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ORIGINAL ARTICLE

Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD

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Objective: The objective of this study was to investigate the association between antioxidant nutrients and markers of oxidative stress with pulmonary function in persons with chronic airflow limitation.
Design: Cross-sectional study exploring the association of antioxidant nutrients and markers of oxidative stress with forced expiratory volume in the first second (FEV_1%) and forced vital capacity (FVC%).
Setting/Subjects: The study data included 218 persons with chronic airflow limitation recruited randomly from the general population of Erie and Niagara counties, New York State, USA.
Results: After adjustment for covariates, multiple linear regression analysis showed that serum β-cryptoxanthin, lutein/zeaxanthin, and retinol, and dietary β-carotene, β-cryptoxanthin, lutein/zeaxanthin, vitamin C, and lycopene were positively associated with FEV_1% ($P<0.05$, all associations). Serum vitamins β-cryptoxanthin, lutein/zeaxanthin, and lycopene, and dietary β-cryptoxanthin, β-carotene, vitamin C, and lutein/zeaxanthin were positively associated with FVC% ($P<0.05$, all associations). Erythrocytic glutathione was negatively associated with FEV_1%, while plasma thiobarbituric acid-reactive substances (TBARS) were negatively associated with FVC% ($P<0.05$).
Conclusion: These results support the hypothesis that an imbalance in antioxidant/oxidant status is associated with chronic airflow limitation, and that dietary habits and/or oxidative stress play contributing roles.
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Introduction

Oxidative stress resulting from an imbalance of reactive oxidant species and antioxidants contributes to a variety of physiological changes, including chronic airflow limitation (MacNee, 2000; Schünemann *et al*., 2001a). Antioxidant vitamins are generally thought to protect tissue from oxidant cellular injury, partly through their ability to quench reactive molecules (Heffner and Repine, 1989, Palace *et al*., 1999). Population-based studies support this notion, given that a majority of these studies report positive associations of pulmonary function and antioxidant vitamins. Most authors measure antioxidant status in serum or by dietary intake assessment (Britton *et al*., 1995; Grievink *et al*., 2000; Schünemann *et al*., 2001b, 2002). In our previous study, biological markers of oxidative stress were associated with pulmonary function in a general population sample (Schünemann *et al*., 1997). Other studies reported that both serum and exhaled breath markers of oxidative stress correlate with asthma (Nadeem *et al*., 2003), COPD (Montuschi *et al*., 2000) and exacerbations of COPD (Dekhuijzen *et al*., 1996; Rahman *et al*., 1996, 1997).

If oxidative stress contributes to and/or causes airflow limitation, the association between antioxidants and pulmonary function may be especially evident in persons with COPD or asthma compared to the general population, comprised mainly of healthy persons. If true, this could result from nutrient deficiency in persons with lung disease, or from an increased demand for antioxidants due to higher levels of oxidative stress that arise from the disease itself. Thus, antioxidant status may be important in the progression of these conditions.

There is limited population-based evidence to support an association of antioxidant nutrients and biomarkers of oxidative stress with pulmonary function in persons with chronic airflow limitation. To the best of our knowledge, no large study has investigated the joint associations among antioxidant nutrients, oxidative stress biomarkers, and pulmonary function parameters. As antioxidant vitamins quench reactive oxygen species generated during inflammation, they are hypothesized to be a mainstay of pulmonary antioxidant defense. Thus, we hypothesized that antioxidant vitamins would be positively associated with pulmonary function, and that oxidative stress markers would be negatively associated with pulmonary function in patients with chronic airflow limitation.

Methods

Subjects and measurements

We randomly selected participants from Erie and Niagara counties in Western New York, USA, as previously described (Schünemann *et al*., 2001b). Approximately 59.4% of individuals who were randomly selected, contacted, and eligible for the study agreed to participate. Among 2537 participants who had a pulmonary function test and nonmissing covariates, 68 patients reported a diagnosis of asthma, 121 reported COPD (including chronic bronchitis and emphysema), and 29 reported asthma and COPD. Thus, the sample consisted of 218 individuals with chronic airflow limitation.

Interview. Interviewers collected information on demographics, smoking behaviors, and physical measurements during in-person interviews.

Pulmonary function tests. Trained personnel performed spirometry between 0630 and 0930. We used multiple linear regression to derive FEV_1 and FVC predicted values, and percent of predicted values based on never-smokers free of respiratory disease ($n = 694$) adjusted for gender, age, height, and race (Schünemann *et al*., 2002). We also computed the percentage of predicted values using prediction equations derived from NHANES III (Hankinson *et al*., 1999).

Blood determinations. Fat-soluble vitamins (μg/ml) in serum were measured using a Shimadzu LC-7A high-pressure liquid chromatography system with a SPD-M6A photodiode array (Shimadzu Scientific Instruments Inc., Braintree, MA) according to methods of Browne and Armstrong (1998a). We measured vitamin C in heparinized plasma after stabilization with metaphosphoric acid using the dinitrophenyl hydrazine method (McCormick and Greene, 1994). Fat-soluble vitamin determinations were quality controlled through the use of standard reference material from the National Institutes of Standards and Technology and participation in the Micronutrients Measurement Quality Assurance Program.

We measured plasma-TBARS (TBARS) and red blood cell glutathione according to the methods of Browne and Armstrong (Armstrong and Browne, 1994; Browne and Armstrong, 1998b). Measurement of glutathione peroxidase was performed using an automated Cobas Mira chemistry analyzer (Pippenger *et al*., 1998). We also measured Trolox equivalent antioxidant capacity (TEAC) (Miller and Rice-Evans, 1993). Intra-assay reproducibility (% coefficient of variation) was assessed for each biomarker of oxidative stress, and the values were as follows: TBARS 7.6%, glutathione 3.3%, glutathione peroxidase 4.4%, and TEAC 8.5%. Inter-assay coefficients of variation were: TBARS 9.2%, glutathione 4.0%, glutathione peroxidase 8.6%, and TEAC 10.0%.

Nutrient intake. We assessed diet over the 12 month period beginning 24 months before the interview and ending 12 months prior to the interview using the self-administered 100 item Health Habits and History Food Frequency Questionnaire (Block *et al*., 1986). We calculated individual mean daily nutrient intake using DietSys (Block *et al*., 1993), and updated using the most recent United States Department of Agriculture information (Holden *et al*., 1999; US Department of Agriculture, 1999).

Statistical analysis

We computed mean values and s.d. for all relevant variables and tested predictor variables for linearity, collinearity, and interaction. We analyzed differences in serum antioxidants and oxidative stress markers by smoking status and type of chronic airflow limitation (asthma versus COPD) using *t*-tests. We then calculated partial correlation coefficients to assess the relation between and among antioxidants and oxidative stress parameters adjusted for age, gender, race, and weight. We then analyzed correlation between serum antioxidants and oxidative stress markers, while adjusting for gender, triglycerides, and pack-years of smoking.

All of the following covariates were considered during multivariable analysis because they are associated with pulmonary function: smoking status, lifetime pack-years, weight, education, and eosinophils. Other adjustments include triglycerides for serum vitamin concentrations, and total energy intake for dietary antioxidant estimates.

We used multiple linear regression analysis to investigate the individual associations of antioxidant vitamins and oxidative stress markers with FEV_1% and FVC% and FEV_1/FVC%. Antioxidant nutrients and oxidative stress markers were not normally distributed, so values were log transformed. Each log-transformed vitamin was entered into multivariate models in the s.d. form in order to investigate relative importance.

Lastly, we analyzed linear regression models containing serum antioxidants that were independently and statistically significantly associated with pulmonary function *and* oxidative stress markers, to determine whether inclusion of antioxidant vitamins and oxidative stress markers could explain a greater proportion of variance in pulmonary function as compared to each entered alone. We repeated these analyses using NHANES predicted values (Hankinson *et al*., 1999). For all analyses, we used the Statistical Package for Social Sciences software (SPSS, 2001).

Results

Table 1 shows the characteristics of individuals with chronic airflow limitation. The sample included more women than men and a small percentage of African Americans. Current and former cigarette smokers constituted approximately 66.5% of the population, with a mean of 24.4 pack-years of smoking. Based upon mean BMI values, individuals were overweight.

There were more reports of physician-diagnosed COPD than asthma. We analyzed how individuals in this sample differed according to asthma or COPD diagnoses. Individuals diagnosed with COPD had greater cigarette exposure and higher levels of glutathione, but we observed no differences in pulmonary function or antioxidant intake. Asthmatics had higher levels of circulating β-cryptoxanthin ($P<0.009$) but all other serum vitamins were not statistically different (data not shown).

Table 1 Demographic, pulmonary function, and antioxidant descriptives ($n=218$)

Variable (unit)	*Mean (s.d.)*
Age (years)	61.7 (10.3)
Gender (% female)	58.7
BMI (kg/m^2)	29.9 (6.7)
Education (% with HS degree or higher)	86.2
African American (%)	7.8
FEV_1 (l)	2.30 (0.93)
FVC (l)	3.27 (1.08)
FEV_1% of predicted	79.70 (24.5)
FVC% of predicted	87.49 (18.7)
FEV_1/FVC	0.70 (0.14)
Current or former smoker (%)	66.5
Pack-years of smoking	24.4 (27.4)
Serum antioxidants	
Vitamin C (mg/dl)	1.23 (0.62)
Vitamin E (μg/ml)	15.52 (8.97)
β-Cryptoxanthin (μg/ml)	0.087 (0.07)
Lutein/zeaxanthin (μg/ml)	0.132 (0.06)
β-Carotene (μg/ml)	0.178 (0.22)
Lycopene (μg/ml)	0.439 (0.26)
Retinol (μg/ml)	0.556 (0.16)
Dietary antioxidants[a]	
Vitamin C (mg/day)	128.3 (80.0)
Vitamin E (mg/day)	9.82 (6.6)
β-cryptoxanthin (μg/day)	224.4 (149.3)
Lutein/zeaxanthin (μg/day)	2188.0 (2887.6)
β-Carotene (μg/day)	3304.5 (2596.2)
Lycopene (μg/day)	2803.5 (2341.4)
Retinol (μg/day)	774.8 (513.7)
Oxidative stress markers[b]	
Plasma-TBARS (nmol/ml)	1.47 (0.5)
Glutathione (ng/dl PRBC)	55.43 (16.7)
Glutathione peroxidase (IU)	619.23 (98.0)
TEAC (%)	72.33 (5.9)

[a]$n=207$.
[b]$n=200$.

Partial correlation coefficients (r) among serum vitamins ranged from 0.16 to 0.44. We observed the strongest correlations between β-cryptoxanthin and lutein/zeaxanthin ($r=0.43$, $P<0.001$), and vitamin E with β-carotene ($r=0.42$, $P<0.001$). Vitamin C was weakly correlated with serum carotenoids (range: -0.08 to 0.20). Among dietary vitamins, we found the strongest correlation between dietary β-carotene and lutein ($r=0.70$, $P<0.001$). Partial correlation coefficients between dietary vitamin C and carotenoids ranged from 0.35 to 0.59.

In addition, we measured the correlation between serum and dietary antioxidant measurements. We observed the highest correlations between serum and dietary values for vitamin C ($r=0.29$, $P<0.001$), β-cryptoxanthin ($r=0.22$, $P<0.002$) and lutein/zeaxanthin ($r=0.18$, $P<0.009$). The smallest correlation among the carotenoids was 0.12, but this correlation was not statistically significant.

When we investigated differences in serum antioxidants and oxidative stress markers by smoking status (current

smoker or not), we found no significant differences in mean oxidative stress markers. The serum antioxidants that were statistically significantly lower in smokers were vitamin C and β-carotene. Smokers also had lower energy-adjusted intakes of vitamin C, β-carotene, lutein/zeaxanthin, and β-cryptoxanthin (data not shown).

Table 2 shows partial correlation coefficients for serum antioxidants and oxidative stress markers. We found inverse correlations of lycopene with glutathione, and a positive correlation of lycopene and glutathione-peroxidase. β-Carotene was inversely correlated with TBARS, and β-cryptoxanthin was positively correlated with glutathione-peroxidase.

Table 3 shows regression coefficients for covariates included in the baseline model and for each antioxidant vitamin entered into separate models. We present separate models because of moderate to high correlation between vitamins. Both serum and dietary intake of β-cryptoxanthin and lutein/zeaxanthin were positively associated with FEV_1% and FVC%. Intake of vitamin C and β-carotene was positively associated with FEV_1% and FVC%. Serum retinol and lycopene intake were positively associated with FEV_1%, while serum lycopene was associated with FVC%. Serum retinol, vitamin C, and lutein/zeaxanthin, as well as dietary β-carotene, lutein/zeaxanthin, and vitamin C were associated with FEV_1/FVC%.

When we analyzed vitamin supplement use, we did not observe additional variance in pulmonary function explained using vitamins C, E, or β-carotene once dietary intakes were considered. We used NHANES III prediction equations for pulmonary function, there were no important changes in the results.

Table 4 shows the association of pulmonary function with oxidative stress markers, and models including antioxidants

Table 2 Partial correlation coefficients for serum antioxidants and oxidative stress markers, adjusted for gender and triglycerides* ($n=200$)

	TBARS	*GSH*	*Glutathione-peroxidase*	*TEAC*
Vitamin C[a]	0.05	−0.12	0.03	−0.02
Vitamin E	0.04	−0.07	−0.04	−0.03
β-Cryptoxanthin	0.06	−0.10	0.14*	−0.01
Lutein/zeaxanthin	−0.02	−0.04	0.01	0.03
β-Carotene[a]	−0.16*	0.02	0.05	−0.02
Lycopene	−0.05	−0.22*	0.17*	0.01
Retinol	−0.08	0.03	−0.05	0.07

[a]Coefficients also adjusted for pack-years of smoking.
*$P<0.05$.

Table 3 Regression coefficients and standard errors for antioxidant vitamins in FEV_1%[a] and FVC%[a] models ($n=218$)

Variable (unit)	*FEV_1%* β (*s.e.*[a])	*FVC%* β (*s.e.*[a])	*FEV_1/FVC%*[b] β (*s.e.*[a])
Baseline model			
Smoking status	−6.63 (2.79)*	−2.41 (2.21)	−3.78 (1.53)*
Pack-years of smoking	−0.15 (0.07)*	−0.08 (0.06)	−0.05 (0.04)
Weight	0.14 (0.08)	−0.04 (0.06)	0.23 (0.05)**
Education	0.70 (0.60)	0.66 (047)	0.09 (0.32)
Eosinophil count	−0.29 (0.75)	—	−0.47 (0.39)
Serum vitamins entered individually[c,d]			
Vitamin C (s.d.)	2.51 (1.65)	0.48 (1.33)	1.81 (0.87)*
Vitamin E (s.d.)	2.11 (1.63)	0.69 (1.31)	1.53 (0.86)
β-Cryptoxanthin (s.d.)	4.18 (1.68)*	4.29 (1.32)**	0.52 (0.89)
Lutein/zeaxanthin (s.d.)	3.81 (1.56)*	3.18 (1.26)*	1.74 (0.85)*
β-Carotene (s.d.)	0.88 (1.72)	1.18 (1.38)	0.12 (0.92)
Lycopene (s.d.)	3.04 (1.56)	2.65 (1.25)*	−0.31 (0.89)
Retinol (s.d.)	3.80 (1.59)*	1.61 (1.27)	2.46 (0.84)*
Dietary vitamins entered individually[c,e]			
Vitamin C (s.d.)	4.45 (1.93)*	3.72 (1.55)*	1.91 (0.91)*
Vitamin E (s.d.)	1.24 (2.36)	1.06 (1.91)	1.21 (0.88)
β-Cryptoxanthin (s.d.)	5.16 (1.81)*	6.01 (1.43)**	0.90 (0.91)
Lutein/zeaxanthin (s.d.)	5.10 (1.86)*	3.44 (1.50)*	2.24 (0.91)*
β-Carotene (s.d.)	6.33 (1.95)**	4.42 (1.59)*	2.59 (0.88)*
Lycopene (s.d.)	4.28 (1.79)*	2.80 (1.45)	1.78 (0.86)*
Retinol (s.d.)	−2.08 (2.29)	−0.28 (1.86)	0.28 (0.87)

[a]FEV_1%, forced expiratory volume in 1 second as percentage of predicted value; FVC%, forced vital capacity as percentage of predicted value; s.e., standard error.
[b]FEV_1/FVC% models also adjusted for age, gender, race, and height.
[c]All models evaluating vitamins also included variables listed in baseline model.
[d]$n=218$.
[e]$n=207$.
*$P<0.05$; **$P\leqslant0.001$.

and oxidative stress markers. We identified negative associations of glutathione with FEV_1%, and TBARS with FVC% ($P<0.035$). Our final models contained antioxidant vitamins and oxidative stress markers, with no evidence for statistical interaction between them. The models that explained the greatest proportion of variance in FEV_1% included baseline variables, one of three positively associated antioxidants (lutein/zeaxanthin, retinol, or β-cryptoxanthin), and inversely associated glutathione. The FVC% model explaining the greatest proportion of variation included baseline variables, positively associated serum antioxidants (lycopene, lutein/zeaxanthin, or β-cryptoxanthin), and inversely associated TBARS.

Table 5 shows differences in antioxidants and oxidative stress markers according to GOLD categories (stage 0–IV) (Pauwels *et al*., 2001). We classified individuals according to prebronchodilator spirometry and compared lipid-adjusted antioxidant concentrations and energy-adjusted dietary intakes across categories, using category 0 as the reference group. We identified lower concentrations of serum vitamin C in category II and retinol in category IV. Dietary vitamin C and β-carotene was significantly lower in categories II and III. Also, β-cryptoxanthin intake was lowest in the most severe category. Plasma TBARS was lower in category II, while TEAC was significantly higher in the most severe category.

When we stratified by asthma and COPD status to analyze associations of antioxidants and oxidative stress markers with pulmonary function, we found regression coefficients that were in the same direction. Lycopene showed a stronger association with pulmonary function in participants with asthma, while lutein/zeaxanthin and retinol was more strongly associated in patients with COPD.

Discussion

In this population-based study, we analyzed the association of antioxidant vitamins (dietary intake and serum levels) and oxidative stress with pulmonary function in individuals with chronic airflow limitation from the general population. We found positive associations of serum and dietary antioxidant vitamins and negative associations of oxidative stress markers with FEV_1% and FVC%, respectively. Our findings contribute evidence for antioxidant and oxidant involvement in reduced pulmonary function in a sample of individuals with chronic airflow limitation.

Several explanations for our findings are conceivable. Individuals with chronic airflow limitation may have lower antioxidant intakes influencing their pulmonary function or may have altered their intake following diagnosis by a physician. However, we did not find statistically significant differences in energy-adjusted intake of any antioxidants between individuals with and without chronic airflow limitation (Schünemann *et al*., 2002). We did, however, find lower serum concentrations of lutein/zeaxanthin, lycopene, β-cryptoxanthin, retinol, and β-carotene in men and women in the CAL group as compared to those without ($P<0.05$) (Schünemann *et al*., 2001b). So, similar intakes and lower

Table 4 Regression coefficients and standard errors for oxidative stress/antioxidant markers in FEV_1%[a], FVC%[a], and FEV_1/FVC[a] models ($n=200$)

Variable (unit)	*FEV_1%*		*FVC%*		*FEV_1/FVC%*	
	β (s.e.[a])	R^2	*β (s.e.[a])*	R^2	*β (s.e.[a])*	R^2
Baseline model		*0.111*		*0.029*		*0.294*
Oxidative stress markers entered individually[b]						
TBARS	−8.91 (5.9)	0.117	−9.50 (4.7)*	0.044	0.01 (3.08)	0.281
Glutathione	−11.86 (5.6)*	0.127	−8.16 (4.5)	0.040	−3.45 (2.98)	0.286
Glutathione-peroxidase	7.09 (10.2)	0.109	13.15 (8.0)	0.037	−9.91 (5.41)	0.293
TEAC	−10.51 (19.5)	0.108	−0.61 (15.7)	0.024	−8.67 (10.46)	0.408
Serum vitamins and oxidative stress markers entered together[b]						
Lutein/zeaxanthin (s.d.)	3.61 (1.6)*	0.141				
Glutathione	−11.93 (5.6)*					
Retinol (s.d.)	4.24 (1.7)*	0.151				
Glutathione	−11.89 (5.5)*					
β-Cryptoxanthin (s.d.)	4.38 (1.8)*	0.170				
Glutathione	−10.93 (5.5)*					
Lycopene (s.d.)			2.68 (1.3)*	0.078		
TBARS			−9.26 (4.6)*			
Lutein/zeaxanthin (s.d.)			3.05 (1.3)*	0.085		
TBARS			−9.42 (4.6)*			
β-Cryptoxanthin (s.d.)			4.58 (1.4)**	0.089		
TBARS			−10.62 (4.6)*			

[a]FEV_1%, forced expiratory volume in 1 second as percentage of predicted value; FVC%, forced vital capacity as percentage of predicted value; s.e., standard error.
[b]Baseline models include smoking status, total pack-years, weight, education, and eosinophils in FEV_1 models only.
*$P<0.05$; **$P\leqslant0.001$.

Table 5 Differences in antioxidants and oxidative stress markers according to GOLD category. Mean (s.d.)

	Category 0 (n = *136*)	*Category I* (n = *15*)	*Category II* (n = *37*)	*Category III* (n = *23*)	*Category IV* (n = *7*)
Pulmonary function					
FEV_1%	91.68 (17.2)	92.40 (9.89)	64.84 (8.24)	40.83 (6.67)	25.86 (5.69)
FVC%	90.57 (16.1)	110.65 (9.27)	85.69 (13.19)	66.93 (16.21)	54.98 (14.10)
FEV_1/FVC%	78.58 (5.15)	64.03 (4.53)	59.42 (8.12)	49.13 (11.60)	37.67 (9.29)
Serum antioxidants					
Vitamin C (mg/dl)	1.31 (0.62)	1.20 (0.52)	1.03 (0.58)*	1.17 (0.69)	1.09 (0.67)
Vitamin E (μg/ml)	15.96 (9.43)	14.37 (6.38)	14.82 (9.48)	16.32 (7.77)	10.45 (2.68)
β-Cryptoxanthin (μg/ml)	0.10 (0.08)	0.08 (0.05)	0.08 (0.07)	0.06 (0.04)	0.07 (0.05)
Lutein/zeaxanthin (μg/ml)	0.13 (0.06)	0.15 (0.06)	0.13 (0.06)	0.12 (0.05)	0.11 (0.03)
β-Carotene (μg/ml)	0.19 (0.23)	0.25 (0.40)	0.14 (0.14)	0.15 (0.11)	0.13 (0.07)
Lycopene (μg/ml)	0.45 (0.29)	0.43 (0.22)	0.39 (0.21)	0.42 (0.18)	0.57 (0.25)
Retinol (μg/ml)	0.56 (0.16)	0.61 (0.16)	0.55 (0.16)	0.54 (0.17)	0.51 (0.12)*
Dietary antioxidants (adjusted for energy intake)					
Vitamin C	0.09 (0.04)	0.08 (0.04)	0.07 (0.04)*	0.06 (0.03)*	0.03 (0.03)
Vitamin E	0.006 (0.003)	0.005 (0.002)	0.005 (0.002)	0.005 (0.003)	0.006 (0.002)
β-Cryptoxanthin	0.14 (0.09)	0.15 (0.08)	0.13 (0.10)	0.13 (0.09)	0.08 (0.02)*
Lutein/zeaxanthin	1.42 (1.22)	1.20 (0.66)	1.19 (0.76)	0.95 (0.52)	0.93 (0.59)
β-Carotene	2.20 (1.44)	2.24 (1.12)	1.65 (0.93)*	1.51 (0.83)*	1.44 (0.85)
Lycopene	1.90 (1.51)	1.83 (1.17)	1.36 (0.97)	1.41 (1.14)	1.46 (0.89)
Retinol	0.44 (0.17)	0.45 (0.17)	0.48 (0.30)	0.46 (0.18)	0.59 (0.26)
Oxidative stress markers:					
Plasma-TBARS (nmol/ml)	1.50 (0.51)	1.21 (0.24)*	1.47 (0.49)	1.45 (0.58)	1.38 (0.39)
Glutathione (ng/dl PRBC)	54.38 (15.07)	54.68 (18.19)	56.73 (20.27)	59.83 (20.07)	52.23 (13.20)
Glutathione peroxidase (IU)	614.68 (96.97)	623.00 (130.73)	622.73 (95.82)	626.71 (106.14)	623.57 (69.55)
TEAC (%)	71.80 (5.77)	74.34 (5.80)	72.11 (5.13)	71.91 (6.24)	78.86 (5.78)*

*$P < 0.05$ for differences in triglyceride-adjusted serum antioxidants, energy adjusted dietary intakes, and oxidative stress markers compared to category 0.

concentrations in peripheral blood suggest that the disease process may deplete antioxidant capacity, perhaps via oxidative stress (Taylor *et al*., 1986), or via other related metabolic processes that are associated with systemic inflammation (Andreassen and Vestbo, 2003). Further evidence supporting antioxidant depletion comes from the low correlations between serum and dietary antioxidant measurements in this study; coefficients ranged from 0.12 to 0.22 for the carotenoids. Previously published correlation coefficients (in individuals without chronic airflow limitation) between carotenoids in serum and diet were larger, ranging from 0.24 to 0.53 (Forman *et al*., 1993; Tucker *et al*., 1999). However, the low correlation between serum and dietary vitamins may also be due to a gap in time between measurements, as dietary intake was estimated for the period 12–24 months prior to blood measurements.

We have previously reported on the association between serum antioxidants and pulmonary function in this population restricted to individuals free of chronic airflow limitation. We found positive associations between serum vitamins C and E, β-cryptoxanthin, and lutein/zeaxanthin with FEV_1%, and β-cryptoxanthin and lutein/zeaxanthin with FVC% (Schünemann *et al*., 2001b). In this sample of individuals with chronic airflow limitation, we found similar associations between pulmonary function and β-cryptoxanthin and lutein/zeaxanthin. The only important difference relates to the association of vitamins C and E; we did not find statistically significant associations of serum vitamins C or E with FEV_1 or FVC in individuals with chronic airflow limitation. This difference may be partly due to our unique sample of individuals with chronic airflow limitation, or because carotenoids may be specific to the inflammatory process of chronic airflow limitation.

In our analysis of the relation between dietary intake of antioxidants and pulmonary function in the general population free of chronic airflow limitation, we found positive associations of dietary vitamin E, lutein/zeaxanthin, and vitamin C with FEV_1% and FVC% (Schünemann *et al*., 2002). Similarly, in the current analysis of individuals with chronic airflow limitation, intake of vitamin C and lutein/zeaxanthin was positively associated with pulmonary function. Other dietary antioxidants associated with pulmonary function included β-cryptoxanthin, β-carotene, and lycopene. Two comprehensive reviews on antioxidants and pulmonary function pointed to limited evidence for an association with β-carotene (Romieu and Trenga, 2001; Schünemann *et al*., 2001a) but carotenoids other than β-carotene have not been examined in detail. This study and our previous analyses suggest that β-cryptoxanthin and lutein/zeaxanthin are more strongly associated with pulmonary function than β-carotene (Schünemann *et al*., 2001b, 2002). Although β-carotene and other carotenoids are highly correlated with

each other, β-cryptoxanthin and lutein/zeaxanthin may exert tissue specific effects that differ from those of β-carotene (Olson, 1999). The only other study investigating carotenoids other than β-carotene and pulmonary function was that of Grievink *et al*., 2000 in a sample of Dutch elderly. The authors described an association between serum β-carotene and FEV_1 and FVC, and lycopene with FEV_1. We found a similar association of FVC and lycopene, but not for β-carotene. Differences in study populations may explain these contradictory findings. Our sample included individuals with chronic airflow limitation who were younger and had greater cigarette smoke exposure. β-Cryptoxanthin and lutein/zeaxanthin showed a stronger association in this sample, suggesting that these particular antioxidants may play a role in the response to oxidative stress in chronic airflow limitation.

The stratified analysis by asthma and COPD status showed regression coefficients that were in the same direction. While lycopene was more strongly associated with pulmonary function in those carrying a diagnosis of asthma, and lutein/zeaxanthin and retinol in those with COPD, we interpret these results with caution due to limited sample size within strata. We did not find evidence for effect medication by smoking, which may be due to the small number of current smokers ($n=48$) in the sample.

We previously also analyzed the relation of oxidative stress and pulmonary function in a sample of nonsmokers free of respiratory disease. We found a negative association of FEV_1% and TBARS, a marker for lipid peroxidation (Schünemann *et al*., 1997). In the current analysis, we found a negative association of TBARS with FVC, but not with FEV_1. Investigators have criticized TBARS for its lack of sensitivity and specificity as a marker of lipid peroxidation. In spite of this limitation, we found a negative association with pulmonary function.

Glutathione is a potent intra- and extra-cellular antioxidant found in large quantities in epithelial lining fluid in the lung (Cantin *et al*., 1989). In this sample of patients with chronic airflow limitation and in our previous study of healthy individuals, we found a statistically significant *inverse* association of glutathione and FEV_1 (Schünemann *et al*., 1997). We speculate that an inverse selection is meaningful, as the body attempts to compensate for lower levels of glutathione in tissues during more severe disease. If serum antioxidant availability declines, it is reasonable to hypothesize that glutathione concentrations are augmented.

We found positive correlations of plasma glutathione-peroxidase with pulmonary function that did not reach statistical significance. Glutathione peroxidase is an important enzyme in the oxidation–reduction pathway of glutathione (Thomas, 1999). It is unclear how this enzyme is regulated in states of chronic inflammation like chronic airflow limitation. Differential regulation of this enzyme may explain our lack of a statistically significant association.

Trolox equivalent antioxidant capacity (TEAC) is a measure of serum antioxidant activity; therefore, we hypothesized a positive association with pulmonary function. Although TEAC levels are lower in patients with COPD as compared to nonsmokers (Rahman *et al*., 1996, 2000), this study and another in patients with COPD found no significant association of TEAC with pulmonary function (Rahman *et al*., 2000).

The major strength of this work lies in the simultaneous measurement of serum antioxidant vitamin levels, oxidative stress markers, dietary antioxidant intake, and measurement of several other potentially confounding factors. We used serum biomarkers to investigate the association of antioxidants potentially reaching tissues, and a food frequency questionnaire to estimate long-term nutritional exposure. We found agreement in serum and dietary results, especially for β-cryptoxanthin and lutein/zeaxanthin. Differences in the relation with pulmonary function by measurement type may be due to inherent inaccuracies in collection and estimation of intake using a food frequency questionnaire. On the other hand, serum antioxidant levels are associated with measurement error and it is not clear whether they are better indicators of antioxidant status. Thus, combining these measures and finding agreement with different exposure measurements strengthens the plausibility of the findings.

The problem inherent in the use of oxidative stress markers as indicators of oxidative stress lies in their validity and reproducibility. When we investigated correlation of serum antioxidants and oxidative stress markers, we found weak but statistically significant correlation between them, suggesting that they may be opposing mediators in oxidative homeostasis.

The most unexpected finding was the lack of a difference in levels of oxidative stress across smoking categories. This may be partly due to our sample of individuals with CAL, who may already have high levels due to the disease process or the absence of an effect of tobacco smoke on the biomarkers we considered in this analysis.

The cross-sectional nature is a limitation of our study, as – just like any other cross-sectional study – it does not provide information about the temporal sequence. As of this study design, we were unable to evaluate whether individuals have increased vitamin intake following their diagnosis of airflow limitation. As described previously, antioxidant intakes were similar to those in the general population sample without chronic airflow limitation; however, we cannot rule out this possibility. This change in intake would bias the observed associations toward the null. Our method of identifying individuals with chronic airflow limitation has limitations. We identified individuals from the general population who self-reported having asthma or COPD and, therefore, they may not be fully representative of these patients in the general population.

Another possible limitation is selection bias resulting from the moderate participation rate of 59.4%. In addition, we investigated antioxidant vitamins individually to evaluate vitamin-specific associations with pulmonary function.

However, uncharacterized or unmeasured antioxidant compounds in food items highly correlated with the antioxidants described here may be responsible for the observed associations.

In summary, we found correlations of serum antioxidants and oxidative stress markers, suggesting that they may be acting as opposing members of the antioxidant/oxidant pathway. We observed positive associations between β-cryptoxanthin and lutein/zeaxanthin with pulmonary function, and negative associations of glutathione and TBARS with pulmonary function. The latter associations persisted after consideration of antioxidant vitamins. These results further strengthen the evidence that the antioxidant/oxidant balance is associated with pulmonary function and that carotenoids other than β-carotene may play a role.

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