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Fractional Exhaled Nitric Oxide Exchange Parameters Among 9-Year-Old Inner-City Children

Maria José Rosa, BA[1,2,3], **Adnan Divjan**[1,2], **Lori Hoepner, MPH**[1,2], **Beverley J. Sheares, MD**[1,4], **Diurka Diaz, MA**[1,2], **Kevin Gauvey-Kern, BA**[1,3], **Frederica P. Perera, DrPH**[1,2], **Rachel L. Miller, MD**[1,2,3], and **Matthew S. Perzanowski, PhD**[1,2,*]

[1] Columbia Center for Children's Environmental Health (CCCEH), Columbia University, New York, New York

[2] Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York

[3] Division of Pulmonary, Allergy, Critical Care Medicine, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York

[4] Pediatric Pulmonary Division, Columbia University College of Physicians and Surgeons, New York, New York

Summary

Objectives and Hypothesis—To determine the feasibility of using a multiple flow offline fractional exhaled nitric oxide (FeNO) collection method in an inner-city cohort and determine this population's alveolar and conducting airway contributions of NO. We hypothesized that the flow independent NO parameters would be associated differentially with wheeze and seroatopy.

Methods—As part of a birth cohort study, 9-year-old children (n = 102) of African-American and Dominican mothers living in low-income NYC neighborhoods had FeNO samples collected offline at constant flow rates of 50, 83, and 100 ml/sec. Seroatopy was defined as having measurable (≥0.35 IU/ml) specific IgE to any of the five inhalant indoor allergens tested. Current wheeze (last 12 months) was assessed by ISAAC questionnaire. Bronchial NO flux (J_{NO}) and alveolar NO concentration (C_{alv}) were estimated by the Pietropaoli and Hogman methods.

Results—Valid exhalation flow rates were achieved in 96% of the children. Children with seroatopy (53%) had significantly higher median J_{NO} (522 pl/sec vs. 161 pl/sec, $P < 0.001$) when compared to non-seroatopic children; however, median C_{alv} was not significantly different between these two groups (5.5 vs. 5.8, $P = 0.644$). Children with wheeze in the past year (21.6%) had significantly higher median C_{alv} (8.4 ppb vs. 4.9 ppb, $P < 0.001$), but not J_{NO} (295 pl/sec vs. 165 pl/sec, $P = 0.241$) when compared with children without wheeze. These associations remained stable after adjustment for known confounders/covariates.

Conclusions—The multiple flow method was easily implemented in this pediatric inner-city cohort. In this study population, alveolar concentration of NO may be a better indicator of current wheeze than single flow FeNO.

Keywords

asthma; atopy; offline FeNO method; alveolar NO; bronchial flux

*Correspondence to: Matthew S. Perzanowski, PhD, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 60 Haven Avenue, B-1, New York, NY 10032. mp2217@columbia.edu.

INTRODUCTION

Fractional exhaled nitric oxide (FeNO) is a useful non-invasive marker of airway inflammation in cohort studies assessing respiratory diseases. Currently, the accepted FeNO collection method utilizes one constant exhalation flow rate, and both online and offline collection methods have been standardized by the American Thoracic Society.[1] However, this method fails to address relative contributions of NO from the peripheral and distal airways. Source attribution is important because FeNO is reduced by inhaled corticosteroid (ICS) use, tobacco smoke exposure, and more importantly, is elevated with atopy, reducing the specificity of this measure for respiratory symptoms in population-based studies.[2,3]

NO concentration in exhaled breath is highly dependent on exhalation flow rate; the higher the flow rate, the lower the FeNO concentration. Taking advantage of this phenomenon, a mathematical model of nitric oxide exchange dynamics was developed that utilizes collection of breath at multiple exhalation flow rates to estimate the NO derived from the alveoli (C_{alv}, alveolar NO concentration) and the airway (J_{NO}, bronchial NO flux).[4] This new method has proved useful in identifying NO production sources in the airways of individuals with cystic fibrosis, chronic obstructive pulmonary disease, and asthma.[5–7] In a study of asthmatic and non-asthmatic children in the UK, when compared to healthy controls, the alveolar concentrations of NO were found to be higher among children with severe or poorly controlled asthma, but not among mild asthmatic or atopic, non-asthmatic children.[8]

The first objective of this study was to demonstrate the feasibility of using a multiple flow offline collection method in 9-year-old children participating in an inner-city cohort study. The second objective was to determine alveolar and conducting airway contributions of NO to exhaled breath in this population-based cohort. We hypothesized that the flow independent NO parameters would be associated differentially with wheeze in the past 12 months and seroatopy.

MATERIALS AND METHODS

Study Population

Participants were enrolled in a prospective birth cohort study conducted by the Columbia Center for Children's Environmental Health (CCCEH). Enrollment has been described previously.[9–11] Briefly, non-smoking, healthy women aged 18–35, who self-identified as being of Dominican ethnicity or African American race and were living in Northern Manhattan and the South Bronx were recruited during pregnancy. Children who had a baseline home visit and a blood sample (cord, maternal or both) were considered fully enrolled (n = 725). Among the children fully enrolled in CCCEH who have reached age 9 (n = 181), 106 children participated in this study (i.e., 41% lost-to-follow-up for this analysis). All Columbia University Institutional Review Board procedures for consent and assent were followed.

Procedures

Breath samples were collected from children using a modified Sievers bag collection and sampling kit (GE Analytical Instruments, Boulder, CO), which allows for inhalation of air from an NO scrubber and exhalation against a back pressure to prevent contamination from ambient NO and the upper airways, respectively.[12] Samples were collected at three different flow rates, 50, 83, and 100 ml/sec, selected because they were considered achievable by a majority of the 9-year-old children in this cohort. The lowest and highest flow rate samples were collected in duplicate and the middle flow rate samples in triplicate. Children were instructed to inhale NO free air through a scrubber. After inhalation, children exhaled for as

long possible while maintaining the desired stable pressure. To account for dead space, the first part of the breath (approximately 1–3 sec) was discarded using the divert valve. The rest of the breath was collected in a 1.5 L Mylar® balloon. The approximate pressure measured in centimeters of water of each breath sample collection was recorded and converted to a flow rate in ml/sec using a conversion equation provided by the device's manufacturer (flow in ml/sec = 21.127 × pressure in cm $H_2O^{0.53306}$).[13] NO was measured with a chemiluminescent analyzer (NOA 280 i-2; Sievers Instruments, Boulder, CO). All balloons were read within 6 hours of collection time.

Mothers were asked if the child had taken any medication on the day of collection or in the previous 3 months. Questionnaires on detailed asthma and allergy related symptoms in the previous year were administered concurrently. Blood from the child was collected the same day. Specific IgE to cat, mouse urine, dog, *Dermatophagoides* farinae, and cockroach were measured as previously described using the Immunocap system (Phadia, Uppsala, Sweden),[14] Children were considered seroatopic if they had measurable IgE (≥0.35 IU/ml) specific to any of the indoor allergens tested. For the 32 children with missing IgE values at age 9, age 7 data were available on 23 children, and was used in the analyses. Data on specific IgE antibodies to mold, grass and ragweed at age seven were also available for 84 of the subjects.

Data Analysis

NO independent parameters were calculated using two previously published methods, the Pietropaoli and Hogman methods.[15,16] Due to a non-normal and non-log-normal distribution of the NO parameters, medians with 25% and 75% are reported and differences between medians were tested by the Mann–Whitney test. Correlations were tested by Spearman Rank test. Parameters also were compared using logistic regression models, adjusting for potential confounders and covariates. For the adjusted models, FeNO collected at 50 ml/sec ($FeNO_{50}$), bronchial flux and alveolar concentrations were dichotomized into highest quartile and the three remaining quartiles. Maternal education, dichotomized on completion of high school at the time of the child's birth, was used as a proxy for socioeconomic status. Children with reported inhaled corticosteroid (ICS) use the day of the test were excluded from these analyses, because these medications are known to decrease both flow dependent and independent exhaled NO concentrations.[8,17] Analyses of the associations were also conducted by excluding children who had a report of inhaled or oral steroid use in the previous 3 months but not on the day of the test. Data were analyzed using Microsoft Excel (Redmond, WA) and SPSS Version 16 (Chicago, IL).

RESULTS

Study subject demographics are detailed in Table 1. Ninety-six percent of children (104/106) achieved a valid test as determined by inhalation through the collection device and exhalation at the desired flow rates. Two children reported ICS use the day of the test and were excluded from the analyses, resulting in 102 children for analyses. Seven additional subjects reported inhaled (n = 7) or oral (n = 4) steroid use the past 3 months but not on the day of the test were included in the analyses (unless otherwise noted).

Correlations Between Flow Independent and Dependent NO Parameters

Despite employing different mathematical models, the Hogman and Pietropaoli methods provided estimates of the flow independent NO parameters that correlated well with one another (Fig. 1). Therefore, only Hogman method parameters are reported in the subsequent analyses. $FeNO_{50}$ correlated with both J_{NO} (r = 0.877, P = <0.001) and C_{alv} (r = 0.388, P = <0.001); however, there was no correlation between J_{NO} and C_{alv} (r = −0.003, P = 0.997).

Flow Dependent and Independent NO Parameters by Demographics, Seroatopy, and Wheeze

No significant differences in flow dependent or independent NO parameters were observed by sex or ethnicity/race. Children whose mother reported having asthma had significantly decreased J_{NO} as compared with those children whose mothers did not report having asthma (Table 2).

Children with seroatopy had significantly elevated FeNO levels at all three flow rates and elevated J_{NO} when compared to children without seroatopy; however, there was no difference in C_{alv} between these two groups (Table 3). Children with reported current wheeze had significantly elevated FeNO at every flow rate when compared to children with no reported wheeze. In contrast to the findings for seroatopy, children with current wheeze had significantly elevated C_{alv} when compared to children with no current wheeze, but there was no difference in J_{NO}. C_{alv} was also significantly elevated in children with reported use of asthma controller medications (e.g., inhaled corticosteroids, leukotriene modifiers) in the past year, and in those who had wheezed with exercise as compared with those who did not. Receiver operation characteristic curves (ROC) further demonstrate the lack of association between atopy and alveolar NO, but show C_{alv} as a better predictor of current wheeze than FeNO or bronchial NO flux (Fig. 2).

The distributions of the FeNO and C_{alv} levels among the seven children who had a report of steroid use in the past 3 months, but not on the day of the test were similar to the "wheeze in the past 12 months" population in general with 3/7 and 4/7 having levels above the median for the wheeze group for $FeNO_{50}$ and Calv, respectively. Similarly, the J_{NO} among this group was similar to that for the seroatopic children with 3/7 having levels above the median for the seroatopic group. The presence or absence of significant differences in NO parameters by the symptoms in Table 3 for the most part were consistent after exclusion of these children from the analyses and are shown in Table 4, with the notable exception that J_{NO} was statistically significantly higher among children with a report of cough at night when compared to those without (428 pl/sec vs. 269 pl/sec, $P = 0.005$). Of the 84 children on whom data on specific IgE to mold, grass, or ragweed was available, only 3 that were not previously classified as allergic were allergic to these allergens. These three children all had $FeNO_{50}$ levels below the median for both the non-wheezers and the children without IgE to the indoor inhalant allergens.

J_{NO} was significantly elevated in both wheezing and non-wheezing atopic children when compared to their non-atopic counterparts (Fig. 3a). C_{alv} medians were not significantly different between atopic and non-atopic children when stratified by reported wheeze (Fig. 3b).

Multivariable Models

Seroatopy was significantly associated with having an $FeNO_{50}$ in the highest quartile, an association which remained after adjustment for wheeze, sex, ethnicity/race, maternal asthma and education, and ambient NO levels (Model 1, Table 5). Seroatopy remained significantly associated with $FeNO_{50}$ when the analyses was limited to the steroid naïve children ($P = 0.02$). In a model controlling for the same covariates, including wheeze, seroatopy was significantly associated with elevated J_{NO} (Table 5), an association also observed among the steroid naïve children ($P = 0.001$). In a third model with C_{alv} in the highest quartile as the dependent variable, only current wheeze was a statistically significant predictor (Table 5), and this association remained when only the steroid naïve children were examined ($P = 0.019$). C_{alv} in the highest quartile was a significant predictor of current

wheeze (OR 7.3; 95% CI 2.4–22.7; P <0.001), after adjustment for seroatopy, J_{NO} and other covariates.

Exclusion of Nonlinear Flow Independent Parameters

Even though the majority of the children in our cohort successfully performed the multiple flow maneuvers, some of the measurements did not fit the linear models employed by Hogman and Pietropaoli. Twenty-two percent (i.e., 22/102) of the children had Hogman models with an r^2 less than 0.8. Having a linear model with an r^2 <0.8 was significantly more common for children with high (>20 ppb) as compared with low (<20 ppb) $FeNO_{50}$ (36.7% vs. 15.35%, respectively, P = 0.017). To determine whether this nonlinearity affected our findings, the adjusted logistic regression models were performed excluding children with an r^2 <0.8. The magnitudes of the associations were similar and in the same direction (Online Supplement E-Tables 1 and 2).

DISCUSSION

In this inner-city cohort of 9-year-old children, we found that the multiple-flow offline collection method was easily implemented, with a majority of the children (96%) achieving the target flow rates. Even though the Hogman and Pietropaoli methods utilize different calculations to estimate bronchial flux and alveolar concentration, their parameters correlated well. All FeNO values were highly correlated with J_{NO} and to a lesser degree with C_{alv}. However, we found that there was no correlation between C_{alv} and J_{NO} values, which has been shown previously in pediatric studies of asthma and cystic fibrosis.[7,8] FeNO values at every flow rate were associated with both seroatopy and respiratory symptoms. J_{NO} rates were strongly associated with seroatopy and less so with respiratory symptoms. In contrast, alveolar NO concentrations were only associated with wheeze and not with seroatopy. These associations held after adjusting for sex, ethnicity/race, maternal asthma and education, seroatopy and ambient NO. Furthermore, C_{alv} was a better predictor of current wheeze than seroatopy or bronchial NO flux in a multivariate model. The lack of correlation between J_{NO} and C_{alv} and the differential associations between these parameters and seroatopy and respiratory symptoms strengthen the proposal that J_{NO} and C_{alv} provide distinct, clinically relevant outcomes for cohort studies.

Our findings of elevated alveolar NO among children with respiratory symptoms but not among children with atopy replicate those from a study of children in the UK.[8] Novelties of our findings come from the population-based study design and the recruitment from high asthma prevalence neighborhoods in New York City. With respect to the former study characteristic, while the prevalence of wheeze in this cohort was relatively high (i.e., 19%), the majority of the asthmatics appeared to be mild asthmatics (e.g., although 90% of wheezing children had a physician diagnosis of asthma, only 2% of the children had wheezed in the past 2 weeks). In the UK study, elevated C_{alv} was observed only among the severe and uncontrolled asthmatics and not the mild asthmatics. Similarly, a clinic based study of adult asthmatics in the UK observed elevated C_{alv} among refractory asthmatics and not among mild asthmatics when compared with controls.[18] So our findings from this inner-city community are in contrast to UK findings since we observed significantly higher alveolar NO among relatively mild asthmatics. While future studies are needed to confirm these differences, they suggest that the level of distal airway inflammation may be higher in the inner-city U.S. mild asthmatic population than in other urban mild asthmatic populations where the prevalence of asthma is also high.

Many studies have demonstrated the importance of distal lung inflammation to asthma morbidity, including its association with airway hyper-responsiveness, symptom exacerbation, and tissue remodeling.[19–21] Support for alveolar NO as a marker of distal lung

airway inflammation comes from studies showing an association between the number of eosinophils in bronchial alveolar lavage (BAL) and alveolar NO in contrast to the number of eosinophils in sputum and bronchial fluid that have been more closely correlated with conducting airway NO.[18] Further evidence of the association between alveolar airway inflammation and distal airway pathology comes from a study of children with refractory asthma that found a correlation between alveolar NO and lung function expiratory mid-flows (MEF_{25-75}).[22] This same study found differential associations between the flow independent parameter of NO and markers of airway remodeling. Alveolar NO correlated with TGF-β in BAL fluid, while airway NO correlated with basement membrane thickness. [22] With all of these studies, the cross-sectional design and study populations consisting of only relatively severe asthmatics limit the interpretation of the role of NO in airway inflammation and remodeling, but do suggest a relevance of anatomical location of the source of NO (i.e., distal vs. proximal airways).

The partitioning of the NO into proximal and distal airway derived NO can provide a measure of inflammation more closely associated with atopy (J_{NO}) and respiratory symptoms (C_{alv}). Because these measures are modeled and not directly measured, we are not certain whether the alveolar NO has solely alveolar sources or also includes NO from the transition zone airways. Given the importance of this latter part of the airway to asthma and the association in our study and others between C_{alv} and respiratory symptoms, it is compelling that the measure of C_{alv} may represent a combination of the alveolar and transition zone airways.

In our study population, none of the FeNO values differed by gender, ethnicity/race, maternal asthma or maternal educational level. While bronchial flux levels were lower among children whose mother had reported asthma and alveolar NO concentrations were higher among children whose mother did not have a high school education, these associations appeared to be driven by associations between maternal asthma and atopy and maternal education and children with wheeze, respectively. The relationships were no longer statistically significant when adjusted for other covariates in a multivariable model.

In our study we collected steroid (inhaled and oral) medication use for asthma for the day of the test and the previous 3 months. Given the findings that steroid medication in the previous week can affect exhaled nitric oxide levels, it is a limitation that we did not ascertain steroid use specifically for that time period.[23] For the current analyses we a priori excluded the children who had taken mediation on the day of the test, but not those that only reported steroid use in the previous 3 months. Based on reports of non-compliance to controller medication use in similar inner-city populations, we felt that it was more likely that children who had not taken steroid medication on the day of the test would also not have taken it in the previous week.[24] The children with a report of steroid use in the past 3 months, but not on the day of the test had similar FeNO and C_{alv} measures to the children with a report of wheeze and J_{NO} levels to the seroatopic children who did not have a report of steroid medication use in the past 3 months. That we did not have a sufficient sample size to test the steroid naïve and steroid exposed groups separately in the current analyses is also a limitation of the current study. We did test differences in the associations with the NO parameters by symptoms after excluding the seven children (Table 4), and for the most part the associations were similar to those with the seven children. The two notable exceptions were an emergence of a significant differences in J_{NO} between the children with and without nighttime cough and wheeze. Given the small number of children with nighttime cough or wheeze and no steroid medication use, we are reluctant to draw substantive conclusions from this finding. However this difference in children with and without a history of steroid use could be related to steroid medication use reducing J_{NO} among children with symptoms.

Clearly, it will be important in the future to examine the response of these NO parameters to steroid medication treatment in this population.

There are other limitations to our study. The use of the offline method does not currently allow for an automated recording of flow rate over the breath collection. We estimated flow rates by recording the approximate average pressure during collection. In general, these 9-year-old children were able to maintain a constant pressure at all of the selected flow rates. The offline method has been shown to be subject to contamination by ambient NO. Nevertheless, adjusting for ambient NO in multivariate models did not alter the associations. Furthermore, to ensure exclusion of ambient NO contamination we had modified the device as to ensure confirmation that the children actually inhaled through the filter before collecting their breath.[12] The online methodology for FeNO collection has been recommended over the offline in clinic settings; therefore, our use of the offline methods may be seen as a limitation. However, we believe that a strength of our study was demonstrating the feasibility of offline multiple flow collection, because unlike currently available online equipment, offline methods can be used to collect multiple flow samples a field setting. Our method could be best described as a modified Hogman method.[15] Nevertheless, we did not utilize the same flow rates stated by this study, but selected flow rates that were easily achievable by the children in our cohort. The Hogman method assumes a linear association between NO output and exhalation flow rate, so the replication of the exact flow rates published in other studies should be less important as long as they are made in the range expected to be linear. Another limitation was that current wheeze was defined broadly as any wheeze within the past year, which would have resulted in children with only one virally associated wheeze episode categorized the same as children with moderate or severe asthma. A more detailed history and the inclusion of a more severe asthmatic population could have allowed us to better understand the association between alveolar NO and asthma severity and control in this inner-city population. Even though we did not correct for multiple comparisons, a priori we only chose two independent health predictors, wheeze and seroatopy, and three FeNO parameters, $FeNO_{50}$, C_{alv}, and J_{NO}. Our main health outcome findings of significant differences in JNO by seroatopy and C_{alv} by wheeze were both highly significant ($P < 0.001$), limiting the likelihood that they were due to chance.

In summary, the multiple flow collection of FeNO proved to be an easily implemented and reproducible method for a study of 9-year-old children living in inner-city communities. The bronchial flux parameter appeared to be conditioned by seroatopy, while alveolar concentrations were only elevated in the presence of respiratory symptoms in the past year. In contrast to previous studies that only found elevated alveolar NO among severe and uncontrolled asthmatics, children with relatively mild asthma symptom frequencies in this inner-city community had higher alveolar NO than those without symptoms. The specificity of alveolar NO for respiratory symptoms and not atopy potentially make it a more suitable outcome than single flow FeNO in cohort studies of pediatric asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

$\mathbf{C_{alv}}$	Alveolar NO concentration
FeNO	Fractional exhaled nitric oxide
$\mathbf{FeNO_{50}}$	Fractional exhaled nitric oxide collected at 50 ml/sec
$\mathbf{J_{NO}}$	Total NO bronchial flux

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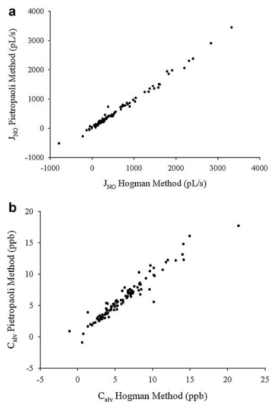

Fig. 1.

Correlation between calculated (a) bronchial fluxes and alveolar concentrations (b) using the Hogman and Pietropaoli methods. There was a significant correlation for estimations of both bronchial fluxes and (R = 0.989, $P<0.001$) and alveolar concentrations (R = 0.962, $P<0.001$).

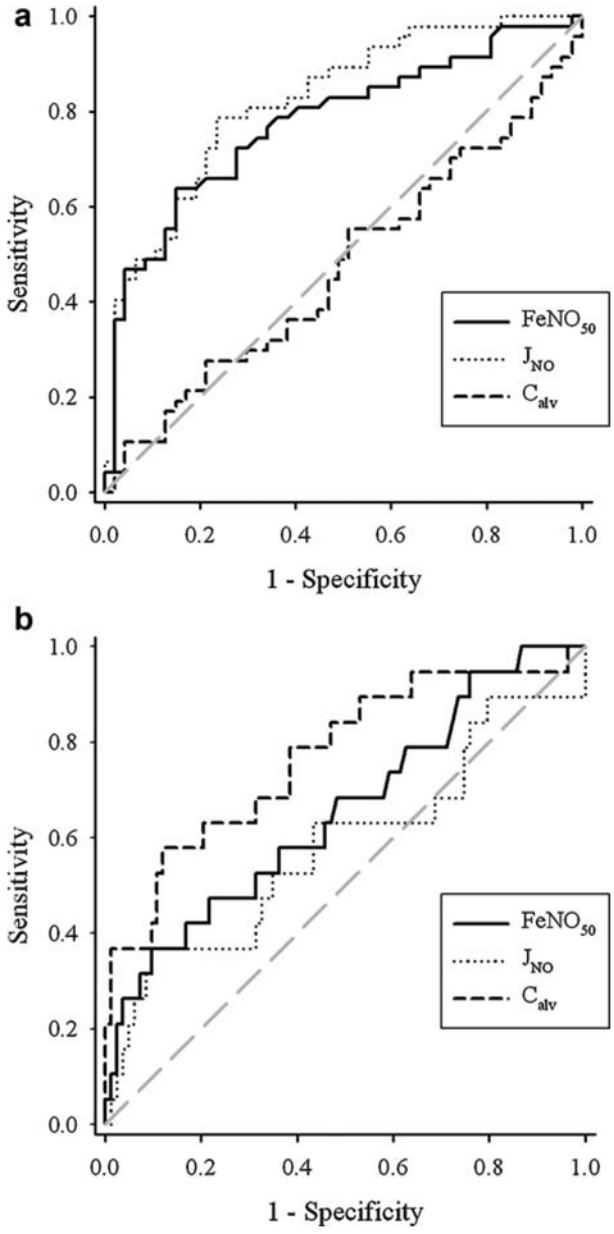

Fig. 2.

Receiver operation characteristics (ROC) curves for (a) seroatopy and (b) current wheeze. For seroatopy, the areas were 0.78 ($P<0.001$), 0.82 ($P<0.001$), and 0.47 ($P= 0.66$), for $FeNO_{50}$, J_{NO}, and C_{alv}, respectively. For wheeze the areas under the curve were 0.66 ($P= 0.034$), 0.58 ($P= 0.29$), and 0.77 ($P<0.001$) for $FeNO_{50}$, Bronchial NO flux (J_{NO}), and alveolar NO (C_{alv}), respectively.

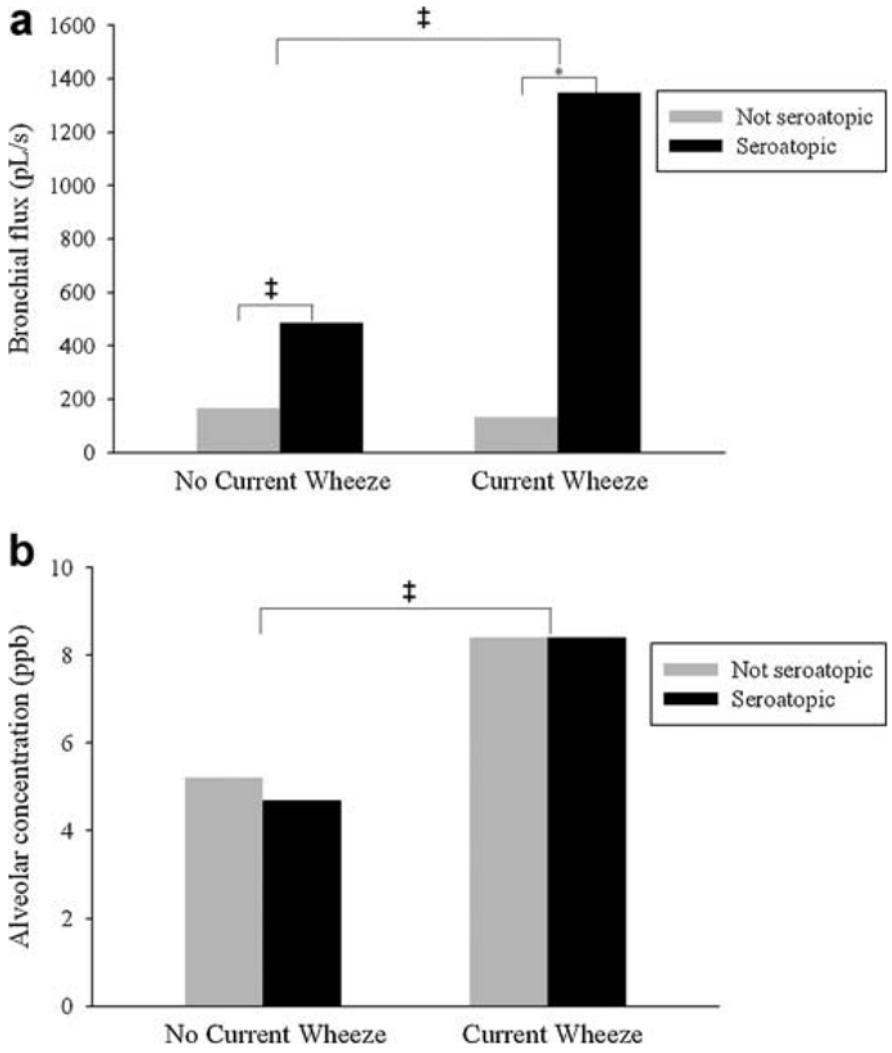

Fig. 3.

Bronchial flux comparison (a) and alveolar concentration comparison (b) by current wheeze and seroatopy status. **P*-value < 0.05, †*P*-value < 0.01, ‡ *P*-value < 0.001.

TABLE 1

Study Cohort Demographic Information

Average age in years (min–max)	9.0 (8.9–9.7)
Male sex, n (%)	45/102 (44)
Ethnicity/race	
Dominican, n (%)	52/102 (51)
African American, n (%)	50/102 (49)
Mother had not completed high school at enrollment, n (%)	35/102 (35.4)
Maternal asthma, n (%)	22/102 (21.6)
Child current wheeze[1], n (%)	19/101 (18.8)
Child wheezed in the past 2 weeks, n (%)	2/101 (2.0)
Child seroatopic[2], n (%)	49/93 (53)

[1] Current wheeze was defined as reported wheeze in the past 12 months by the ISAAC questionnaire.

[2] Seroatopy was defined as specific IgE ≥ 0.35 IU/ml to cockroach, mouse urine, *D. farinae*, cat dander, or dog.

TABLE 2

Flow Dependent and Independent NO Parameters Medians (25th–75th Percentile) by Demographic Characteristics

		FeNO by flow rate (ppb)			Flow independent parameters [1]	
	n	50 ml/sec	83 ml/sec	100 ml/sec	J_{NO} (pl/sec)	C_{alv} (ppb)
Sex						
Male	45	10.7 (7.9–22.8)	9.6 (6.5–17.2)	9.5 (6.5–14.1)	326 (137–810)	5.3 (3.7–7.5)
Female	57	9.9 (7.9–21.5)	8.9 (7.9–21.5)	8.9 (6.0–14.5)	273 (141–695)	5.8 (3.8–7.9)
Ethnicity/race						
Dominican	52	10.4 (8.0–20.1)	8.7 (6.6–12.7)	8.8 (6.5–13.4)	265 (135–635)	6.2 (4.5–8.2)
African-American	50	12.5 (7.5–22.6)	9.9 (6.0–18.4)	9.8 (5.7–14.9)	80 (−38–153)	4.7 (3.1–7.5)
Maternal asthma						
No	80	11.8 (8.0–22.1)	9.4 (6.3–16.1)	9.8 (6.3–15.4)	308 (172–782)*	5.2 (3.8–7.4)
Yes	22	9.2 (6.7–13.9)	8.7 (6.5–12.3)	8.8 (5.8–11.0)	143 (52–469)	6.5 (4.5–8.3)
Mother completed high school						
No	35	9.9 (8.0–16.5)	7.6 (6.0–12.9)	7.6 (6.3–10.7)	265 (158–685)	4.8 (3.3–6.7)
Yes	64	11.8 (7.9–24.5)	9.6 (6.6–19.3)	9.9 (6.3–16.9)	304 (141–884)	6.0 (3.9–8.3)*

[1] Flow independent NO parameters calculated by the Hogman method.[15]

* *P*-value < 0.05.

TABLE 3

Flow Dependent and Independent Parameter Medians (25th–75th Percentile) by Medication Use, Respiratory Symptoms[1] and Seroatopy

		FeNO (ppb) by flow rate			Flow independent parameters	
	N	**50 ml/sec**	**83 ml/sec**	**100 ml/sec**	**J_{NO} (pl/sec)**	**C_{alv} (ppb)**
Current wheeze						
No	82	10.2 (7.7–19.6)	8.6 (6.2–13.9)	8.2 (5.9–13.2)	269 (135–675)	4.9 (3.5–7.1)
Yes	19	16.2 (9.1–41.1)*	13.2 (8.6–28.2)†	17.0 (9.5–26.5)‡	389 (140–1595)	8.4 (5.9–13.1)‡
Asthma controller medication[2]						
No	90	10.7 (7.9–20.4)	8.8 (6.3–14.6)	8.8 (6.1–13.3)	280 (155–693)	5.2 (3.7–7.2)
Yes	11	10.6 (7.9–32.8)	12.1 (6.6–25.0)	17.0 (7.9–19.5)*	306 (107–1595)	8.4 (5.2–14.1)*
Woken up at night by cough						
No	85	10.4 (7.8–20.7)	8.7 (6.3–14.7)	8.4 (6.0–13.4)	273 (141–679)	5.2 (3.7–7.4)
Yes	13	16.8 (9.2–40.4)*	13.8 (9.6–28.9)*	16.5 (10.9–23.1)†	522 (137–1681)	6.7 (4.8–10.3)
Wheeze after exercise						
No	84	10.6 (7.9–21.5)	9.1 (6.3–15.7)	8.9 (6.1–14.1)	295 (159–727)	5.3 (3.7–7.2)
Yes	14	10.0 (7.8–34.5)	10.5 (6.7–25.8)	10.9 (6.6–21.2)	165 (103–1488)	6.9 (4.9–10.2)*
Seroatopy[3]						
No	44	9.0 (7.3–11.5)	7.4 (6.1–9.7)	7.5 (5.8–10.1)	161 (80–271)	5.5 (3.8–7.8)
Yes	49	19.5 (10.2–30.4)‡	13.8 (7.3–22.9)‡	12.3 (7.4–19.0)‡	522 (269–1360)‡	5.8 (3.2–8.2)

[1] All symptom data were assessed through ISAAC questionnaire. Current wheeze was defined as reported wheeze in the past 12 months in ISAAC questionnaire.

[2] Asthma controller medications include leukotriene modifiers, inhaled or oral steroids.

[3] Seroatopy was defined as specific IgE ≥ 0.35 IU/ml to cockroach, mouse urine, *D. farinae*, cat dander, or dog. Among the 92 children with IgE measured at age 7 and age 9 years, total IgE values for the same children correlated well (R = 0.829, $P < 0.001$) and 88% of the children considered seroatopic at age nine also were considered seroatopic at age 7.

* P-value < 0.05.

† P-value < 0.01.

‡ P-value < 0.001.

TABLE 4

Flow Dependent and Independent Parameter Medians (25th–75th Percentile) by Medication Use, Respiratory Symptoms[1] and Seroatopy Including Only Steroid Naïve Subjects

		Flow dependent parameters: FeNO (ppb) by flow rate			Flow independent parameters	
	N	50 ml/sec	83 ml/sec	100 ml/sec	J_{NO} (pl/sec)	C_{alv} (ppb)
Current wheeze						
No	81	9.9 (7.7–18.1)	8.6 (6.2–13.8)	8.2 (5.9–12.8)	265 (134–618)	4.9 (3.5–7.1)
Yes	13	21.5 (9.4–44.1)*	15.9 (8.9–29.0)†	18.1 (8.2.5–27.6)†	467 (244–1620)*	8.3 (5.6–12.6)†
Asthma controller medication[2]						
No	90	10.7 (7.9–20.4)	8.8 (6.3–14.6)	8.8 (6.1–13.3)	280 (155–693)	5.2 (3.7–7.2)
Yes	4	15.6 (7.0–49.5)	14.4 (5.7–35.4)	12.9 (4.8–30.7)	401 (181–1858)	8.4 (2.3–14.2)
Woken up at night by cough						
No	82	9.9 (7.7–20.1)	8.5 (6.2–14.1)	8.2 (5.9–13.2)	269 (143–590)	5.2 (3.7–7.4)
Yes	9	27.7 (14.9–40.4)†	19.6 (11.9–28.9)†	16.5 (11.0–23.1)†	1256 (427–1828)†	5.9 (4.2–7.2)
Wheeze after exercise						
No	82	10.5 (7.9–21.5)	8.8 (6.3–15.4)	8.8 (6.1–13.9)	295 (160–721)	5.2 (3.7–7.2)
Yes	9	10.8 (7.4–28.9)	10.4 (6.6–21.0)	9.6 (6.1–19.2)	183 (125–987)	6.0 (4.8–9.0)
Seroatopy[3]						
No	40	8.7 (7.1–12.3)	7.1 (5.9–9.6)	7.4 (5.7–10.0)	180 (85–291)	5.2 (3.8–7.2)
Yes	46	16.6 (9.9–28.1)‡	13.3 (7.2–21.9)‡	12.0 (6.9–18.2)‡	482 (261–1094)‡	5.8 (3.1–8.2)

[1] All symptom data were assessed through ISAAC questionnaire. Current wheeze was defined as reported wheeze in the past 12 months in ISAAC questionnaire.

[2] Asthma controller medications include leukotriene modifiers, inhaled or oral steroids.

[3] Seroatopy was defined as specific IgE ≥ 0.35 IU/ml to cockroach, mouse urine, *D. farinae*, cat dander, or dog. Among the 92 children with IgE measured at age 7 and age 9 years, total IgE values for the same children correlated well (R = 0.829, $P < 0.001$) and 88% of the children considered seroatopic at age nine also were considered seroatopic at age 7.

* P-value < 0.05.

† P-value < 0.01.

‡ P-value < 0.001.

TABLE 5

Adjusted Logistic Regression Models[1] for Flow Dependent and Independent Parameters

Covariates	Model 1 $FeNO_{50}$ OR (95% CI)	Model 2 J_{NO} OR (95% CI)	Model 3 C_{alv} OR (95% CI)
Current wheeze	3.3 (0.76–14.7)	2.0 (0.50–8.4)	5.8 (1.7–19.6)†
Seroatopy	40.0 (4.7–338.6)†	19.2 (3.9–94.6)‡	1.0 (0.34–2.9)
Sex	1.2 (0.36–4.0)	1.5 (0.49–4.8)	0.7 (0.22–2.0)
Ethnicity/race	3.3 (0.97–11.4)	3.4 (1.1–10.8)*	1.1 (0.39–3.2)
Maternal asthma	0.6 (0.11–3.3)	0.57 (0.11–2.8)	1.1 (0.28–4.0)
Maternal education at enrollment	2.1 (0.53–8.4)	1.2 (0.33–4.2)	2.6 (0.72–9.0)
Ambient NO	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)

[1] All models adjusted for all listed covariates.

* *P*-value < 0.05.

† *P*-value < 0.01.

‡ *P*-value < 0.001.