

Biomarkers of oxidative stress and antioxidants in severe asthma A Prospective Case-Control Study

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ABSTRACT

Background: Bronchial airway inflammation is the hallmark of asthma, which may be driven by an imbalance between oxidative stress and antioxidant defenses. Antioxidants deficiency may play a role, but this has remained unconfirmed.

Objective: To evaluate the oxidative stress burden and antioxidants defenses in patients with increasing asthma severity.

Methods: This prospective case-control study compared fractional exhaled nitric oxide (FeNO), exhaled breath condensate nitrite/nitrate (EBC-NOx), spirometry, and serum vitamins and trace elements among patients with and without asthma.

Results: Sixty participants were recruited (30 with severe asthma number; 23 women [76.7%]; mean age, 41.4 years; mean forced expiratory volume in 1 second [FEV₁], 2.2 L [72.2% predicted]; mean inhaled corticosteroid dosage, 2,540 µg/d; 18/30 [60%] receiving maintenance oral corticosteroids; 15 with mild asthma; all corticosteroids naïve; 9 women [60%]; mean age, 34.6 years; mean FEV₁, 3.48 L [100.5% predicted]; 15 healthy controls; 12 women [80%]; mean age, 37.6 years; and mean FEV₁, 3.53 L [111.7% predicted]). The mean FeNO levels increased significantly with increasing asthma severity ($P = .01$), but the EBC-NOx levels did not change significantly ($P = .90$). Paradoxically, vitamin A and vitamin E increased with increased disease severity, with vitamin E levels increasing significantly ($P = .07$ and $P < .001$, respectively). There was no significant difference between groups in the levels of copper ($P = .37$), zinc ($P = .97$), or selenium ($P = .90$).

Conclusion: FeNO but not EBC-NOx is increased significantly with asthma severity with no evidence of vitamins or trace elements deficiency in severe asthma. Impaired oxidative stress defenses in severe asthma may be driven by factors other than vitamins or trace elements deficiency.

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Introduction

Asthma is a chronic inflammatory disorder of the airways associated with airway hyperresponsiveness, leading to recurrent episodes of wheezing, breathlessness, chest tightness, and cough. Although airway inflammation is the hallmark of asthma,

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several phenotypes have been described that may differ in their response to treatment.¹

Oxidative stress represents increased production of oxidizing species attributable to inflammation and/or a reduction in antioxidant capacity, which can trigger a cascade of events, resulting in oxidant-mediated cellular injury and death. The respiratory tract is at particular risk of increased oxidative stress because of environmental factors alongside physiologic production. It has been suggested that patients with asthma are deficient in antioxidants, with the resultant oxidative stress burden leading to pulmonary dysfunction.^{2,3}

Although reactive oxygen species (ROS) play essential roles in maintaining health, increased levels can become damaging.⁴ The

lung is exposed to exogenous ROS, generated from air pollutants and smoking, and endogenous ROS, produced by inflammatory cells, such as activated eosinophils, neutrophils, and macrophages in asthmatic airways. High levels of ROS are generated primarily through the NADPH oxidase–dependent complex and the cytosolic xanthine oxidase system.⁵ Reactive nitrogen species are generated through interaction of nitrite and myeloperoxidase or eosinophil peroxidase–derived ROS.⁶ ROS are difficult to measure directly because they are highly reactive and short lived; therefore, products of ROS reactions are measured instead to estimate the oxidative stress burden and inflammation. Fractional exhaled nitric oxide (FeNO) has been used as a marker of oxidative stress and eosinophilic airways inflammation. Consequently, it has been used in the assessment of asthma diagnosis and control.⁷

Exhaled breath condensate (EBC) collection has been used to measure nitrate and nitrite (EBC-NOx),⁸ which are stable end products of nitric oxide after reaction with oxygen.⁹ Previous studies reported correlation between EBC-NOx and airway inflammation,⁸ with increased levels in patients with asthma compared with healthy controls.¹⁰ Levels of EBC-NOx decreased after treatment with inhaled corticosteroids and have been found to relate to asthma control.¹¹ EBC-NOx is closely correlated with other common markers of oxidative stress.¹² However, the EBC-based methods of oxidative stress assessment proved difficult to replicate, and the procedure of EBC collection and analysis suffered from lack of standardization.¹³ In contrast, FeNO measurement was robust and reproducible.

Antioxidants counteract the damaging effect of oxidative stress and play a vital role in maintaining the health of the airways. Enzymatic antioxidants, such as the superoxide dismutases, catalase, and glutathione peroxidases, require copper, zinc, selenium, and others to catalyze oxygen radicals to hydrogen peroxide and then to water and oxygen.¹⁴ Other antioxidant defenses include nonenzymatic compounds, such as vitamins A, C, and E, ceruloplasmin, ferritin, and bilirubin, among others, which react directly with oxygen species, thereby reducing their potency as a radical.¹⁵ Vitamin A has a role in promoting proliferation and prolonging survival of T cells, enhancing their antigen-presenting capacity and promoting T_H2 cell response, thereby playing a central role in asthma and allergy.¹⁶ Although it has demonstrable antioxidant properties *in vitro*, its role *in vivo* remains uncertain. Observational studies reported no association between asthma and vitamin A intake or a potential benefit.¹⁷ In children, serum vitamin A was negatively correlated with asthma severity.¹⁸ Interventional studies, however, have found no benefit with dietary vitamin A supplementation,¹⁹ and conversely high dietary vitamin A supplementation was associated with increased risk of asthma onset and severity in a mouse model.²⁰ Similarly, observational studies reported no association between asthma and vitamin E¹⁷ or improvement in asthma symptoms and severity²¹ with increased dietary intake. However, dietary supplementation has produced no benefit.¹⁹

The role played by trace elements in the severity of asthma remains uncertain. Although increased selenium levels in maternal and cord blood were associated with reduced incidence of wheeze, this effect disappeared by 5 years of age.²² In adults with asthma, selenium levels were lower than in controls,²³ but this was not confirmed in other studies.^{24,25} In a mouse model, worse outcomes were associated with both low and high selenium levels when compared with a medium group,²⁶ suggesting the influence of selenium on asthma may be more complex than a simple dose-response relationship. Zinc studies have been mixed, finding either no effect²⁷ or an association between lower zinc levels and asthma.²⁸ Copper studies have found either no effect²⁷ or increased serum copper levels in asthma.²³

The association between asthma severity and oxidative-antioxidant balance remains unclear. Studies have reported

increased oxidative stress burden with increasing asthma severity; however, the evidence for this is weak, with no clear-cut documented evidence of antioxidants deficiency in severe asthma. This study assessed the oxidative stress and antioxidants defences in a well-characterized severe asthma cohort compared with mild corticosteroid–naïve patients with asthma and healthy, nonatopic, nonasthmatic controls.

Methods

This prospective case-control study compared patients with severe asthma (British Thoracic Society/Scottish Intercollegiate Guidelines Network Guidance steps 4/5, representing patients treated with high-dose inhaled corticosteroids [ICSs] and other controller asthma medication or those receiving maintenance oral corticosteroids [OCSs], respectively²⁹) with mild corticosteroid-naïve patients with asthma and nonatopic healthy controls. The local research and ethics committee approved the study, and all participants who met the study protocol inclusion and exclusion criteria signed a written informed consent form before their inclusion in the study.

Study Participants

All patients and healthy volunteers were screened using pre-designed study questionnaire for the following inclusion and exclusion criteria; age range of 18 to 60 years, nonsmokers or ex-smokers with less than 10 pack-years of tobacco smoke, and no other active respiratory disease, autoimmune disease, infection, or malignant tumor. Other exclusion criteria were pregnancy, lactating patients taking corticosteroid-sparing agents (eg, cytotoxic medications), and those taking dietary antioxidant or trace elements supplements.

Thirty patients with uncontrolled severe asthma despite treatment with 2,000 µg/d or greater of beclomethasone dipropionate or equivalent and a second controller medication were recruited from the Birmingham Regional Severe Asthma Service (BRSAS) at Heartlands Hospital. Fifteen atopic and corticosteroid-naïve patients with mild asthma were recruited from the local clinics and primary care practices. Atopy was defined as a positive skin prick test result to at least one of the common aeroallergens. All patients with mild asthma were corticosteroid naïve and treated with intermittent short-acting β-adrenergic agonist bronchodilator not exceeding 3 doses per week. Patients with mild asthma had a forced expiratory volume in 1 second (FEV₁) of 80% predicted or greater and evidence of reversible airflow obstruction (12% increase in FEV₁ after 400 µg of inhaled salbutamol, diurnal peak expiratory flow variation of ≥20% on least 50% of the time during a 2-week period, or positive methacholine bronchial provocation test result). Patients with mild asthma were free from severe asthma attacks or hospital admissions in the preceding 5 years to recruitment.

The 15 healthy controls were never-smokers recruited from the general public through local advertisements in the hospital and university. Healthy controls had no personal or familial history of asthma and had no history of asthma symptoms. Their lung function was normal, including negative peak expiratory flow variability after 2 weeks of recordings. All healthy controls had a negative skin prick test results, confirming their nonatopic status.

Clinical Assessment and Study Visits

All participants attended 3 visits, 3 weeks apart, each lasting 60 minutes, in which they completed a predesigned questionnaire that detailed their asthma and allergy history, symptoms, medical and social history, medications, and use of vitamin and trace elements supplementation. Physiologic measurements conducted included spirometry and reversibility, skin prick testing, FeNO, and EBC.

Spirometric parameters (FEV₁ and forced vital capacity) were measured according to European Respiratory Society guidelines using the Masterlab Spirometry Program (Jaeger, Viasys Healthcare, Warwick, England). Three comparable forced vital capacity maneuvers were completed with the best of 3 attempts recorded. FEV₁ values were required to be within 100 mL of each attempt to ensure reproducibility. Reversibility testing was conducted by remeasuring FEV₁ 20 minutes after inhalation of 400 µg of salbutamol. Positive reversibility was considered at 12% or greater increase in post-bronchodilator FEV₁. Skin prick testing to cat dander, dog dander, house dust mite, *Aspergillus fumigatus*, tree mix, grass pollen, and latex was conducted in all participants. A wheal size 3 mm larger than the negative control represented positive sensitization (atopy). FeNO was measured using the NIOX MINO system (Aerocrine AB, Solna, Sweden) at a steady exhalation rate of 50 mL/s. EBC was collected using a previously described collection system³⁰ and nitrate/nitrite levels were measured using total nitric oxide and nitrate/nitrite assay (R and D Systems, Abingdon, England).

Measurement of serum antioxidants was performed after collection of 20 mL of venous blood. Vitamin A and E levels were measured using high-performance liquid chromatography and fluorometric detection (ClinRep, Waters, England). Serum levels of copper, selenium, and zinc were measured using inductively coupled plasma mass spectrometry with an Agilent 7500c instrument (Agilent Technologies, Santa Clara, California). Commercial reference materials (Institut National de Santé Publique, Montreal, Quebec, Canada) were used to check batch accuracy, and the assay performance was monitored by participation in 2 international External Quality Assurance schemes (International Comparison Program, Montreal, Quebec, Canada, and UK National External Quality Assessment Service for Trace Elements, Sheffield, England).

Statistical Analysis

Parametric and nonparametric statistics were performed according to data distribution as determined by the Shapiro-Wilk normality test. Data were presented as means (SDs) unless specified otherwise. Comparisons were performed using Kruskal-Wallis tests, *t* tests, 1-way analysis of variance, or multiple analysis of variance. A χ^2 cross-tabulation was used to test for significance in categorical parameters. Correlations were explored using the Pearson coefficient. Cut-off level for statistical significance was

considered at $P < .05$ after allowing for multiple comparisons using Bonferroni correction.

Results

Demographics

Seventy-nine individuals had consented to participate in the study. Twelve were excluded because they did not meet the study inclusion and exclusion criteria on screening (4 healthy controls were excluded because of positive skin prick test results, whereas 7 other individuals withdrew consent or failed to attend study visits). Sixty individuals completed the study as per the protocol and formed the analysis population.

Demographics and clinical characteristics of the study population are presented in Table 1. Women comprised most of the participants in all 3 comparator groups. The severe asthma group had a significantly higher mean body mass index and lower mean lung function than the mild asthma and healthy control groups. All the healthy controls and patients with mild asthma were corticosteroid naive (none received ICSs or systemic corticosteroids). In contrast, all patients with severe asthma were taking high doses of ICSs at a mean (SD) of 2,540 (897.7) µg/d, and 18 (60%) of 30 were taking maintenance OCSs with a mean (SD) prednisolone dose of 9.7 (11.3) mg/d for the whole group (30 patients) and 16.17 (10.40) mg/d for the 18 patients taking maintenance OCSs only. Mild asthma diagnosis was confirmed through positive reversibility test results in 11 participants, peak expiratory flow variability in 2 participants, and positive methacholine bronchial provocation results in the remaining 2 participants.

Oxidative Stress and Inflammation Markers

The mean FeNO level was significantly higher in the severe asthma than in the mild asthma and healthy control groups ($P = .01$). This difference was confirmed in 3 prospective study visits conducted at 3 weekly intervals (Fig 1). For the total study population and using FeNO of 25 ppb or higher as a cut-off level for a high vs low FeNO values,³¹ 33 cases (55%) cases were FeNO high and 25 (45%) were FeNO low. There were 2 (13%) of 15 FeNO-high individuals in the healthy control group, 9 (60%) of 15 in the mild asthma group, and 22 (73%) of 30 in the severe asthma group ($\chi^2 = 14.75$, $P < .001$). The 2 FeNO-high individuals in healthy control group had borderline readings of 25 and 26 ppb.

Table 1
Demographics and Characteristics of the Study Cohort^a

Variable	Healthy controls (n = 15)	Mild asthma (n = 15)	Severe asthma (n = 30)	P value
Females	12/15 (80)	9/15 (60)	23/30 (77)	.39
Age, mean (range), y	37.6 (25–52)	34.6 (19–57)	41.4 (19–56)	.15
Weight, mean (SD), kg	66.8 (10.2)	72.57 (16.9)	87.24 (18.6)	<.001
BMI, mean (SD)	23.7 (2.8)	24.8 (4.5)	32.5 (7.9)	<.001
Smoking ^b				
Never	11/15 (73)	15/15 (100)	23/30 (77)	
Ex-smokers	4/15 (27)	0/15 (0.0)	7/30 (23)	
Current	0/15 (0.0)	0/15 (0.0)	0/30 (0.0)	
Atopy ^c	0/15 (0.0)	15/15 (100)	26/30 (87)	
ICS, mean (SD), µg/d	0.00 (0.00)	0.00 (0.00)	2,540 (897.7)	
OCS, % maintenance	0.0	0.0	18/30 (60)	
All group OCS, mean (SD), mg/d	0.00 (0.00)	0.00 (0.00)	9.7 (11.3)	
Maintenance OCS only, mean (SD), mg/d	0.00 (0.00)	0.00 (0.00)	16.17 (10.40) (18/30)	
FEV ₁ , mean (SD), L	3.53 (0.56)	3.48 (1.06)	2.2 (0.93)	
FEV ₁ , mean (SD), % predicted	111.75 (8.99)	100.49 (15.79)	72.21 (20.94)	<.001
FEV ₁ /FVC, mean (SD), %	81.90 (5.71)	82.17 (11.80)	72.64 (11.95)	.005

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by square of height in meters); FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroid; OCS, oral corticosteroid.

^aData are presented as number/total number (percentage) of patients unless otherwise indicated.

^bAll patients were noncurrent smokers. Ex-smokers did not smoke in the 12 months preceding the recruitment and overall had less than 10 pack-years of tobacco smoke.

^cAtopy was defined as a positive skin test result to at least one allergen (a wheal size of ≥ 3 mm than the negative saline control).

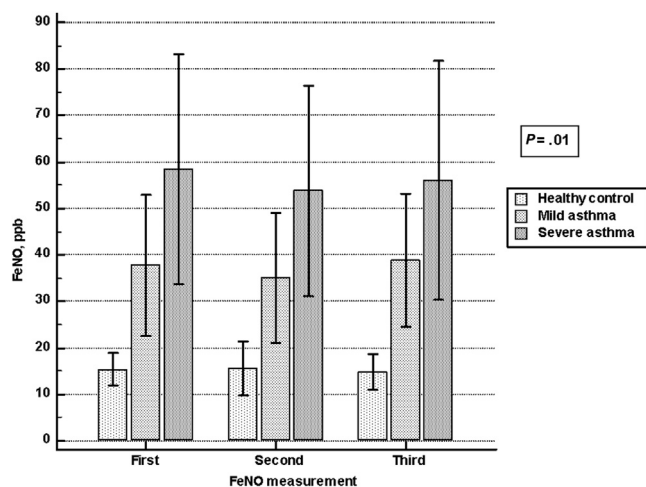


Figure 1. Mean fractional exhaled nitric oxide (FeNO) levels in healthy controls, patients with mild asthma (corticosteroid naive), and patients with severe asthma. Measurements were conducted at baseline, week 3, and week 6 and reveal significant and consistent elevation in the mean FeNO level in the severe asthma group compared with the mild asthma and healthy control groups. Statistical significance was measured using analysis of variance. Error bars indicate SD.

The mean (SD) EBC-NOx level did not differ among the 3 groups (8.30 [6.84], 8.04 [7.93], and 8.24 [5.66] $\mu\text{mol/L}$ in the severe asthma, mild asthma, and healthy control groups, respectively; $P = .90$). However, in the whole study cohort, the EBC-NOx level was significantly higher in the FeNO-high subgroup ($n = 32$ mean [SD], 10.69 [6.65] $\mu\text{mol/L}$) than in the FeNO-low subgroup ($n = 25$; mean [SD], 5.03 [4.49] $\mu\text{mol/L}$; $P = .001$). An increase in EBC-NOx in the FeNO-high individuals was also observed in mild asthma and severe asthma as separate groups (Fig 2).

Antioxidants

Serum levels of copper, selenium, and zinc did not differ significantly among the 3 groups (Table 2). We observed a statistically nonsignificant increase in serum vitamin A level ($P = .07$)

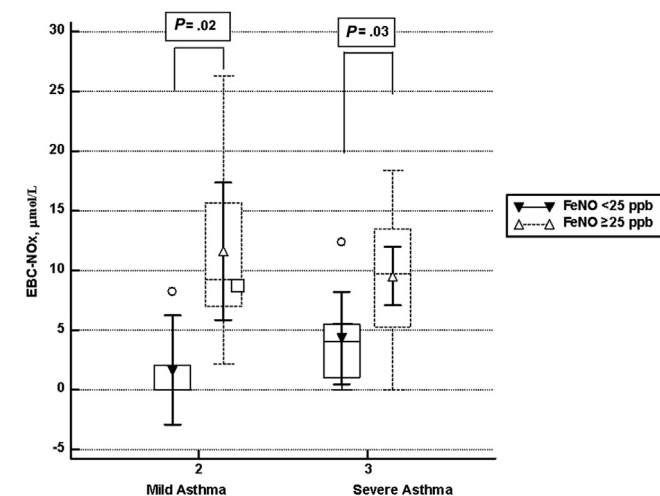


Figure 2. Comparisons of exhaled breath condensate–nitrate/nitrite (EBC-NOx) in patients with mild and severe asthma among the fractional exhaled nitric oxide (FeNO)–high (≥ 25 ppb) and FeNO–low groups. In the mild asthma FeNO-high group ($n = 9$), the mean (SD) FeNO level was 11.60 (7.46) $\mu\text{mol/L}$ vs 1.64 (3.67) $\mu\text{mol/L}$ in the FeNO-low group ($n = 5$) ($P = .02$) and 9.53 (5.41) $\mu\text{mol/L}$ in the severe asthma FeNO-high group ($n = 21$) 4.36 (4.16) $\mu\text{mol/L}$ in the FeNO-low group ($n = 7$) ($P = .03$). Error bars indicate SD.

Table 2

Comparisons of Mean (SD) Values of Vitamins A and E and Trace Elements in the Study Groups

Element	Healthy controls (n = 15)	Mild asthma (n = 15)	Severe asthma (n = 30)	P value
Copper, ($\mu\text{g}/100$ mL)	1.06 (0.31)	1.04 (0.20)	1.15 (0.24)	.37
Zinc, $\mu\text{g}/100$ mL	0.85 (0.12)	0.85 (0.12)	0.85 (0.28)	.97
Selenium, $\mu\text{g}/100$ mL	76.40 (10.60)	76.87 (10.03)	78.23 (11.86)	.90
Vitamin A, mg/L	0.70 (0.26)	0.82 (0.20)	0.91 (0.31)	.07
Vitamin E, mg/L	13.27 (2.48)	15.05 (3.22)	17.77 (3.580)	<.001

and a significant increase in serum vitamin E in patients with severe asthma compared with patients with mild asthma and healthy controls ($P < .001$) (Table 2). The serum vitamin E level also significantly changed with body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) (normal weight BMI of 18–25 [$n = 25$]; mean [SD], 14.85 [3.20] mg/L; overweight BMI of >25 –30 [$n = 14$]; mean [SD], 15.43 [3.49] mg/L; and obese BMI of >30 [$n = 19$]; mean [SD], 17.64 [4.07] mg/L; $P = .04$). However, within the severe asthma group, the serum vitamin E level was elevated regardless of the BMI category (normal weight [$n = 6$]; mean [SE], 17.5 [1.48] mg/L; overweight [$n = 5$]; mean [SE], 17.2 [1.61] mg/L; obese $n = 17$: mean [SE], 18.04 [0.87] mg/L) and higher than that of mild asthma regardless of BMI categories. Similarly, in patients with mild asthma, vitamin E was not influenced by the BMI category (normal weight [$n = 10$]; mean [SE], 15.03 [1.15] mg/L; overweight [$n = 3$]; mean [SE], 15.65 [2.09] mg/L; obese [$n = 2$]; mean [SE], 14.23 [2.56] mg/L). Therefore, the observed increase in serum vitamin E level in patients with severe asthma persisted even when the BMI effect was accounted for and demonstrates a paradoxical association between vitamin E and asthma severity.

The mean serum vitamin A, vitamin E, copper, selenium, or zinc levels did not differ between FeNO-high or FeNO-low, EBC-NOx–high or EBC-NOx–low groups, or composite groups of FeNO and EBC-NOx, indicating no association between these vitamins or trace elements and these oxidative stress markers in this cohort (data not shown).

Correlation Analyses

To explore the associations among BMI, lung function, oxidative stress markers, and antioxidants, we conducted correlations analyses (Table 3). BMI correlated negatively with FEV₁ and positively with vitamin E and copper levels, whereas FEV₁ correlated negatively with BMI, vitamin A, and vitamin E. There was also a significant correlation between vitamins A and E. The severe asthma group had significantly higher BMI, lower lung function, and higher vitamin E. However, within the severe asthma group, mean vitamin E level was similar in those with mild, moderate, and severe FEV₁ reduction (the mean [SE] vitamin E levels were 17.8 [1.3] mg/L, 17.9 [1.3] mg/L, and 17.1 [1.5] mg/L for FEV₁ $\geq 80\%$ predicted, 60%–80%, and $<60\%$, respectively). Similarly, in obese individuals, the mean vitamin E level did not differ significantly among the 3 lung function groups (data not shown). Therefore, the observed correlation between vitamin E and low lung function seems to be an effect of asthma severity and BMI rather than a direct association.

Discussion

In this study, we found evidence of increased oxidative stress burden in severe asthma as measured by FeNO but not EBC-NOx and paradoxical elevation of vitamins E and A in the severe asthma group compared with the mild asthma and healthy control groups. In addition, we observed no evidence of trace elements

Table 3
Correlation Analysis of Oxidative Stress Biomarkers, Antioxidants, and Lung Function

Variable	FEV ₁ , % predicted	FeNO	EBC-NOx	Vitamin A	Vitamin E	Copper	Selenium	Zinc
BMI								
CC	−0.34	0.09	0.07	0.17	0.38	0.33	0.09	0.13
P value	.008*	.50	.63	.20	.003*	.01*	.48	.32
n	60	60	57	58	58	60	60	60
FEV ₁ , % predicted								
CC		−0.23	0.04	−0.30	−0.44	−0.20	−0.22	−0.09
P value		.08	.80	.02*	<.001*	.12	.09	.47
n		60	57	58	58	60	60	60
FeNO								
CC			0.007	0.11	0.21	−0.15	0.04	−0.05
P value			.96	.43	.12	.26	.76	.70
n			57	58	58	60	60	60
EBC-NOx								
CC				−0.11	−0.07	−0.21	0.12	−0.06
P value				.41	.60	.13	.38	.66
n				55	55	57	57	57
Vitamin A								
CC					0.37	0.10	0.22	−0.07
P value					.004*	.44	.10	.59
n					58	58	58	58
Vitamin E								
CC						0.07	0.13	−0.08
P value						.58	.33	.57
n						58	58	58
Copper								
CC							0.21	0.23
P value							.11	.08
n							60	60
Selenium								
CC								−0.017
P value								.90
n								60

Abbreviations: BMI, body mass index; CC, correlation coefficient; EBC-NOx, exhaled breath condensate nitrite/nitrate; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; n, number of individuals included in the analysis.

*Statistical significance of $P < .05$.

(copper, selenium, zinc) deficiency in severe asthma or mild asthma compared with healthy controls.

We observed increased EBC-NOx levels in the FeNO-high groups only regardless of asthma severity. EBC-NOx has previously been observed to correlate with FeNO and other markers of oxidative stress^{8,10,12}; however, it is also susceptible to confounding factors, such as diet and external contamination, and as such is liable to sampling errors.³² Our data suggest that FeNO is more sensitive as an inflammatory marker and correlates better with asthma severity than EBC-NOx. FeNO measurement is more standardized and easier to measure than EBC, which is technically challenging to conduct and standardize.

The lack of vitamin or trace element deficiency in the severe asthma group in our study contrasts the findings of previously published studies, which, although inconsistent, have reported no significant difference or a negative correlation with severity of asthma. Our results suggest that the association between antioxidant levels and asthma is not as straightforward as a simple dose-response model with the impaired oxidative stress defenses in severe asthma may be attributable to factors that affect use and function rather than absolute deficiency.

The severe asthma cohort in this study was recruited from a tertiary severe asthma center with 60% of patients taking maintenance OCSs. The intake of dietary vitamins or trace elements supplementation was an exclusion criterion within the study; however, this was self-reported, and health behavioral modification as a potential cause of the observed elevated vitamins E and A levels could not be entirely excluded. Nonetheless, our findings do not support the notion of supplementing vitamins or trace elements to combat oxidative stress in severe asthma.

A plausible explanation for the observed increased vitamins A and E in the severe asthma cohort is the effect of obesity. Within

our study, the severe asthma group had a higher mean BMI than the mild asthma and healthy control groups. Obesity influences a number of metabolic pathways, including vitamins A³³ and D,³⁴ although the exact nature of its influence has not been established. Serum vitamin A levels were increased in obese patients with type 2 diabetes but reduced in patients with type 1 diabetes,³⁵ although this was an inconsistent observation.³⁶ Trasino et al³⁷ found increased serum vitamin A levels in obese mice and vitamin A deficiency in the tissues of the liver, lungs, pancreas, and kidneys because impaired vitamin A transcription signaling. With weight loss, both signaling and serum and tissue levels returned to the normal range³⁸ The association between vitamin E and obesity has been less well studied; however, lower serum levels have been observed in nonobese individuals.³⁹

Obese individuals have been reported to have higher levels of systemic oxidative stress,³⁹ with hypertrophied adipose tissue producing proinflammatory cytokines and adipokines resulting in metabolic inflammation.⁴⁰ Obesity is also a risk factor for asthma, with weight loss improving the clinical course of the disease.⁴¹ The precise interaction between obesity and asthma is unclear, although there are many different hypotheses, including the effects of inflammatory adipokines on airway inflammation, hormonal influences during lung development in obese children, bronchial remodeling in response to mechanical stresses, and genetic pleomorphism influencing asthma and obesity risk.⁴²

We observed that even after adjusting for BMI as a confounding factor, vitamin E levels increased with increasing severity of asthma. Vitamin E consists of 8 natural isomers, which included 4 forms of tocopherol, the most abundant of which are α -tocopherol and γ -tocopherol. Mammals do not interconvert the different isoforms; however, levels are subject to genetic variation, with normal metabolism leading to preferential transfer of α -tocopherol in

the liver⁴³ and a higher rate of production of metabolites of γ -tocopherol for excretion.⁴⁴ Mutations in liver transfer proteins can result in α -tocopherol deficiency.⁴⁵ These different isoforms demonstrate opposing regulatory functions, with the α -tocopherol isoform having anti-inflammatory effects that block airway hyperreactivity and the γ -tocopherol isoform having proinflammatory effects that increase airway hyperreactivity during eosinophilic allergic lung inflammation in mice.^{37,46} It may therefore be the case that it is the use and function of antioxidants that are altered in higher levels of oxidative stress seen in severe asthma rather than an absolute deficiency. Furthermore, the observed correlation between reduced FEV₁ and increased BMI and vitamins A and E in this study also seemed to be a function of asthma severity rather than a direct effect.

The evidence of an influence of trace elements on the severity of asthma has been inconclusive. Lower serum levels of copper, selenium, and zinc have been associated with asthma in some studies but not consistently.²³ In animal models, both high and low selenium levels have been associated with worsened asthma outcomes.²⁶ A large, 14-center study conducted by the Global Allergy and Asthma European Network found that selenium status was not significantly associated with asthma severity,⁴⁷ which is consistent with our findings. Overall, this finding also supports the notion that impaired defenses are related to function and use of antioxidants rather than an absolute deficiency.

Finally, the lack of available data on allergen exposure and blood or sputum eosinophils may have limited the exploratory ability of this study in assessing the oxidative stress burden and antioxidant defenses through comparing eosinophilic vs noneosinophilic phenotypes in the context of the atopic status and allergen exposure. Although all patients with mild asthma were atopic, the severe asthma group had atopic and nonatopic individuals who may have also being noneosinophilic. However, this did not influence our results, which continued to show a dose-response increase in FeNO with increased asthma severity, so the severe group with mixed atopic and nonatopic patients had higher mean FeNO than the mild asthma group, who were all atopic. In addition, despite the association between blood eosinophils and FeNO, the association is not mutually exclusive (ie, patients may have high FeNO and normal blood eosinophils or vice versa).

In conclusion, this study found an elevated oxidative stress burden with increased asthma severity as measured by FeNO but not EBC-NOx with no evidence of vitamins A and E or trace elements deficiency in asthma. FeNO measurement was reproducible and was a reliable marker of airway inflammation and oxidative stress that was increased with asthma severity. However, EBC-NOx did not correlate with asthma severity despite its increased level in the FeNO-high patients. Future studies on oxidative stress in asthma require the development of more specific and reliable biomarkers, whereas exploring the roles of antioxidants should focus on its function and use perhaps locally within the airways.

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