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Original Contribution

Oxidative Stress and Pulmonary Function in the General Population

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Studies have shown increased oxidative stress in patients with chronic airflow limitation; however, the population-based evidence for the association of oxidative stress with pulmonary function is limited. The authors analyzed the association of plasma thiobarbituric acid-reactive substances (TBARS), glutathione, glutathione peroxidase, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)-equivalent antioxidant capacity with forced expiratory volume in 1 second and forced vital capacity using data collected from 1996 to 2000 in a general population sample from western New York State ($n = 2{,}346$). After adjustment for covariates including smoking status, lifetime pack-years of smoking, education, weight, and eosinophils, multivariate analysis showed an inverse association of TBARS with forced expiratory volume in 1 second and forced vital capacity as the percentage of the predicted value (FEV_1% and FVC%, respectively), positive associations of glutathione peroxidase with FEV_1% and FVC%, and an inverse association of glutathione with FEV_1% in men ($p < 0.05$). The associations of TBARS and glutathione peroxidase with FVC% in men remained statistically significant after adjustment for serum carotenoid levels. There were no statistically significant associations of oxidative stress with pulmonary function in women. These results suggest that oxidative stress may be associated with airflow limitation in men, and that gender differences may exist in the relation of oxidative stress to pulmonary function.

forced expiratory volume; glutathione; glutathione peroxidase; oxidative stress; respiratory function tests; thiobarbituric acid reactive substances; vital capacity

Abbreviations: FEV_1, forced expiratory volume in 1 second; FEV_1%, forced expiratory volume in 1 second as the percentage of the predicted value; FVC, forced vital capacity; FVC%, forced vital capacity as the percentage of the predicted value; TBARS, thiobarbituric acid-reactive substances; TEAC, Trolox-equivalent antioxidant capacity.

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Oxidative stress resulting from an imbalance of reactive oxidant species and antioxidants (1, 2) plays a role in a number of disease processes including impaired pulmonary function (3, 4). Lung tissues are particularly susceptible to oxidative damage, because of direct contact with oxidants in ambient air (5, 6). An individual's ability to alleviate oxidative stress in tissues depends on endogenous enzymatic and nonenzymatic pathways, as well as exogenous factors including the intake of antioxidant nutrients (1).

Although there is abundant evidence that oxidative stress increases in a number of pulmonary diseases (7–12), there is limited population-based evidence for the relation of oxidative stress biomarkers to pulmonary function. We previously analyzed this relation in a pilot study of 132 nonsmokers (predominantly male) from the general population and found an inverse association of plasma thiobarbituric acid-reactive substances (TBARS), a biomarker of lipid peroxidation, with forced expiratory volume in 1 second (FEV_1), suggesting that oxidative damage is associated with lower pulmonary function (3).

While oxidative stress may be a contributing factor to impaired pulmonary function, the ability of the body to counteract oxidative stress may be equally important. Thus, exposure to antioxidant nutrients, which can quench oxidizing radicals (13), would be important to consider. We and others have reported a positive association of antioxidant vitamin status (including carotenoids) with pulmonary function in the general population (4, 14–18), in particular for carotenoids. Positive associations of individual antioxidant vitamins with pulmonary function suggest that the underlying mechanism is one of oxidative stress.

In this article, we expand our preliminary observations and investigate whether higher levels of oxidative stress biomarkers are associated with worse pulmonary function in a general population sample. We further hypothesize that these associations may be attenuated when antioxidant nutrient status is considered.

MATERIALS AND METHODS

We randomly selected participants ($n = 4{,}041$) from the general population in Erie and Niagara counties in western New York State between 1995 and 2000 as previously described (4). We excluded individuals from the sample who reported diagnosis of chronic lung disease ($n = 348$), missing or not acceptable pulmonary function tests ($n = 1{,}047$), missing oxidative stress biomarkers ($n = 117$), and missing covariates ($n = 183$), resulting in 1,197 men and 1,149 women for the analyses. We compared individuals who were excluded but for whom some data were available for analysis in terms of age, education, smoking status, pulmonary function, and oxidative stress markers. We found no differences in age, body mass index, lifetime pack-years of smoking, FEV_1 (l), forced expiratory volume in 1 second as the percentage of the predicted value (FEV_1%), TBARS, glutathione, or 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)-equivalent antioxidant capacity (TEAC) ($p > 0.05$). However, excluded individuals had fewer years of education and lower forced vital capacity (FVC) (l) and forced vital capacity as the percentage of the predicted value (FVC%), as well as higher concentrations of glutathione peroxidase ($p < 0.05$). However, we have no additional data for those whom we contacted but who refused to give further information. In terms of classifying according to chronic lung disease, we used positive answers to questions about physician-diagnosed lung disease.

Pulmonary function tests

Trained personnel performed spirometry tests between 6:30 and 9:30 a.m. We used multiple linear regression to derive FEV_1 and FVC prediction equations for men and women using lifelong nonsmokers free of respiratory disease ($n = 1{,}033$) from our sample. We then calculated the percentage of the following prediction equations for pulmonary function in men:

$$\text{Predicted FEV}_1 = -1.022 - 0.0302\,(\text{age}) + 3.640\,(\text{height}) - 0.555\,(\text{race})$$

$$\text{Predicted FVC} = -2.976 - 0.0334\,(\text{age}) + 5.432\,(\text{height}) - 0.690\,(\text{race})$$

and in women:

$$\text{Predicted FEV}_1 = 0.0003244 - 0.0272\,(\text{age}) + 2.601\,(\text{height}) - 0.357\,(\text{race})$$

$$\text{Predicted FVC} = -0.788 - 0.0291\,(\text{age}) + 3.600\,(\text{height}) - 0.564\,(\text{race})$$

We computed the correlation of FEV_1 and FVC as the percentages of predicted values with published prediction equations from the National Health and Nutrition Examination Survey (19) and obtained coefficients of 0.97 for FEV_1% and 0.98 for FVC%.

Blood determinations

The protocol prescribed that a trained phlebotomist collected blood samples between 7:30 a.m. and 9:30 a.m. after an overnight fasting. Samples were stored on ice and processed within 30 minutes for glutathione measurement and within 2 hours for other oxidative stress biomarkers. We stored the samples at −70°C until measurement in batches.

We measured the following blood parameters: plasma TBARS (nmol/ml of malondialdehyde equivalents) (20); erythrocytic glutathione (mg/dl of packed red blood cells) (21); plasma glutathione peroxidase using a Cobas Mira automated chemistry analyzer (22); and fat-soluble vitamins (μg/ml) using a Shimadzu LC-7A high-pressure liquid chromatography system with a SPD-M6A photodiode array (Shimadzu Scientific Instruments, Inc., Braintree, Massachusetts) (23). Intraassay coefficients of variation for TBARS, glutathione, glutathione peroxidase, and TEAC were 7.55, 3.26, 4.35, and 8.5 percent, respectively. Interassay coefficients of variation for TBARS, glutathione, glutathione peroxidase, and TEAC were 9.20, 3.97, 8.57, and 9.95 percent, respectively (3).

Interview

Interviewers collected demographic information, smoking behaviors, and physical measurements during in-person interviews. We classified individuals as current smokers if they were smoking at the time of the study and as never smokers if they had smoked less than 100 cigarettes in their lifetime. We classified respondents as former smokers if they had smoked more than 100 cigarettes but were not currently smoking. We also collected lifetime smoking histories by decade to compute lifetime pack-years (multiplying the number of years smoked by the number of cigarettes smoked per day divided by 20). Trained interviewers measured height and weight according to a strict protocol.

Statistical analysis

We computed means and standard deviations for all relevant variables and tested predictor variables for linearity and interaction. Oxidative stress variables were normally distributed except for the TBARS measurement, which exhibited a slight deviation from normal. We conducted the bivariate correlations and linear regression analyses with and without transforming TBARS but observed no important differences. Therefore, we presented all results using nontransformed TBARS.

Partial correlation coefficients were used to assess the relation among oxidative stress parameters, adjusted for gender, smoking, and age. The following variables were considered as covariates in multivariable models because they are associated with pulmonary function: smoking status, lifetime pack-years of tobacco smoking, education, weight, and eosinophils. We modeled smoking using smoking status as a three-level variable and lifetime pack-years as a continuous variable. We considered interactions statistically significant at the 10 percent significance level.

Because of the biologic pathways of glutathione as a substrate for glutathione peroxidase and the anticipated correlation between the two variables that might cause collinearity, we entered each oxidative stress biomarker individually in multivariable models. A priori we decided to stratify the sample by smoking status, although the interaction terms for smoking status and total pack-years with oxidative stress markers were not statistically significant ($p > 0.10$). Our regression models based on FEV_1 and FVC as the percentages of the predicted value include an adjustment for height. Therefore, weight is a more appropriate term to adjust for compared with body mass index. However, when we replaced weight with body mass index in the regression models to explore whether adjustment for body mass index would alter the results, we found no differences in the relation between oxidative stress biomarkers and pulmonary function.

We used raw FEV_1 and FVC values to investigate modification of the oxidative stress and pulmonary function association by gender and body mass index, while adjusting for age, height, race, and other covariates. We analyzed trends in FEV_1% and FVC% across oxidative stress quartiles using general linear models and adjustment for the previously mentioned covariates. Using multiple linear regression analysis, we investigated the associations of oxidative stress biomarkers with FEV_1% and FVC%. In a subsample with available serum vitamin data ($n = 1{,}605$), we examined the differences in serum antioxidants and the influence of antioxidants on the association of oxidative stress with pulmonary function. We used software from SPSS, Inc. (Chicago, Illinois), for the analyses (24).

TABLE 1. Demographics and descriptors for individuals aged 35–79 years ($n = 2{,}346$), Erie and Niagara counties, New York, 1996–2000

	Men† (mean (SD‡) or %)	Women† (mean (SD) or %)
Variable (unit)		
Age (years)	57.8 (11.4)	60.8 (11.9)**
African American (%)	7.5	7.0
Education (% high school degree or higher)	90.3	91.8
Body mass index (kg/m^2)	28.4 (4.5)	27.8 (6.0)*
FEV_1‡ (liter)	3.33 (0.83)	2.57 (0.57)**
FVC‡ (liter)	4.37 (0.97)	3.30 (0.69)**
FEV_1%‡	95.41 (17.69)	98.24 (15.11)**
FVC%‡	97.89 (16.13)	99.51 (14.78)*
FEV_1/FVC (%)	76.11 (8.27)	77.89 (7.05)**
Smoking status (%)		
Current smoker	12.4	14.8
Former smoker	51.1	34.6
Never smoker	36.4	50.7
Lifetime smoking (pack-years)	17.57 (22.45)	9.06 (15.39)**
Oxidative stress biomarkers		
TBARS‡ (nmol/ml)	1.44 (0.45)	1.33 (0.39)**
Glutathione (ng/dl of PRBC‡)	51.52 (15.43)	56.84 (15.12)**
Glutathione peroxidase (IU)	614.85 (90.14)	610.23 (89.62)
TEAC‡ (%)	72.76 (4.99)	71.45 (5.28)**

* $p < 0.05$; **$p < 0.001$ for sex differences.

† Men: $n = 1{,}197$; women: $n = 1{,}149$.

‡ SD, standard deviation; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEV_1%, forced expiratory volume in 1 second as the percentage of the predicted value; FVC%, forced vital capacity as the percentage of the predicted value; TBARS, thiobarbituric acid-reactive substances; PRBC, peripheral red blood cells; TEAC, Trolox-equivalent antioxidant capacity.

RESULTS

Table 1 shows the characteristics of individuals included in this study. Women were slightly older than men ($p < 0.001$). The sample contained slightly more men than women and few African Americans. Approximately 56.6 percent were current or former cigarette smokers, with a mean of 13.4 pack-years of smoking. Raw FEV_1 and FVC values were higher for men than women ($p < 0.001$).

TABLE 2. Trends in FEV_1%* and FVC%* by oxidative stress biomarkers for individuals aged 35–79 years, Erie and Niagara counties, New York, 1996–2000

	Men†									
	Quartile I (lowest)		Quartile II		Quartile III		Quartile IV (highest)		Difference between quartiles IV and I	p_{trend}‡
	Mean	95% confidence interval	Mean	95% confidence interval	Mean	95% confidence interval	Mean	95% confidence interval		
					FEV_1%					
TBARS*	97.4	95.5, 99.2	94.8	92.9, 96.6	95.0	93.2, 96.9	94.5	92.6, 96.4	− 2.874	0.13
Glutathione	96.8	94.9, 98.7	96.6	94.7, 98.5	94.8	93.0, 96.7	93.4	91.6, 95.3	− 3.383	0.04
Glutathione peroxidase	93.4	91.5, 95.3	96.3	94.4, 98.2	95.7	93.8, 97.5	96.3	94.4, 98.2	2.917	0.10
TEAC*	94.4	92.6, 96.3	95.2	93.3, 97.1	95.8	93.8, 97.7	96.3	94.4, 98.2	1.862	0.54
					FVC%					
TBARS	100.2	98.4, 102.0	97.9	96.2, 99.7	96.7	95.0, 98.5	96.7	94.9, 98.4	−0.551	0.02
Glutathione	98.7	97.0, 100.5	99.2	97.4, 100.9	97.4	95.7, 99.2	96.2	94.5, 98.0	0.521	0.09
Glutathione peroxidase	96.2	94.4, 97.9	98.1	96.3, 99.8	98.7	97.0, 100.5	98.6	96.8, 100.3	2.383	0.17
TEAC	97.0	95.2, 98.7	98.3	96.5, 100.0	97.4	95.6, 99.2	99.0	97.2, 100.7	2.002	0.39
	Women†									
	Quartile I (lowest)		Quartile II		Quartile III		Quartile IV (highest)		Difference between quartiles IV and I	p_{trend}‡
	Mean	95% confidence interval	Mean	95% confidence interval	Mean	95% confidence interval	Mean	95% confidence interval		
					FEV_1%					
TBARS	98.2	96.5, 99.9	98.5	96.8, 100.2	98.5	96.8, 100.2	97.7	96.0, 99.4	−0.507	0.90
Glutathione	99.3	97.6, 101.0	97.5	95.8, 99.2	97.1	95.4, 98.7	99.1	97.5, 100.8	−0.187	0.14
Glutathione peroxidase	98.5	96.8, 100.2	98.3	96.6, 100.0	98.2	96.6, 99.9	98.0	96.3, 99.6	−0.541	0.98
TEAC	98.4	96.7, 100.1	99.1	97.4, 100.8	97.5	95.9, 99.1	98.0	96.3, 99.7	−0.357	0.59
					FVC%					
TBARS	99.9	98.2, 101.6	100.0	98.4, 101.7	99.8	98.1, 101.5	98.3	96.6, 100.0	−1.609	0.45
Glutathione	100.7	99.0, 102.3	99.2	97.5, 100.8	98.4	96.7, 100.0	99.9	98.2, 101.6	−0.742	0.26
Glutathione peroxidase	99.5	97.8, 101.1	99.4	97.7, 101.1	99.6	98.0, 101.3	99.6	97.9, 101.2	0.112	0.99
TEAC	98.8	97.1, 100.5	99.9	98.2, 101.6	100.4	98.8, 102.1	98.8	97.1, 100.5	0.019	0.43

* FEV_1%, forced expiratory volume in 1 second as the percentage of the predicted value; FVC%, forced vital capacity as the percentage of the predicted value; TBARS, thiobarbituric acid-reactive substances; TEAC, Trolox-equivalent antioxidant capacity.

† Men: $n = 1{,}197$; women: $n = 1{,}149$.

‡ Trends adjusted for smoking status, pack-years of smoking, weight, education, and eosinophils.

However, once we adjusted for age, height, and race, women had higher values of FEV_1% and FVC% than did men ($p < 0.01$). Men had higher serum levels of TBARS, lower levels of glutathione, and higher TEAC levels ($p < 0.001$). We found no differences in oxidative stress biomarkers by smoking status (data not shown). Serum concentrations of raw and triglyceride-adjusted β-cryptoxanthin and vitamin E were higher in women ($p < 0.001$) (data not shown).

Partial correlation coefficients (r) among oxidative stress biomarkers ranged from −0.064 to −0.210. We observed the strongest inverse correlation of glutathione and glutathione peroxidase ($r = -0.210$; $p < 0.001$) and the weakest inverse correlation between TBARS and TEAC ($r = -0.064$; $p < 0.002$) (data not shown).

We explored the interaction of gender and oxidative stress biomarkers using FEV_1% and FVC%. We found statistically significant interaction terms for gender and each of the biomarkers in FVC models but not in FEV_1 models. Therefore, we stratified further analyses by gender.

Table 2 summarizes trends in pulmonary function by quartiles of oxidative stress biomarkers in men and women. We found a borderline-significant negative trend in glutathione and FEV_1 ($p_{trend} < 0.04$) and a negative association of TBARS and FVC ($p_{trend} < 0.02$) in men. We did not observe

TABLE 3. Regression coefficients for oxidative stress biomarkers in FEV_1† and FVC† models for individuals, stratified by sex, aged 35–79 years ($n = 2,346$), Erie and Niagara counties, New York, 1996–2000‡

Variable (unit)	FEV_1%† (β (SE†))	FVC%† (β (SE))
Men ($n = 1,197$)		
TBARS† (nmol/ml)	−2.560 (1.07)*	−3.566 (1.00)**
Glutathione (ng/dl of PRBC†)	−0.078 (0.03)*	−0.049 (0.03)
Glutathione peroxidase (IU)	0.012 (0.01)*	0.013 (0.01)*
TEAC† (%)	0.028 (0.10)	0.030 (0.09)
Women ($n = 1,149$)		
TBARS (nmol/ml)	0.252 (1.11)	−1.023 (1.10)
Glutathione (ng/dl of PRBC)	−0.016 (0.03)	−0.013 (0.03)
Glutathione peroxidase (IU)	−0.001 (0.01)	0.004 (0.01)
TEAC (%)	−0.102 (0.08)	−0.053 (0.08)

* $p < 0.05$; **$p \leq 0.001$.

† FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEV_1%, forced expiratory volume in 1 second as the percentage of the predicted value; SE, standard error; FVC%, forced vital capacity as the percentage of the predicted value; TBARS, thiobarbituric acid-reactive substances; PRBC, peripheral red blood cells; TEAC, Trolox-equivalent antioxidant capacity.

‡ Models adjusted for smoking status, lifetime pack-years, education, weight, and eosinophils.

statistically significant trends in pulmonary function for any oxidative stress biomarkers in women (all $p_{trend} > 0.14$).

Table 3 summarizes linear regression coefficients for oxidative stress biomarkers entered individually into the baseline model. We identified inverse associations of TBARS and glutathione with FEV_1% and a positive association of glutathione peroxidase with FEV_1% (all $p < 0.02$). Similarly, TBARS was also inversely associated with FVC, and glutathione peroxidase was positively associated with FVC% in men ($p < 0.01$). In women, we found no statistically significant associations of any oxidative stress biomarkers with FEV_1% or FVC% (all $p > 0.21$).

Table 4 shows the association of TBARS with FEV_1 and FVC stratified by smoking status and gender. The inverse association of TBARS with FEV_1 was strongest in never smoking men, while the association of glutathione peroxidase with FEV_1 and FVC was strongest in former smokers. In women, we observed no statistically significant associations of oxidative stress biomarkers with pulmonary function after stratification by smoking status.

Table 5 summarizes results from a subsample, which was used to investigate simultaneously the associations of serum β-cryptoxanthin, vitamin E, and oxidative stress biomarkers with pulmonary function. There was no important collinearity between serum antioxidants and the oxidative stress biomarkers. When we entered β-cryptoxanthin into FEV_1 models for men, the inverse association with TBARS and glutathione, while attenuated, remained in the same direction. In other models adjusted for serum antioxidants, TBARS was a statistically significant negative predictor of FVC, and glutathione peroxidase was a statistically significant positive predictor of FVC. In women, we found evidence for a statistically significant interaction between β-cryptoxanthin and TBARS. In models adjusted for serum antioxidants, there was little or no association of glutathione, glutathione peroxidase, or TEAC with pulmonary function in women.

The interaction terms for body mass index and TBARS, as well as weight and TBARS, in women were statistically significant for FVC. When we stratified women by body mass index to further investigate this interaction, we found an inverse association of TBARS with FVC in women with a body mass index of more than 30 kg/m^2 that did not reach statistical significance. All other interaction terms for men and women and other oxidative stress markers were not statistically significant.

When we stratified women by menopausal status, we found no differences in pulmonary function, higher TBARS and TEAC in postmenopausal women ($p < 0.02$), and borderline-significant lower glutathione concentrations in postmenopausal women ($p < 0.05$). However, we found no statistically significant associations of oxidative stress biomarkers with pulmonary function after stratification by menopausal status (data not shown).

DISCUSSION

In this population-based study, we found gender differences in the association of oxidative stress with pulmonary function. We identified inverse associations of TBARS and glutathione with pulmonary function and positive associations of glutathione peroxidase with pulmonary function in men. We did not observe significant associations between oxidative stress biomarkers with pulmonary function in women.

''Oxidative stress'' is a term used to describe an imbalance of antioxidants and prooxidants favoring prooxidants. We chose to investigate the relation of oxidative stress in the context of this study using a marker for lipid peroxidation (TBARS), members of the glutathione pathway (reduced glutathione, glutathione peroxidase), and antioxidant capacity (TEAC) in peripheral blood as biomarkers of oxidative stress.

Similar to the observations in our previous analyses, the observations in this analysis included an inverse association of TBARS with FEV_1 and FVC in men without chronic lung disease who were randomly selected from the general population. Other investigators measured TBARS in individuals with chronic airflow limitation. One study reported a higher level of TBARS during chronic obstructive pulmonary disease exacerbation that approached lower levels similar to those of healthy subjects at the time of hospital discharge (25). In another study, investigators found higher concentrations of TBARS in exhaled breath condensate of asthmatics as compared with healthy controls (26). Together, the findings of increased oxidative stress in chronic obstructive pulmonary disease and asthma and the inverse association of TBARS with pulmonary function in the general

TABLE 4. Regression coefficients for oxidative stress biomarkers entered individually into FEV_1† and FVC† models, stratified by smoking status, Erie and Niagara counties, New York, 1996–2000‡

Variable (unit)	Men		Women	
	FEV_1%† (β (SE†))	FVC%† (β (SE))	FEV_1% (β (SE))	FVC% (β (SE))
Never smokers§				
TBARS† (nmol/ml)	−4.751 (1.81)*	−6.032 (1.84)**	0.158 (1.42)	−0.980 (1.44)
Glutathione (ng/dl of PRBC†)	−0.078 (0.05)	−0.049 (0.05)	−0.049 (0.03)	−0.045 (0.04)
Glutathione peroxidase (IU)	0.007 (0.01)	0.006 (0.01)	0.010 (0.01)	0.010 (0.01)
TEAC† (%)	0.109 (0.16)	0.089 (0.16)	−0.032 (0.11)	−0.0009 (0.11)
Former smokers¶				
TBARS (nmol/ml)	−1.624 (1.45)	−2.429 (1.32)	1.520 (2.03)	−1.079 (2.08)
Glutathione (ng/dl of PRBC)	−0.085 (0.05)#	0.060 (0.04)	0.030 (0.06)	0.019 (0.06)
Glutathione peroxidase (IU)	0.019 (0.01)*	0.019 (0.01)*	−0.003 (0.01)	−0.002 (0.01)
TEAC (%)	−0.048 (0.13)	0.022 (0.12)	−0.249 (0.14)	−0.163 (0.15)
Current smokers††				
TBARS (nmol/ml)	−0.931 (3.24)	−2.115 (2.8)	−0.596 (3.20)	−0.497 (2.95)
Glutathione (ng/dl of PRBC)	−0.051 (0.08)	−0.022 (0.07)	0.022 (0.09)	0.052 (0.08)
Glutathione peroxidase (IU)	0.001 (0.02)	0.006 (0.01)	−0.023 (0.01)	0.001 (0.01)
TEAC (%)	0.124 (0.29)	0.107 (0.25)	0.107 (0.67)	0.090 (0.23)

* $p < 0.05$; ** $p \leq 0.001$.

† FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEV_1%, forced expiratory volume in 1 second as the percentage of the predicted value; SE, standard error; FVC%, forced vital capacity as the percentage of the predicted value; TBARS, thiobarbituric acid-reactive substances; PRBC, peripheral red blood cells; TEAC, Trolox-equivalent antioxidant capacity.

‡ All models include lifetime pack-years, weight, education, and eosinophils.

§ Never smokers: men, $n = 436$; women, $n = 582$.

¶ Former smokers: men, $n = 612$; women, $n = 397$.

# $p < 0.06$.

†† Current smokers: men, $n = 149$; women, $n = 170$.

population support the generally accepted idea that oxidative stress plays a key role in the etiology of obstructive lung disease and lung damage (27).

Alterations in the glutathione oxidation-reduction system may indicate depleted antioxidant capacity and oxidative stress (28, 29). We identified a negative association of glutathione with FEV_1 in this study, similar to that in our previous study (3). Some studies suggest that cellular concentrations of glutathione are reduced in response to oxidative stress (30, 31). However, increased plasma concentrations of glutathione may be interpreted as a signal for depleted glutathione in tissues followed by glutathione release from the liver (29). Another indicator of the glutathione oxidation-reduction system is the levels of the enzyme, glutathione peroxidase, which can consume hydrogen peroxide and catalyze the reaction of glutathione into oxidized glutathione (32, 33). In this study, we observed a positive association of glutathione peroxidase with pulmonary function, which is consistent with depletion of glutathione during oxidative stress and airway injury (3).

TEAC is a biomarker of antioxidant activity in peripheral blood (28, 34). The total antioxidant capacity in the sample is the amount that can inhibit free radicals in the assay, as compared with that of a water-soluble form of vitamin E, called Trolox. Rahman et al. (8) reported lower TEAC levels in healthy smokers and chronic obstructive pulmonary disease patients as compared with healthy nonsmokers, but they found no statistically significant correlations of TEAC with FEV_1 or FVC in smokers or chronic obstructive pulmonary disease patients. Similarly, in this study and our pilot study of nonsmokers, we found no statistically significant associations of TEAC with FEV_1 or FVC (3).

One finding in this study points out gender differences in the association of oxidative stress with pulmonary function. In our study, men had higher TBARS levels than did women, indicating increased oxidative stress or decreased antioxidant capacity. We found inverse associations of TBARS and glutathione with pulmonary function and a positive association of TEAC with pulmonary function in men. Women in our sample had better pulmonary function, lower levels of TBARS, higher levels of glutathione, and lower TEAC in peripheral blood than did men. In spite of these differences, we found no statistically significant associations of oxidative stress with pulmonary function in women. One possible explanation for lower levels of TBARS in women is that women had higher serum levels of the antioxidant vitamins C and E, β-carotene, and β-cryptoxanthin, which may be protective against oxidative stress, via the quenching of oxygen radicals. Women had statistically significant higher levels of triglyceride-adjusted serum

TABLE 5. Regression coefficients for oxidative stress biomarkers in FEV_1† and FVC† models, adjusted for serum antioxidants, for individuals aged 35–79 years ($n = 1,605$), Erie and Niagara counties, New York, 1996–2000‡

Variable (unit)	FEV_1%† (β (SE†))	FVC%† (β (SE))
Men ($n = 784$)‡		
β-Cryptoxanthin	1.71 (0.70)*	1.68 (0.66)*
Vitamin E	−0.002 (0.01)	0.003 (0.01)
Each biomarker entered separately into model containing antioxidants		
TBARS† (nmol/ml)	−2.01 (1.22)	−3.29 (1.14)*
Glutathione (ng/dl of PRBC†)	−0.05 (0.04)	−0.03 (0.03)
Glutathione peroxidase (IU)	0.01 (0.01)§	0.003 (0.01)*
TEAC† (%)	0.02 (0.11)	0.001 (0.10)
Women ($n = 821$)‡		
β-Cryptoxanthin	0.61 (0.63)	0.60 (0.64)*
Vitamin E	0.01 (0.01)	0.02 (0.01)*
Each biomarker entered separately into model containing antioxidants		
TBARS (nmol/ml)	0.06 (1.25)	−1.63 (1.25)
Glutathione (ng/dl of PRBC)	−0.03 (0.03)	−0.013 (0.03)
Glutathione peroxidase (IU)	−0.003 (0.01)	−0.001 (0.01)
TEAC (%)	−0.07 (0.10)	−0.07 (0.10)
Significant interactions		
β-Cryptoxanthin	−3.11 (1.75)¶	−4.47 (1.74)*
TBARS (nmol/ml)	−2.82 (1.77)	−5.55 (1.77)**
β-Cryptoxanthin × TBARS	2.71 (1.18)*	3.69 (1.18)**

* $p < 0.05$; ** $p \leq 0.002$.

† FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEV_1%, forced expiratory volume in 1 second as the percentage of the predicted value; SE, standard error; FVC%, forced vital capacity as the percentage of the predicted value; TBARS, thiobarbituric acid-reactive substances; PRBC, peripheral red blood cells; TEAC, Trolox-equivalent antioxidant capacity.

‡ Models adjusted for smoking status, lifetime pack-years, education, weight, and eosinophils. β-Cryptoxanthin and vitamin E were adjusted for serum triglycerides.

§ $p < 0.06$.

¶ $p < 0.07$.

β-cryptoxanthin, but the association of β-cryptoxanthin was not stronger in women. One could also hypothesize that gender differences in oxidative stress are mediated via a hormonal mechanism, namely, the antioxidant activity of estrogen (35). After we stratified by menopausal status, we found higher levels of TBARS in postmenopausal women, but we did not observe statistically significant associations of oxidative stress with pulmonary function. We could not fully control for estrogen levels through adjustment for menopausal status, as we did not have information on hormone replacement therapy. To our knowledge, there are very limited data on how estrogen is related to oxidative stress in the general population. The only study that investigated oxidative stress and estrogen in premenopausal women concluded that plasma estrogen could not explain the gender differences in oxidative stress (TBARS) (36). As evidence suggests that some forms of estrogen may function as antioxidants (35), further investigation of the action of estrogen and its association with oxidative stress is warranted.

When we investigated effect modification by body mass index, we identified a statistically significant interaction term for body mass index and TBARS in women. Once we stratified by body mass index (stratified at 30 kg/m^2), we found an inverse association of TBARS with FVC in women with a body mass index of 30 kg/m^2 or more versus less than that. However, this estimate of TBARS in the model did not reach statistical significance. These results suggest that body mass index may be modifying the association of oxidative stress with pulmonary function in women, but there is no conclusive evidence.

We analyzed smoking as a contributor to oxidative stress and as a potential effect modifier, as cigarette smoke contains a number of oxidants (37). Interestingly, we found no differences in the concentrations of any oxidative stress biomarkers by smoking status. We did, however, find stronger associations of oxidative stress (TBARS and glutathione peroxidase) with pulmonary function in never and former smokers. One possible explanation involves uncontrolled confounding by

passive smoking, an exposure that we did not consider. Another explanation may be that TBARS as a marker of lipid peroxidation does not measure oxidative stress due to smoking. In another related study, we identified an inverse association of TBARS and glucose and a positive association of glutathione peroxidase and glucose (38). Insulin resistance, which is recognized as an inflammatory condition (39, 40), may be related to oxidative stress and pulmonary function in never smokers. These results support the idea that there are other factors associated with oxidative stress.

Once we considered the antioxidant β-cryptoxanthin measured in serum, which we previously reported had a positive association with pulmonary function (41), the inverse associations of TBARS and glutathione with FEV_1 in men were no longer statistically significant. However, the inverse association of TBARS with FVC and the positive association of glutathione peroxidase with pulmonary function in men did remain statistically significant. In women, we identified a statistically significant inverse association of TBARS once β-cryptoxanthin entered the model, suggesting that there may be a stronger association of TBARS with pulmonary function in women who had lower levels of serum β-cryptoxanthin. However, once we categorized women by serum concentrations of β-cryptoxanthin, we reduced the sample size within strata and therefore lacked statistical power to detect important associations of oxidative stress with pulmonary function.

When we investigated differences in serum antioxidant concentrations by smoking status, we found lower levels of vitamin C, β-cryptoxanthin, and β-carotene in current smokers as compared with nonsmokers. However, these differences did not explain differences in the strength of the association between oxidative stress markers and FEV_1 or FVC.

Concurrent investigation of antioxidants and oxidative stress biomarkers poses a challenge, as they may be opposing members of the same pathway. On the other hand, the antioxidants and oxidative stress biomarkers that we investigated may represent only a fraction of oxidant homeostasis. We point to the complexity of the association between oxidative stress and antioxidants and to differences in the regulation of oxidant/antioxidant homeostasis as possible explanations for the low correlations among markers and differences in the strength of association between pulmonary function and each oxidative stress marker.

The major strength of this study is the use of highly standardized procedures for the measurement of oxidative stress biomarkers. In addition, we were able to control for a number of factors that influence pulmonary function, including antioxidant vitamin serum levels, using a large sample of men and women from the general population.

A limitation of this study relates to the cross-sectional design, as we cannot determine whether oxidative stress negatively influenced pulmonary function or vice versa. We collected blood for measurements of oxidative stress just before spirometry. Therefore, in this analysis we investigated the association of current oxidative status with pulmonary function, and these measurements may not be representative of long-term oxidative stress status in tissues. Longitudinal studies would better elucidate how oxidative stress and pulmonary function are related. Antioxidant/oxidant status is determined by an individual's ability to quench oxidative reactions, and it is likely that genetic factors and other lifestyle factors that we did not consider also confer susceptibility to oxidative damage. Furthermore, the moderate participation rate and the missing data leave the possibility for selection bias. Because of these limitations, cautioned generalization is warranted.

Evidence regarding reproducibility over time of oxidative stress markers in epidemiologic studies is sparse. Similarly, there is little evidence about the correlation between blood markers and markers in end-organ tissue. Thus, our results should be interpreted in the context of this lack of evidence. In addition, the correlation of peripheral blood concentrations and lung tissue is not well understood (42, 43). It is also not clear which peripheral tissue would be most representative of tissue levels. For example, erythrocyte glutathione may not be representative of glutathione levels in the lung lining fluid.

In summary, we found negative associations of glutathione and TBARS with pulmonary function and positive associations of glutathione peroxidase with pulmonary function in men. We did not find statistically significant associations of oxidative stress with pulmonary function in women, which may be due to higher serum antioxidant levels in women. These results strengthen the evidence that the antioxidant/oxidant balance is associated with reduced pulmonary function, but they warrant further investigation into gender differences in oxidative stress.

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