.66)	−1.01	(0.66)	−1.00	(0.66)
Pack–years of smoking	−0.082*	(0.020)	−0.080*	(0.020)	−0.090*	(0.020)
Weight, kg	−0.125†	(0.022)	−0.105†	(0.022)	−0.104†	(0.023)
Education, degree	0.362†	(0.133)	0.356†	(0.132)	0.353†	(0.132)
Eosinophil count, cells/μl	−0.099	(0.197)	−0.089	(0.196)	−0.096	(0.196)
Vitamin C (SD)	0.491	(0.374)	—	—	0.177	(0.380)
Vitamin E (SD)	—	—	1.657*	(0.382)	1.621*	(0.390)

* $p < 0.001$.
† $p < 0.01$.

TABLE 5a. MULTIPLE LINEAR REGRESSION COEFFICIENTS (SE) FOR REGRESSION OF FEV_1% AGAINST SERUM CAROTENOIDS AND RETINOL

	Dependent Variable FEV_1%			
	Antioxidant Vitamins Added Individually to Baseline Model*		Antioxidant Variables Added Simultaneously to Baseline Model*	
Variable Serum antioxidants	β	(SE)	β	(SE)
β-cryptoxanthin (SD)	1.518§	(0.408)	1.330‡	(0.471)
Lutein/zeaxanthin (SD)	0.900†	(0.392)	0.337	(0.437)
β-carotene (SD)	0.674†	(0.399)	0.112	(0.429)
Lycopene (SD)	0.207	(0.386)	−0.146	(0.406)
Retinol (SD)	0.919‡	(0.385)	0.760	(0.389)

Definition of abbrevition: β = β-coefficient.

*Baseline model includes pack-years of smoking, smoking status, education, eosinophil count, and weight.

† $p < 0.05$.

‡ $p < 0.01$.

§ $p < 0.001$.

Because of the proposed physiologic interaction, we will discuss the findings for vitamin C and E first (9). Few studies have measured serum vitamin C in relation to pulmonary function in adults. Hu and Cassano observed in their analysis of the Third National Health and Nutrition Examination Survey (NHANES III) a similar effect size compared with our study for vitamin C alone (31). These researchers reported an increase in FEV_1 of approximately 28 ml per standard deviation increase in vitamin C for a person 1.70 m tall. Our corresponding estimates are approximately 21 ml. In an ecological study in rural China that related vitamin C in serum samples pooled from 120 men and women in each of 69 counties to their average pulmonary function levels, Hu and coworkers observed an increase of 143 ml in FEV_1 and 94 ml in FVC per mg/dl increase in serum vitamin C after adjustment for several confounders (21). Similar to our results, the association of serum vitamin C with FEV_1 was stronger than with FVC, but the corresponding increase in our study was smaller, amounting to an increase of approximately 35 ml in FEV_1 and 30 ml in FEV_1. However, these results are not directly comparable because an ecological analysis does not provide data on individuals. In addition, in the Chinese population, baseline vitamin C intake was approximately 50% higher than in the average U.S. population (21, 33).

TABLE 5b. MULTIPLE LINEAR REGRESSION COEFFICIENTS (SE) FOR REGRESSION OF FVC% AGAINST SERUM CAROTENOIDS AND RETINOL

	Dependent Variable FVC%			
	Antioxidant Vitamins Added Individually to Baseline Model*		Antioxidant Variables Added Simultaneously to Baseline Model*	
Variable Serum antioxidants	β	(SE)	β	(SE)
β-cryptoxanthin (SD)	1.631†	(0.390)	1.180‡	(0.450)
Lutein/zeaxanthin (SD)	1.380†	(0.375)	0.921‡	(0.419)
β-carotene (SD)	1.021‡	(0.382)	0.442	(0.410)
Lycopene (SD)	0.082	(0.371)	−0.531	(0.388)
Retinol (SD)	0.657§	(0.370)	0.424	(0.372)

* Baseline model includes pack-years of smoking, smoking status, education, and weight.

† $p < 0.005$.

‡ $p < 0.05$.

§ $p < 0.1$.

TABLE 6. MULTIPLE LINEAR REGRESSION COEFFICIENTS (SE) FOR REGRESSION OF FEV_1% AND FVC% AGAINST SERUM ANTIOXIDANT VITAMINS

	Antioxidant Dependent Variable FEV_1%	
Variables Added Simultaneously to Baseline Model*	β	(SE)
β-cryptoxanthin (SD)	1.217	(0.475)†
Vitamin E (SD)	0.894	(0.434)‡
Retinol (SD)	0.838	(0.393)‡
Vitamin C (SD)	0.302	(0.402)
Lutein/zeaxanthin (SD)	0.192	(0.441)
β-carotene (SD)	−0.167	(0.446)
Lycopene (SD)	−0.178	(0.408)
	Dependent Variable FVC%	
	β	(SE)
Vitamin E (SD)	1.369	(0.416)†
β-cryptoxanthin (SD)	1.091	(0.453)‡
Lutein/zeaxanthin (SD)	0.734	(0.422)$^{p = 0.083}$
Retinol (SD)	0.579	(0.375)
β-carotene (SD)	0.055	(0.426)
Vitamin C (SD)	−0.082	(0.384)
Lycopene (SD)	−0.446	(0.389)

* For FEV_1% the baseline model was adjusted for pack-years of smoking, smoking status, education, eosinophil count, and weight. For FVC% the baseline model was adjusted for baseline model including pack-years of smoking, education, and weight.

† $p < 0.01$.

‡ $p < 0.05$.

A few other studies have reported vitamin E blood levels in relation to spirometry. Grievink and coworkers (29) found only a small and statistically nonsignificant difference of 23 ml in FEV_1 and 36 ml in FVC between participants at the 10th and 90th percentile of serum vitamin E in 367 Dutch men and women. In a more recent study, Grievink and coworkers observed a larger difference of 94 ml in FEV_1 in a sample of 528 elderly adults using similar statistical methods (30). Compared with our results, the magnitude of the effect for vitamin E was similar in the analysis of NHANES III (31). Morabia and coworkers compared 28 men with airway obstruction ($FEV_1/FVC < 75\%$) with 55 men without airway obstruction and found no association between vitamin E serum levels and airway obstruction (14). In this latter study, however, individuals with airway obstruction had approximately 6% lower vitamin E levels than those without but this difference did not reach statistical significance.

It has been described that vitamin C and vitamin E have a close physiologic interaction (9). Information regarding the interrelation of vitamins C and E is limited to studies that measured dietary intake of these vitamins. Contradictory evidence exists from these dietary studies as to which of the two antioxidant vitamins may be more strongly associated with pulmonary function, although most (19, 20, 29), but not all (31, 39) studies report a stronger association of pulmonary function with vitamin C intake. We found that low levels of both vitamin C and vitamin E in serum are associated with lowest lung function but when we examined the overall effect of these two antioxidant vitamins, only vitamin E appeared to be related to pulmonary function independently.

The stronger association of pulmonary function with vitamin E may be the result of either biologic mechanisms or methodological effects. Our findings suggest that the function of vitamin E is, at least in part, independent of vitamin C. Recent work indicates that in addition to vitamin C there are other substances that can function as co-antioxidants for vitamin E (e.g., bilirubin and possibly glutathione [both found in the lung]), supporting an antioxidant role of vitamin E inde-

pendent of vitamin C (40, 41). Biologically, vitamin E, predominantly bound to the cell membrane, could play a more significant role in antioxidant defense in the lung, because the tissue concentrations may be more stable and may exert greater long-term protective effects than the water-soluble vitamin C (7).

It is also possible that serum levels of vitamin C may be poorer indicators of tissue antioxidant activity than serum levels of vitamin E, especially in the lung. It has been suggested, in fact, that one possible response to oxidative stress may be the greater transfer of vitamin C than vitamin E from serum into respiratory epithelial lining fluid of the lung (41); this localized action in the lung may not be well reflected by serum levels. In addition, the transfer of vitamin C to the respiratory epithelial lining fluid may result in lower serum vitamin C levels (40, 43). Consequently, serum levels of vitamin C may be less important as indicators of localized tissue antioxidant mechanisms.

Finally, measurement error or biologic variability could be responsible for the weaker association of vitamin C with lung function compared with other antioxidant vitamins. It is known that both laboratory error and biologic variability for serum vitamin C are higher than for the other serum vitamins we measured (27). Therefore, characterization of individuals with regard to their vitamin C status may be more problematic than for vitamin E, retinol, and carotenoids.

In general, our results regarding carotenoids and retinol confirm previous findings that serum carotenoids and retinol are positively associated with pulmonary function. Grievink and coworkers found differences of approximately 52 ml in FEV_1 and 125 ml in FVC between participants at the 10th and those at the 90th percentile of serum β-carotene in one study (29). Our corresponding estimates are approximately 60 ml in FEV_1 and 95 ml in FVC. In another study, Grievink and coworkers measured several carotenoids in serum and found a positive association of α-carotene, β-carotene, and lycopene with FEV_1 and of α-carotene and β-carotene with FVC (30). Lutein, β-cryptoxanthin, and lutein/zeaxanthin were not significantly associated with FEV_1 or FVC. α-carotenoid contributed only 4% to the total carotenoid concentration in serum, and carotenoids were measured in nonfasting blood samples, which may contribute to the differences compared with our study. Chuwers and coworkers found slightly higher estimates (approximately 100 ml for both FEV_1 and FVC between participants at the 25th and 75th percentile), but these investigators studied smokers exposed to asbestos (25). This latter study also observed a positive and significant association between serum retinol and FVC (approximately 70 ml raising serum retinol from the 25th to the 75th percentile) and a weaker and not significant association with FEV_1. Finally, a smaller study in 83 men observed no significant association between airway obstruction (defined as $FEV_1/FVC < 75\%$) and low serum β-carotene or lycopene but found a significant inverse association with low serum retinol and a nonsignificant inverse trend for a measure of total carotenoids other than β-carotene and lycopene (14).

Results of studies focusing on dietary intake of retinol and carotenoids are inconsistent, and many have failed to separate between effects of retinol and the various carotenoids. Two previous studies examined dietary intake of carotene in relation to FEV_1 and FVC and observed a positive association (19, 31). Two large epidemiologic studies investigated dietary vitamin A intake in relation to airway obstruction. One of these studies observed an inverse relation of vitamin A intake with airway obstruction enhanced by smoking (13) whereas the other found this association only in heavy smokers, although similar analytical methods were used (15).

Because most of these studies could not separate effects of vitamin A and β-carotene on lung function from those of other carotenoids, our study adds important information to current knowledge about antioxidant vitamins and lung function by considering measurement of different carotenoids. We focused on carotenoids with the highest concentrations in human plasma, demonstrated antioxidant properties, and presence in lung tissue (44, 45). It may be important to distinguish among the effects of the many specific carotenoids (45), because their concentration and distribution differ among different tissues and organs (46, 47) although little is known about the concentration of carotenoids in the lung (48).

When we analyzed all antioxidant vitamins together, our findings indicate that among these antioxidants, β-cryptoxanthin and vitamin E have the strongest relation to both FEV_1% and FVC%. This finding is somewhat surprising because lycopene and β-carotene have higher blood levels and because lycopene has been considered to be a more powerful antioxidant than β-cryptoxanthin (11).

Nevertheless, a number of reasons can be hypothesized to explain these findings. First, it is possible that the blood concentrations of at least some of these biomarkers do not adequately reflect the specific conditions in the lung or that β-cryptoxanthin, lutein/zeaxanthin, and retinol have stronger antioxidant activity in the lung tissue compared with other carotenoids. It is known that serum levels of the carotenoids measured in our study are correlated with their lung tissue concentration (45), but limited information exists with regard to relative antioxidant activity of carotenoids in the lung. In addition, it has been emphasized that carotenoid antioxidant properties, including their relative power compared with other carotenoids, *in vitro* may not translate to complex *in vivo* conditions (49). For example, in comparison to other carotenoids a stronger negative association with prevalence of angina pectoris and carotid atherosclerosis has been reported for serum β-cryptoxanthin (50, 51), but, in general, limited data are available for carotenoids other than β-carotene.

The potential confounding effects of nutritional and life-style habits can be part of the explanation of our findings. It is possible that food items rich in β-cryptoxanthin are associated with high levels of other important antioxidants (52). As a result, it is possible that the β-cryptoxanthin serum concentration reflects intake of nutrients other than β-cryptoxanthin that positively influence lung function. For example, β-cryptoxanthin is derived from fruits that are also rich in vitamin C (53) and several studies have found a positive relation between lung function and both fresh fruit and vitamin C intake (18, 20). Vitamin C, however, showed a weaker association with pulmonary function, and inclusion of serum vitamin C in the multiple linear regression analysis did not significantly influence the association between β-cryptoxanthin and lung function.

On the other hand, it is possible that for some of the carotenoids the strength of their association is weakened by dietary and life-style correlates that may be associated with poorer lung function. For instance, tomato products represent the major source of lycopene, but in the Western diet this food item can also be associated with a dietary pattern characterized by saturated fat intake and low intake of important sources of antioxidants (fruits and vegetables) and life-style habits linked to poor lung health (i.e., smoking) (54). Therefore, it is possible that discrepancies in dietary and other habits between the Dutch and the U.S. population explain the differences in our results compared with the study by Grievink in Dutch elderly (30).

The results of our study also suggest that high levels of one antioxidant could overcome, at least partially, low levels of the

other. After cross-classification by vitamins C and E, the results of the analysis in quartiles indicated that there was a trend toward higher pulmonary function within quartiles of each vitamin associated with increased levels of the other antioxidant vitamin. However, conclusions have to be drawn with caution because of the limited power available for this type of analysis. We also may have failed to observe a statistically significant interaction of serum antioxidants with smoking, because of limited statistical power available for these analyses (owing to the low prevalence of current smoking). Cigarette smoke is a major source of oxidants (42). Thus, a stronger association of antioxidant vitamins with pulmonary function would be expected in smokers if the effect is, in fact, a result of protection against oxidative stress.

The magnitude of the observed association of serum β-cryptoxanthin and vitamin E with pulmonary function appears to be of clinical significance. After allowing for the effects of other factors related to FEV_1, the difference in FEV_1 associated with a reduction of 1 SD of serum vitamin E or β-cryptoxanthin was equivalent to the negative effect of approximately 1 to 2 yr of aging on FEV_1. In general, factors other than serum vitamins identified in this study as significant independent predictors of FEV_1 and FVC were consistent with those described in other studies (34). Both the inverse association of blood eosinophil count with FEV_1 independent of asthma and the effect of current smoking in addition to the cumulative effect of lifetime smoking on FEV_1 have been described previously and were not unexpected (55, 56).

Our study has both strengths and weaknesses. A limitation of our study is the cross-sectional nature and the subsequent uncertainty about the cause–effect relation. Did the antioxidant vitamins affect pulmonary function, or did lower pulmonary function lead to increased oxidative stress and reduced serum antioxidant levels? Longitudinal studies may provide an answer to this question.

One of the strengths of our study is that we measured serum vitamin levels according to a highly standardized protocol, which may, at least for vitamin E and β-carotene, be better indicators of lung antioxidant activity than dietary intake, as reported by Redlich and coworkers who found that lung tissue levels of α-tocopherol and β-carotene correlated more strongly with blood levels than a history of dietary intake in humans (45). Another strength is that we enrolled participants randomly selected from the general population and, therefore, our results may be generalizable, although the participation rate of 51% in our study is only moderate. Furthermore, we obtained detailed information on several important life-style factors related to pulmonary function, enabling us to adjust for their effects in the analysis.

In conclusion, we have observed positive associations of several antioxidant vitamins with pulmonary function. Of these antioxidants, β-cryptoxanthin and vitamin E appear to be a stronger correlate of lung function than other vitamins. We identified β-cryptoxanthin as the carotenoid with the strongest association to pulmonary function, and after adjustment for this nutrient only lutein/zeaxanthin had an additional effect on FVC and retinol on FEV_1. Our findings indicate that in addition to vitamin E, carotenoids and retinol may play a role in respiratory health and that the most important carotenoid may not be β-carotene. Longitudinal and intervention studies are necessary to demonstrate a temporal relation and whether antioxidants can slow the decline in pulmonary function with aging. The evaluation of the association of antioxidant vitamins with pulmonary function is important because reduced pulmonary function is a risk factor for chronic disease mortality and antioxidant vitamins could help to reduce the risk.

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