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Nitric Oxide Oxidation Products are Increased in the Epithelial Lining Fluid of Children with Persistent Asthma

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Abstract

Background—Children with severe allergic asthma have persistent airway inflammation and oxidant stress.

Objectives—We hypothesized that children with severe allergic asthma would have increased concentrations of the NO oxidation products nitrite, nitrate, and nitrotyrosine in the proximal and distal airway epithelial lining fluid (ELF). We further hypothesized that NO oxidation products would be associated with higher exhaled nitric oxide (F_{ENO}), greater allergic sensitization, and lower pulmonary function.

Methods—Bronchoalveolar lavage (BAL) was obtained from 15 children with mild-to-moderate asthma, 30 children with severe allergic asthma, 5 non-asthmatic children and 20 non-smoking adults. The BAL was divided into proximal and distal portions and nitrite, nitrate, and nitrotyrosine were quantified.

Results—Children with mild-to-moderate and severe allergic asthma had increased concentrations of nitrite (adult control: 15 ± 3; pediatric control: 23 ± 4; mild-to-moderate asthma: 56 ± 26; severe asthma: 74 ± 18 μM), nitrate (37 ± 13 vs. 145 ± 38 vs. 711 ± 155 vs. 870 ± 168 μM) and nitrotyrosine (2 ± 1 vs. 3 ± 1 vs. 9 ± 3 vs. 10 ± 4 μM) in the proximal ELF. Similar results were seen in the distal ELF although the concentrations were significantly lower (p < 0.05 for each). Although univariate analyses revealed no associations between NO oxidation products and clinical features, multivariate analyses revealed F_{ENO} to be a significant predictor of NO oxidation in asthmatic children.

Conclusions—NO oxidation products are increased in the ELF of asthmatic children. The relationship between F_{ENO} and airway nitrosative stress is complicated and requires further study.

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This manuscript has an on-line data repository

CLINICAL IMPLICATIONS

Symptomatic children with mild-to-moderate and severe allergic asthma have significant nitrosative stress despite corticosteroid treatment. Additional therapies to decrease airway nitrosative stress may be warranted in these children.

CAPSULE SUMMARY

Symptomatic children with persistent asthma have significant oxidation of nitric oxide (i.e., "nitrosative stress") in the airways despite corticosteroid treatment. Nitrosative stress may account for ongoing symptoms in this group of children.

Keywords

Asthma; Children; Nitric oxide; Nitrogen oxides; Nitrosation; Nitrosative Stress; Reactive nitrogen species

INTRODUCTION

Severe allergic asthma in school-age children is a complex disorder characterized by persistent airway inflammation, ongoing symptoms, and increased exhaled nitric oxide (F_{ENO}) concentrations despite treatment with high doses of inhaled and oral corticosteroids.^{1,2} Although airway nitric oxide (NO) is essential for epithelial signaling and host defense, excessive NO production results in NO oxidation and potential toxicity.³ This process of excessive NO oxidation is commonly referred to as “nitrosative stress”⁴ and ultimately promotes protein nitration, resulting in structural and functional protein alterations that may enhance the inflammatory response.⁵ Thus, excessive airway NO concentrations in children with severe allergic asthma may contribute to an ongoing cycle of airway destruction with airway injury.⁶

In the human airway, the most readily detectable NO oxidation products include nitrite (NO_2^-) and nitrate (NO_3^-), which can be derived from NO through a series of reactions involving superoxide anion (O_2^-) and oxygen (Figure 1). Nitrotyrosine is also easily measured in airway samples and reflects the overall degree of protein nitration.⁷ Indeed, previous studies have noted increased nitrite, nitrate and nitrotyrosine concentrations in the exhaled breath condensate of asthmatic children^{8–10} and in the epithelial lining fluid (ELF) of adults with mild-to-moderate and severe asthma.^{11,12} However, no study to date has examined NO oxidation products in the ELF of asthmatic children. Because children with severe asthma have profound airway oxidant stress,¹³ the purpose of this study was to quantify NO oxidation products in the ELF of children with mild-to-moderate and severe allergic asthma. The secondary purpose of this study was to determine the association between increased ELF NO oxidation products and clinical features of asthma severity in children. We hypothesized that children with severe allergic asthma would have increased concentrations of the NO oxidation products nitrite, nitrate, and nitrotyrosine in the proximal and distal airway epithelial lining fluid (ELF). We further hypothesized that these increased NO oxidation products would be associated with increased F_{ENO} , greater allergic sensitization, and lower pulmonary function.

METHODS

Sample

Children 5–17 years of age with symptomatic asthma attending an asthma clinic at Emory University were invited to participate in this study. Asthmatic children met published criteria for persistent asthma¹⁴ and had a history of at least a 12% change in the forced expiratory volume in one second (FEV_1) after albuterol administration.¹⁵ Severe asthma was diagnosed according to criteria developed by the NIH/NHLBI Severe Asthma Research Program,¹ which were adapted from the American Thoracic Society’s Consensus Panel Report (Online Repository, Table E1).¹⁶ Thresholds for high-dose inhaled corticosteroids (ICS) were defined as ≥ 440 mcg of fluticasone equivalent per day for children less than 12 years and ≥ 880 mcg for children 12–17 years of age.¹⁴ Children with severe allergic asthma were treated with a stable dose of ICS or oral corticosteroids for at least 6 months prior to recruitment. Adherence to ICS therapy was monitored by an analysis of prescription refills. Informed consent was obtained from all caregivers. Children also provided verbal and written assent.

Children who fit criteria for severe allergic asthma underwent flexible bronchoscopy with bronchoalveolar lavage (BAL) as indicated for persistent asthma symptoms despite appropriate treatment with high-dose inhaled and systemic corticosteroids.¹⁷ Children with mild-to-moderate asthma underwent bronchoscopy for suspected foreign body aspiration, recurrent pneumonia, persistent cough, and suspected congenital anomalies. Controls for this study included children with psychogenic (habit) cough or vocal cord dysfunction undergoing bronchoscopy for definitive diagnosis and healthy, non-smoking adult volunteers. Control subjects were nonsmokers with no family history of asthma and a negative bronchodilator response.

Procedures

Spirometry was performed before and after 2 inhalations of albuterol sulfate (90 µg/inhalation) with a portable spirometer (KoKo® Legend, Ferraris, Louisville, CO). The results fulfilled ATS criteria for reproducibility¹⁸ and were interpreted according to reference standards.¹⁹ Atopic sensitization was assessed by skin prick testing using a standard kit (Multi-Test® II, Lincoln Diagnostics, Decatur, IL) containing tree pollen, grass pollen, ragweed pollen, weed pollen, dog hair, cat epithelium, alternaria, cladosporidium, aspergillus, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cockroach, normal saline, and histamine extracts (Greer Laboratories, Lenoir, NC). The application site was examined 15 minutes after application and considered positive if both a wheal ≥ 3 mm diameter and erythema ≥ 10 mm diameter were present.

On the day of bronchoscopy, participants submitted F_{ENO} samples and underwent venipuncture. F_{ENO} was collected with a reservoir bag within 1 hour prior to bronchoscopy. For this procedure, subjects took two tidal breaths of NO-free air through a scrubbing filter, followed by a 6-second exhalation at a fixed flow rate of 0.35 L/second.²⁰ The first 150 mL exhaled were discarded. Subjects repeated this procedure three times. The resulting samples were analyzed offline by chemiluminescence (Sievers NOA™ 280-I, Ionic Instruments, Boulder, CO) within 1 hour of collection. The data were averaged to reflect mean F_{ENO}. Serum immunoglobulin (IgE) concentrations and plasma urea were determined after venipuncture.

Bronchoscopy in pediatric participants was performed by pediatric pulmonologists using a laryngeal mask airway. BAL fluid was collected from the right middle lobe with three 1 mL/kg (50 mL maximum) saline lavages flushed through the suction channel of a flexible bronchoscope (Olympus BF-3C160 [3.7 mm] or BF-P160 [4.9 mm], Olympus America Inc., Melville, NY). Bronchoscopy was performed in adults using a flexible bronchoscope (Olympus BF-1T20D) passed trans-nasally into the right middle lobe. Three 50 mL saline aliquots were instilled and immediately aspirated. The first lavage from all participants was reserved for evaluation of proximal airway constituents.²¹ The second and third lavages were pooled for distal airway constituent analysis. In children, the BAL return volume was divided between the research and clinical laboratories.

BAL was centrifuged at 1200 rpm within 1 hour of collection for 7 minutes at 4° C to separate the supernatant and cellular fractions. Given the limited number of cells present in the proximal airway lavage, the cell pellets from the proximal and distal airway lavages were pooled and resuspended in 10 mL of Dulbecco's Modified Eagles Medium with 10% fetal calf serum for cell counting. Total cell counts were performed manually with a hemocytometer and cellular differentials were determined from 300 consecutive cells after Wright staining.

The protein content of the BAL supernatant was assessed using a Coomassie (Bradford) protein assay (Pierce Biotechnology, Rockford, IL) read at an absorbance of 595 nm with a detection limit 1 µg/mL. Urea nitrogen was measured in plasma and BAL supernatant using a quantitative

colorimetric assay (Pointe Scientific, Canton, MI) with sensitivity of 0.05 to 150 mg/dL. The dilution of the proximal and distal BAL was calculated from $[\text{urea}]_{\text{plasma}}/[\text{urea}]_{\text{BAL}}$.²²

Nitrite and total nitrite + nitrate concentrations were determined from the BAL supernatant using a colorimetric assay (Cayman Chemical, Ann Arbor, MI) analyzed at 540 nm with a lower detection limit of 0.1 μM . All samples were analyzed in duplicate. For this assay, nitrate was converted to nitrite with nitrite reductase, followed by the addition of Griess reagent. Nitrate concentrations were determined by subtracting the concentration of nitrite from total nitrite + nitrate. To minimize false nitrate and nitrite readings during the assay, samples were analyzed immediately after thawing. The background nitrite and nitrate content in the saline lavage fluid was pre-determined and subtracted from the final concentration values.

Nitrotyrosine concentrations were determined spectrophotometrically using a microplate sandwich ELISA (Oxis International, Foster City, CA) with sensitivity of 2.0 nM and inter-assay precision of 11%. Samples were analyzed in duplicate and corrected for the background levels of nitrotyrosine in the saline lavage fluid. Absorbance was measured at 450 nm.

Statistical analysis

Data were analyzed with SPSS® software (Version 15, SPSS Inc., Chicago, IL). Nitrite, nitrate, and nitrotyrosine from the proximal and distal airway lavage were adjusted according to the urea dilution²² and were logarithmically transformed. Nitrotyrosine concentrations were further adjusted for the total protein content of the BAL supernatant. Differences between groups and post-hoc tests were assessed by Kruskal-Wallis tests and Mann-Whitney U tests, respectively. Pearson correlations were used to examine associations between NO oxidation products and clinical features. To evaluate factors that might affect NO oxidation in the ELF of asthmatic children, multivariate backward elimination linear regression was performed using total nitrite + nitrate concentrations in the proximal and distal ELF as dependent variables and age, gender, ethnicity, ICS dose, FEV₁, FEV₁ bronchodilator reversibility, serum IgE, history of hospitalization, F_{ENO}, and the percentage of airway eosinophils and neutrophils as predictors. Multicollinearity between predictors was assessed with tolerance statistics. Entry and removal probabilities were set at 0.05 and 0.10, respectively. Significance was defined as a two-tailed $\alpha \leq 0.05$ for all tests.

RESULTS

Initially, 49 asthmatic children (severe asthma, n = 32), 7 pediatric controls, and 20 healthy adult controls were recruited for this study. However, five children, including 2 pediatric controls, 2 mild-to-moderate asthmatics, and 2 severe asthmatics were infected with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and/or *Moraxella catarrhalis* and were excluded from data analysis due to potential denitrification.²³ The features of the excluded children appear in the online repository (Online repository, Tables E2–E3). Thus the final sample included in data analysis contained 30 children with severe allergic asthma, 15 children with mild-to-moderate asthma, 5 pediatric controls, and 20 adult controls.

Because bronchoscopy was performed only for clinical indications, all of the asthmatics were symptomatic. None of the children with mild-to-moderate asthma had evidence of airway infection or chronic aspiration syndromes. The features of the final sample are presented in Table I. Whereas all (100%) children with severe asthma had allergic sensitization, allergic sensitization was present in only half (53%) of the children with mild-to-moderate asthma (Online repository Table E4). Children with severe allergic asthma were also treated with higher doses of ICS but had significantly lower baseline pulmonary function and increased bronchodilator reversibility. Whereas F_{ENO} was elevated in both groups of asthmatics, there

were no differences in F_{ENO} between children with mild-to-moderate and severe allergic asthma (Table I).

The characteristics of the BAL fluid are presented in the online repository (Table E5). Although larger lavage volumes were used for adult controls, the percentage of BAL return was similar between adult and pediatric controls (proximal lavage: 23 vs. 27%; distal lavage: 49 vs. 35% for adult vs. pediatric controls). However, the BAL samples from adult controls were characterized by higher total cell counts (adult control: 7.81 ± 3.61 ; pediatric control: 3.53 ± 2.32 ; mild-to-moderate asthma: 3.81 ± 3.06 ; severe asthma: $3.32 \pm 2.02 \times 10^6$, $p < 0.01$). Whereas severe asthmatics had the highest percentage of BAL eosinophils (adult control: 0.4 ± 0.5 ; pediatric control: 0.3 ± 0.5 ; mild-to-moderate asthma: 0.7 ± 0.7 ; severe asthma: $1.9 \pm 3.2\%$, $p = 0.03$), mild-to-moderate and severe asthmatics had higher percentages of neutrophils compared to both groups of controls (adult control: 3.5 ± 3.2 ; pediatric control: 3.2 ± 1.4 ; mild-to-moderate asthma: 5.3 ± 3.9 ; severe asthma: 5.2 ± 3.2 , $p = 0.04$).

NO oxidation products in the proximal and distal airway lavage

Compared to controls, children with mild-to-moderate and severe allergic asthma had significantly higher concentrations of nitrite, nitrate and nitrotyrosine in the ELF (Figure 2). However, no significant differences in NO oxidation products were observed between children with mild-to-moderate and severe allergic asthma. In each group, nitrate was the most abundant NO oxidation product measured, with concentrations nearly 10-fold higher than those of nitrite. Furthermore, nitrite, nitrate and nitrotyrosine concentrations were also consistently higher in the proximal versus the distal airway ELF (Figure 2). Similar increases in NO oxidation products were also apparent in the raw BAL samples without adjustment for the urea dilution (Online repository Figure E1). Analysis of the entire sample (all asthmatics and controls) revealed strong correlations between proximal and distal airway ELF concentrations of total nitrite + nitrate ($r = 0.76$, $p < 0.01$), nitrite ($r = 0.50$, $p < 0.01$), nitrate ($r = 0.76$, $p < 0.01$), and nitrotyrosine ($r = 0.34$, $p = 0.02$). When this analysis was restricted only to asthmatic children, similar correlations between the proximal and distal ELF NO oxidation products were observed (total nitrite + nitrate: $r = 0.44$, $p < 0.01$; nitrite: $r = 0.58$, $p < 0.01$; nitrate: $r = 0.31$, $p = 0.05$; nitrotyrosine: $r = 0.35$, $p = 0.05$). Within the proximal and distal airway ELF, high agreement was further observed between the measured concentrations of total nitrite + nitrate and nitrotyrosine (Figure 3).

Relationship of NO oxidation products to F_{ENO} and other clinical features in asthmatic children

To determine the clinical implications of increased ELF oxidation products in children with mild-to-moderate and severe asthma, correlational analysis was first performed between NO oxidation products and clinical features of asthma severity, including F_{ENO} , serum IgE, the number of skin prick responses, FEV_1 , FEV_1 bronchodilator reversibility, and the percentage of BAL eosinophils and neutrophils. This analysis was restricted to children with mild-to-moderate and severe asthma and did not include controls. No significant correlations were observed between NO oxidation products and any the clinical features measured, including F_{ENO} (Online repository Table E6). However, F_{ENO} was significantly associated with the percentage of BAL eosinophils ($r = 0.35$, $p = 0.04$) and serum IgE ($r = 0.30$, $p = 0.02$).

To further evaluate factors that might affect NO oxidation in the ELF of asthmatic children, multivariate backward elimination linear regression was performed using total nitrite + nitrate concentrations in the proximal and distal ELF as the dependent variables and age, gender, ethnicity, ICS dose, FEV_1 , FEV_1 bronchodilator reversibility, serum IgE, history of hospitalization, F_{ENO} , and the percentage of airway eosinophils and neutrophils as predictors. Control data was excluded. In the proximal ELF, age ($p < 0.01$), gender ($p = 0.02$), and

F_{ENO} ($p = 0.06$) were significant predictors of nitrite + nitrate concentrations (final model $R^2 = 0.49$, $p = 0.01$, online repository Table E7). Likewise, gender ($p = 0.05$) and F_{ENO} ($p = 0.05$) were significant predictors of total nitrite + nitrate concentrations in the distal ELF of mild-to-moderate and severe asthmatic children (final model $R^2 = 0.25$, $p = 0.05$, online repository Table E8). In both the proximal and distal airway ELF, the relationship between F_{ENO} and NO oxidation was negative, such that higher F_{ENO} concentrations were associated with lower NO oxidation product formation.

DISCUSSION

To our knowledge, this is the first study to directly measure NO oxidation products in the ELF of children with persistent asthma. Compared to controls, children with mild-to-moderate and severe allergic asthma had increased concentrations of nitrite, nitrate, and nitrotyrosine in the ELF which were consistently higher in the proximal versus the distal airways. Contrary to our hypothesis, we failed to detect significant differences in NO oxidation products between mild-to-moderate and severe asthmatic children. Furthermore, no associations between NO oxidation products and clinical features such as F_{ENO} were detected using univariate analyses. However, with multivariate modeling to control for the potential confounding effects of ICS and atopy on NO synthesis, F_{ENO} was identified as a modest predictor of NO oxidation product formation. While the clinical relevance of this finding is yet unclear, these data highlight the complexity of NO biology in children with asthma and suggest that the relationship between F_{ENO} and NO oxidation is not directly proportional. Thus in children with severe asthma, lower F_{ENO} concentrations may not necessarily indicate the absence of airway inflammation, but instead may reflect decreased NO bioavailability from increased NO oxidation.

Airway NO biochemistry is complex and the exact contribution of NO to the pathogenesis of asthma is not fully understood. NO is produced by nitric oxide synthases (NOS) in a variety of cell types and serves as an important signaling molecule both within and outside of the cell.³ NO production is also vital to the epithelial antiviral and immune defenses of the airways.²⁴ While the generation of NO oxidation products from NO is important for transcription factor activation and the regulation of airway inflammation,³ excessive airway NO production from altered NOS isoforms or lack of endogenous NOS inhibition can lead to the oxidation of NO and potential nitrogen oxide toxicity.²⁵ The resulting nitrosative stress may ultimately contribute to protein dysfunction and airway cellular destruction.²⁶ Our findings of increased nitrite, nitrate and nitrotyrosine in the ELF of asthmatic children confirm that nitrosative stress is a distinguishing feature of the asthmatic airway. However, the underlying mechanisms responsible for this finding are unclear and warrant further study.

Although this is the first study to directly measure NO oxidation products in the ELF of children with mild-to-moderate and severe allergic asthma, our findings support previously-reported observations in the exhaled breath condensate. In these previous studies, baseline concentrations of nitrite, nitrate and nitrotyrosine were significantly higher in the exhaled breath condensate of asthmatic children.^{8,10,27} Whereas others have shown reductions in nitrite and nitrate after 8 weeks of ICS therapy,²⁸ we observed NO oxidation in the ELF of children with mild-to-moderate and severe allergic asthma despite ICS treatment. This observation is intriguing and may reflect decreased sensitivity to ICS in this population. Alternatively, NO oxidation products in the ELF may reflect complex biochemical abnormalities that are distinct from other types of airway inflammation and are not necessarily influenced by ICS treatment.²⁹

While there is increasing evidence of distal airway inflammation in human³⁰ and experimental³¹ models of asthma, our results show that airway nitrosative stress is consistently higher in the proximal versus the distal airways. For this study, we performed sequential BAL

of the right middle lobe to separate proximal and distal airway constituents.²¹ Because this method of lavage was adapted for children to account for different body weights, our data may not accurately reflect nitrosative stress in the bronchial versus alveolar airspace. Thus our distal airway samples may have contained a pooling of bronchial and alveolar NO oxidation products. However, our findings are similar to those of others showing increased inflammation in the bronchial versus alveolar space in asthmatic adults³² and lend support to the more proximal involvement of the airways in asthmatic children.

Our data do not show clear linear associations between ELF NO oxidation products and clinical features of asthma in children, which may be a function of our limited sample size or our patient selection. In addition, it is possible that our measurements of F_{ENO} and NO oxidation products were confounded by ICS and atopy. In steroid-naïve asthmatics, F_{ENO} falls in a dose-dependent manner after the initiation of ICS.³³ Allergic sensitization is also associated with increased F_{ENO} independent of asthma,³⁴ a finding which may be attributable to a late-phase influx of eosinophils. In the present study, all of the children with severe asthma were treated with ICS and had objective evidence of aeroallergen sensitization. Furthermore, 80% (n = 12) of the children mild-to-moderate asthma were taking daily ICS and 53% (n = 8) had positive skin prick responses. Whereas NO metabolites were not associated with any clinical features, like others,³⁵ we did observe an association between F_{ENO} and airway eosinophils. This finding may explain the utility of F_{ENO} in guiding ICS reduction and evaluating asthma control.³⁶ Because there may also be neutrophilic or other patterns of airway inflammation in children with severe asthma, our findings also may reflect the marked heterogeneity of this group of patients. Alternatively, the differences in F_{ENO} among asthmatics may be due to airway pH³⁷ or altered s-nitrosothiol metabolism³⁸ and not NO oxidation.

This study had a number of limitations. Because bronchoscopy cannot be ethically performed in healthy children, our pediatric control group was limited to non-asthmatic children with significant respiratory symptoms. The inclusion of these children may have resulted in inadvertent selection of a group of children with significant nitrosative stress. It is also possible that some of our mild-to-moderate asthmatics were under-treated. Thus, the NO oxidation products measured in our group of children with mild-to-moderate asthma may not be reflective of the larger population and may have been reduced with more aggressive ICS treatment.

In summary, we have demonstrated significant increases in the formation of NO oxidation products in the proximal and distal airway ELF of children with persistent asthma. Contrary to our hypothesis, NO oxidation products did not differ between children with mild-to-moderate and severe allergic asthma. While these data highlight the magnitude of oxidant stress that is present in the airways of children with symptomatic asthma, the relationship of this nitrosative stress to asthma severity is yet unclear. Additional studies are warranted to determine the clinical utility of measuring NO oxidation products in asthmatic children, particularly given the marked heterogeneity of the disease. It may be that targeted interventions to reduce nitrosative stress are indicated in children with significant nitrosative stress despite ICS treatment.

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ABBREVIATIONS

BAL, Bronchoalveolar lavage
 ELF, Epithelial lining fluid
 F_{ENO} , Fraction of exhaled nitric oxide
 FEF_{25-75} , Forced expiratory flow
 FEV_1 , Forced expiratory volume in one second
 FVC, Forced vital capacity
 ICS, Inhaled corticosteroid
 IgE, Immunoglobulin E
 NO, Nitric oxide
 NO_2^- , Nitrite
 NO_3^- , Nitrate

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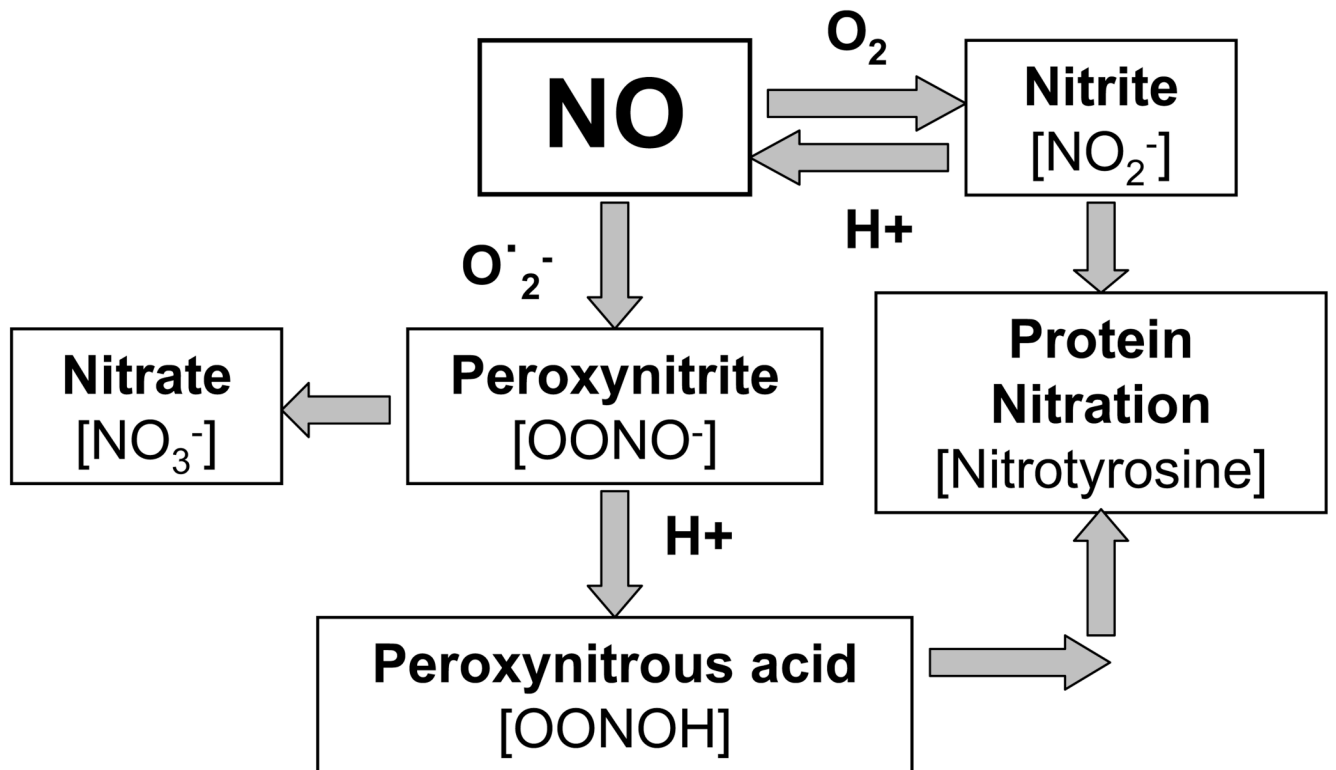


Figure 1.
Diagram of nitric oxide (NO) metabolite formation in the airways.

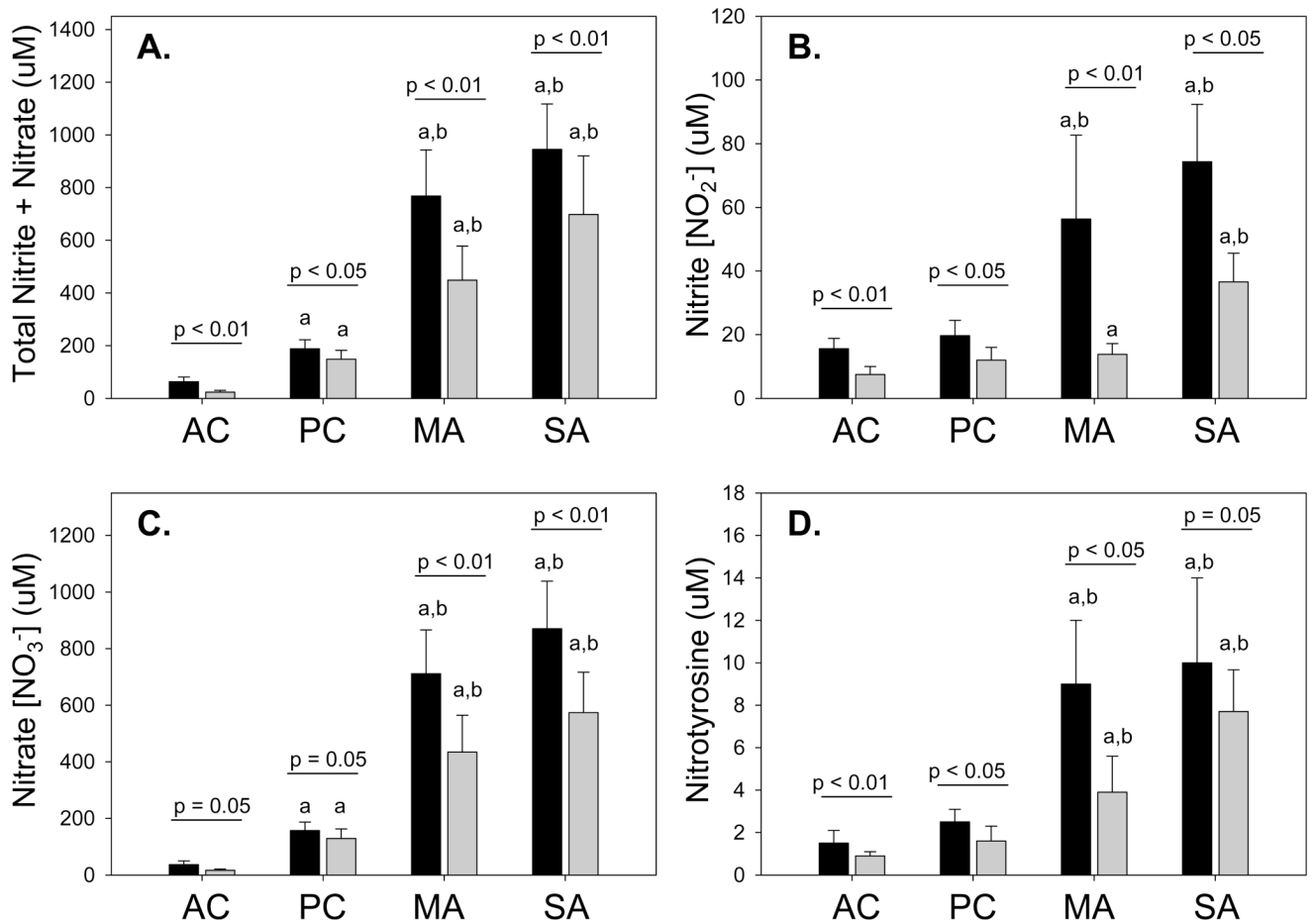


Figure 2.

(A) Total nitrite + nitrate, (B) nitrite, (C) nitrate, and (D) nitrotyrosine concentrations (μM) in the proximal (dark bars) and distal (light bars) airway ELF. Data represent the mean \pm SEM with AC = adult control, PC = pediatric control, MA = mild-to-moderate asthma, and SA = severe asthma. ^ap < 0.05 versus AC, ^bp < 0.05 versus PC.

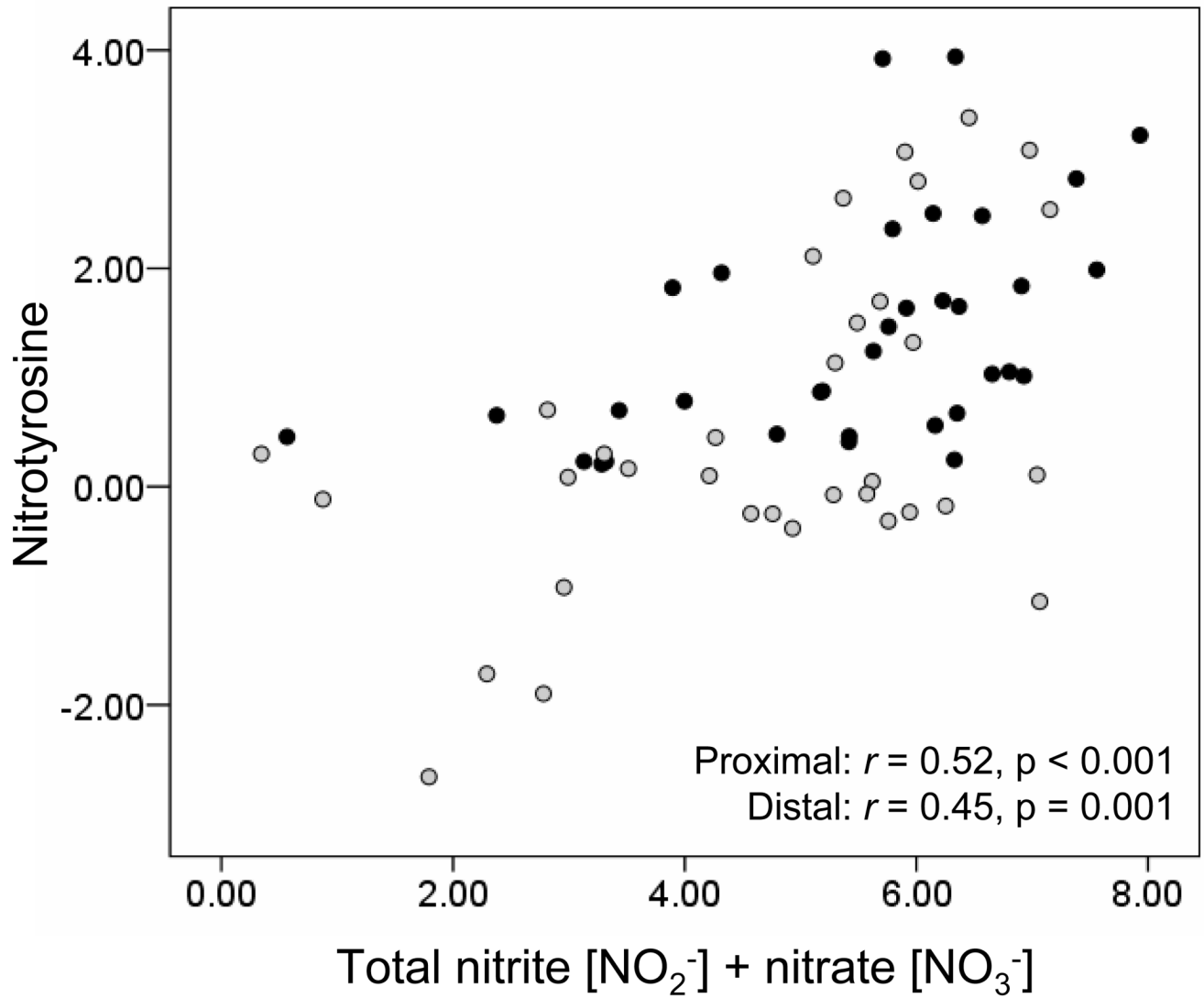


Figure 3. Scatterplot depicting the relationship between total nitrite + nitrate and nitrotyrosine concentrations (μM) in the proximal (dark circles) and distal (light circles) airway ELF. Data were logarithmically transformed.

Table IFeatures of the sample. Data represent the mean \pm SD or the frequency (%).

	Adult control (n = 20)	Pediatric control (n = 5)	Mild-to-Moderate asthma (n = 15)	Severe asthma (n = 30)
Age (in years)	39 \pm 10	11 \pm 4 ^a	10 \pm 4 ^a	10 \pm 4 ^a
Male gender	8 (40)	3 (60)	10 (67)	15 (50)
Caucasian	8 (40)	4 (80)	14 (93) ^a	9 (30) ^{b,c}
African-American	11 (55)	1 (20)	1 (7) ^a	20 (67) ^{b,c}
ICS dose (μ g fluticasone/day)	0	0	262 \pm 189 ^{a,b}	917 \pm 236 ^{a,b,c}
Asthma medications				
Budesonide	0	0	3 (20)	7 (23)
Fluticasone	0	0	1 (7)	1 (3)
Fluticasone/salmeterol	0	0	8 (53) ^{a,b}	22 (73) ^{a,b,c}
Montelukast	0	0	10 (67) ^{a,b}	28 (93) ^{a,b,c}
Prednisone	0	0	0	11 (37) ^{a,b,c}
Emergency room visit (previous year)	0	0	3 (20)	28 (93) ^{a,b,c}
Hospitalization (previous year)	0	0	1 (7)	26 (87) ^{a,b,c}
Intensive Care Unit admission (ever)	0	0	0	14 (47) ^{a,b,c}
Intubation (ever)	0	0	0	6 (20) ^{a,b,c}
FVC (% predicted)	98 \pm 16	102 \pm 18	102 \pm 15	87 \pm 19 ^{a,b,c}
FEV ₁ (% predicted)	103 \pm 16	101 \pm 15	100 \pm 15	73 \pm 20 ^{a,b,c}
FEV ₁ FVC	0.86 \pm 0.07	0.89 \pm 0.03	0.87 \pm 0.06	0.74 \pm 0.12 ^{a,b,c}
FEF ₂₅₋₇₅ (% predicted)	121 \pm 32	92 \pm 16 ^a	94 \pm 23 ^a	51 \pm 25 ^{a,b,c}
FEV ₁ bronchodilator reversibility (%) ^I	3 \pm 6	6 \pm 5	9 \pm 11	23 \pm 17
F _{ENO} (offline, ppb)	5 \pm 3	7 \pm 4	11 \pm 12 ^a	13 \pm 10 ^{a,b}
Elevated baseline F _{ENO} (> 10 ppb)	4 (20)	2 (40)	3 (20)	20 (67) ^{a,b,c}
Reported allergies	Not assessed	2 (40)	9 (60)	25 (83)
Reported atopic dermatitis	Not assessed	0	5 (33)	21 (70)
Number of skin prick responses	Not assessed	0	2 \pm 2	5 \pm 3 ^{b,c}
Serum IgE (kU/L)	100 \pm 194	80 \pm 64	94 \pm 139	487 \pm 730 ^{a,b,c}

^I Calculated by: [(FEV₁; post-bronchodilator – FEV₁ pre-bronchodilator)/predicted FEV₁]*100^a p < 0.05 vs. adult control^b p < 0.05 vs. pediatric control^c p < 0.05 vs. mild-to-moderate asthma