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Exhaled NO Among Inner-city Children in New York City

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Summary

Background—Fractional Exhaled Nitric Oxide (FeNO) has been proposed as a biomarker of airway inflammation for cohort studies of asthma.

Objectives—To assess the association between FeNO and asthma symptoms among seven-year old children living in an inner-city community. To test the association between ETS exposure (previous and current) and FENO among these children.

Methods—As part of a longitudinal study of asthma, children recruited in Head Start centers at age 4 years had offline FeNO and lung function testing at age 7 years. Children with allergen specific IgE (≥0.35 IU/ml) at age 7 were considered seroatopic. Environmental tobacco smoke (ETS) exposure at ages 4 and 7 was assessed by questionnaire.

Results—Of 144 participating children, 89 had complete questionnaire data and achieved valid FeNO and lung function tests. Children with reported wheeze in the previous 12 months (n=19) had higher FeNO than those without wheeze (n=70) (geometric means 17.0 vs. 11.0ppb, p=0.005). FeNO remained significantly associated with wheeze (p=0.031), after adjusting for seroatopy and FEV_1 in multivariable regression. FeNO at age 7 was positively associated with domestic ETS exposure at age 4 (29%)(β=0.36, p=0.015) but inversely associated with ETS exposure at age 7 (16%) (β= −0.74, p<0.001).

Conclusions—Given its association with current wheeze, independent of seroatopy and lung function, FeNO provides a relevant outcome measure for studies in inner-city communities. While compelling, the positive association between ETS exposure at age 4 and a marker of airway inflammation at age 7 should be confirmed in a larger study.

Keywords

Exhaled Nitric Oxide; IgE; Inner-city; Wheeze; Environmental Tobacco Smoke

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Introduction

Because of the episodic nature of its symptoms and the subjectivity of self- and parentally reported symptoms, asthma is a difficult disease to diagnose in young children and outcome to study in research settings.[1, 2] Inflammation of the airways is a virtually universal component of asthma exacerbations, and the measurement of fractional exhaled nitric oxide (FeNO) has emerged as a non-invasive method for measuring airway inflammation.[3] FeNO has been approved for use in clinical settings to assist in monitoring the response to steroid therapy, although the benefit to inner-city asthmatics has been questioned. [4–7] Nevertheless, FeNO may provide a useful measure of airway inflammation to study the inflammatory response in the lungs to environmental exposures.[8–13]

It is well established that FeNO levels are lower in active smokers than in non-smokers.[14] In studies of non-smoking adults and children, those currently exposed to environmental tobacco smoke (ETS) were found to have lower FeNO than non-ETS exposed individuals, and acute ETS exposure was shown to transiently lower FeNO.[12, 15, 16] Both active smoking and passive ETS exposure have been related to long-term detrimental effects on the lung;[17] but the long-term effect of ETS on FeNO in children has not been widely evaluated.

We collected FeNO by offline methods as an outcome measure in a study of seven year-old children living in low-income NYC communities. The aim of this study was to assess FeNO as a predictor of asthma symptoms in this cohort and to test the association between previous and current ETS exposure and FENO among these children.

Methods

Study cohort

Children were recruited through Head Start Centers at age 4 years as part of a previously described study.[18, 19] Briefly, a baseline questionnaire was completed by caregivers of children attending Head Start Centers in New York City. Three years later when the children were 7 years old, participating caregivers brought their children to the clinic for lung function testing. A convenience sample of children provided exhaled breath samples for FeNO measurement. We obtained informed consent for participation for all participating children from their parents or legal guardians. This study was approved by the Institutional Review Board of the Columbia University Medical Center.

Questionnaire

Baseline questionnaires were administered in the Head Start center, by telephone or in the home. For the current analyses, the study cohort included the children for whom a caregiver answered baseline questions regarding wheeze and ETS. The questionnaire included detailed assessment of the child's history of respiratory and other illnesses from birth and frequency of symptoms and clinical visits in the previous year. Information was also collected about home environment characteristics. Questions were asked regarding whether anyone in the home smoked cigarettes and a child living in the home with at least one smoker was considered exposure to ETS. More detailed questions about the amount of smoking were administered but were not included in the analyses because of limited statistical power to further stratify children into categories of smoke exposure.

Three years later a follow-up questionnaire was administered either in the home, in the clinic or over the phone. The follow-up questionnaires included questions about current respiratory symptoms and environmental exposures including ETS. Any child with a report of wheeze or whistling in the chest in the past 12 months at the age 7 interview was considered to have

"current wheeze." For most of the children (94% at baseline, >89% at follow-up) the mother answered the questionnaire at both age 4 and 7.

On a subset of the children (n=58/89 of those in the final analyses) a brief questionnaire was administered at the time of FeNO collection. Caregivers were asked about the child's consumption of food or drink in the previous two hours, current colds or respiratory infections, colds or respiratory infections in the previous 7 days, medications currently taken for asthma or allergies and any medications taken on the day of the FeNO test. Children who had taken medication on the day of the test which could influence FeNO (e.g. inhaled corticosteroids) were excluded from the analyses.

Offline FeNO

At age 7 years, children were brought to our medical center's pediatric pulmonology clinic for lung function testing and collection of exhaled breath for NO analysis. Prior to lung function testing, exhaled breath was collected by the offline method, according to the ATS guidelines,[3] with a previously described modified version of a commercially available collection device (#CBSK 01400-01, GE Instruments, Boulder, CO).[20] The sampler used in the current study had modifications related to exhalation flow, but not inhalation. Samples were collected at a flow of 83 ml/s, which is a higher flow rate than the currently recommended 50 ml/s.[3] This will lead to lower FeNO levels in our cohort than if we had collected the breath sample at 50 ml/s, and thus limits the comparison of levels to other studies that have used the lower flow rate.[21] Exhaled breath from airway dead space was excluded by use of a diversion valve for the first 1–3 seconds of collection. Children were instructed to exhale for as long as they could.

We collected two room air samples on each day for measurement of ambient NO. We then collected breath samples from each child in individual Mylar balloons, monitoring exhalation flow and inhalation through the device. Breath samples were considered valid only if: 1) the child exhaled at a consistent, correct flow *AND*, 2) *EITHER* the child inhaled correctly through the exhaled breath collection device (and thus the charcoal scrubber) *OR* the ambient NO was less than 20 ppb. When children did not provide a valid exhalation, we attempted to collect more samples until we had at least three per child. A mean of the valid tests was calculated for analyses. Among the children in the final analyses, 73% had at least two tests. The correlation between the first two tests was good (R^2=0.75, P<0.001) and the geometric mean difference between the 1st and 2nd test was 1.3 ppb. Within 4 hours of collection, samples were assayed using an NO analyzer (GE Instruments, Boulder, CO).

Lung function

Spirometry was conducted according American Thoracic Society (ATS) guidelines[22] with the children standing and using nose clips. Percent predicted values for FVC, FEV_1, and $FEF_{25–75}$ were calculated using NHANES predicted values for age, height, and race.[23]

Body mass index

Heights and weights were measured at the time of FeNO collection. Data on height, weight, age, and gender were used to calculate body mass index (BMI) percentile using the Centers for Disease Control 2000 growth charts in Epi Info (CDC, Atlanta, GA).[24]

Serum antibodies

During the clinic visit or during a home visit at age 7, serum was collected from the child. Total IgE and IgE antibodies against *Dermatophagoides farinae*, German cockroach, mouse, and cat were measured by ImmunoCAP (Phadia, Portage, MI). Children with specific IgE ≥0.35 IU/ml to any of the four allergens tested were considered seroatopic.

Statistics

Because the distributions of FeNO and total IgE data in this sample were log-normal, we analyzed their logarithmically transformed values. We used ANOVA to compare the means of these values, and exponentiated the means of log-transformed values to report the geometric mean values with 95% confidence intervals. Because the percent predicted values of FVC, FEV_1, and $FEF_{25–75}$ were normally distributed, we report their arithmetic means. Pearson's correlation coefficients were used to test the associations between lung function and log-transformed FeNO values. Multivariable logistic and linear regression models were used to analyze predictors of wheeze and logarithmically transformed FeNO, respectively. Data were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL).

Results

Of the 976 children whose caregivers provided answers to the relevant questions in the baseline questionnaire when the children were age 4 years, a convenience sample of 144 participated in FeNO testing at the clinic at age 7 years. Children who returned for testing differed in race/ethnicity but not in asthma-related symptoms, seroatopy, or ETS exposure at baseline, from the 831 children who did not have FeNO assessed (Table 1).

Of the 144 children, 125 (87%) had a valid FeNO test. Two of the children were excluded from the analyses because they had taken fluticasone propionate on the day of the test. Of the remaining 123 children, 9 lacked an IgE measurement at age 7, 21 did not have a valid lung function test, and 12 did not have completed questionnaires regarding wheezing in the previous year. (Some children were missing more than one component.) The 89 children who had a complete age 7 data set (valid FeNO and lung function tests, specific IgE, and wheeze questionnaires) did not differ in age, gender, or ethnicity from the 55 who were tested for FeNO, but were excluded from the analyses because of incomplete data (Table 1). The mean FeNO (11.6 ppb [9.7–13.9]) of the 36 children who had a valid FeNO test but were not included in the analyses, was not different from that of the included children (12.0 ppb [10.6–13.7], P=0.78).

Respiratory outcome and FeNO

The overall geometric mean [95% CI] FeNO was 12.0 ppb [10.6–13.7]. In univariate analyses, FeNO concentration was not associated with gender, race/ethnicity, or BMI (Table 2). Mean FeNO concentrations were significantly higher among children who were seroatopic, had asthma-like symptoms in the last year (wheeze, nighttime cough), or had acute medical visits for asthma-like symptoms than among other children. Common lung function parameters were lower among children with respiratory symptoms in the past 12 months than among children without recent symptoms, but were not associated with seroatopy.

FeNO concentrations were not correlated with the percent predicted lung function measures, BMI percent predicted for age and sex, or ambient concentrations of NO (P values=0.16–0.55). Total IgE concentrations were significantly correlated with FeNO concentrations (r=0.38, P<0.001), but not with lung function measures (P=0.38–0.85).

In logistic regression analysis controlling for gender, maternal asthma, and presence of a smoker in the home, FeNO was significantly associated with current wheeze (OR 3.7; 95% CI 1.3–10.3; P=0.011) and with being awakened at night by wheeze (OR 3.7; 95% CI 1.5–9.4; P=0.005). These associations remained statistically significant after adjustment for total IgE and FEV_1% predicted (Table 3) or the other lung function measures (data not shown).

ETS and FeNO

The frequency of ETS exposure in the home decreased from 29% at age 4 to 16% at age 7. Mean FeNO and lung function measures by ETS exposure are reported in Table 2. Children who were currently exposed to domestic ETS at age 7 had significantly lower FeNO concentrations (7.8 ppb [5.9–10.2]) than those who were not currently exposed (13.1 ppb [11.4–15.0], P=0.004). Among those children who were not currently exposed to ETS at age 7, the children who had been exposed at age 4 had significantly higher FeNO and lower FEV1 and $FEV_{25–75\%}$ than those not exposed at age 4. In linear regression analyses including adjustment for sex, maternal asthma, ambient NO, and seroatopy, current ETS exposure was significantly inversely related to FeNO and previous ETS exposure was significantly positively associated with FeNO (Table 4). The association was not altered by inclusion of the variable “ever asthma,” distinguishing children described by their caregiver either at baseline as having ever been diagnosed by physician with asthma or at age 7 as currently under the care of a physician for asthma, from children not so described. Similarly, inclusion of reported wheeze symptoms at either age did not alter the associations between ETS exposure and FeNO.

Discussion

In this inner-city cohort of 7-year-old children, we found significantly higher levels of FeNO among children with recent respiratory symptoms than among those without such symptoms. This association remained statistically significant after adjustment for total and specific IgE and common lung function metrics, indicating that FeNO provided a unique, useful respiratory outcome measure in this research setting. Children exposed to ETS at baseline (three years before FeNO assessment) had higher FeNO than non-exposed children, suggesting that increased airway inflammation may be a long-term detrimental effect of tobacco smoke exposure. However, children currently exposed to ETS had lower FeNO than other children, demonstrating the importance of determining previous ETS exposure when measuring FeNO in children.

For this study, FeNO was measured using a previously described modification of a commercially available offline collection device.[20] With this improved method, we obtained a valid test from 87% of the children in this cohort of 7-year-olds. The offline method allows for collection in a clinic or field setting with minimal equipment, making it ideal for use in epidemiology studies including those of inner-city populations.

A recent large study reported that FeNO measured in the field by the offline method correlated well with FeNO measured by the online method, but that the offline collection was influenced by ambient NO.[25] In our cohort, ambient NO was not correlated with FeNO; however, we collected our ambient NO samples in a clinic, and only 5% were above 50 ppb. Among adults, we have observed a correlation between offline FeNO and ambient NO, but only at levels above 50 ppb (unpublished data). Given its potential influence on FeNO, ambient NO should be measured whenever FeNO is collected offline. Samples collected in homes with gas stoves may be more prone to contamination by ambient NO than those collected in the clinic because stove use can raise ambient NO levels considerably higher than 50 ppb (unpublished observation).

Like many other investigators, we observed no association between FeNO and lung function.[26] FeNO and FEV_1 represent measures of airway inflammation and obstruction, respectively. Because they were independently associated with wheeze, including both measures in epidemiologic studies may help to distinguish asthma phenotypes or exposure pathways.

The strong association between seroatopy and FeNO, reported in several other studies, has been attributed to the up-regulation of inducible nitric oxide synthase (iNOS) by proinflammatory cytokines as a consequence of the allergic reponse.[9, 26–29] Because, in our multivariable model, FeNO and seroatopy were independently associated with wheeze, including both in future cohort studies could also be informative about phenotypes and pathways.

It has been proposed that tobacco smoke exposure lowers FeNO because it increases arginase activity and therefore decreases arginine availability for NO production.[30] NO is formed by the conversion of arginine to citrulline by nitric oxide synthase (NOS), principally iNOS, in the airways. Arginine is also converted by arginase to ornithine, which a precursor of prolines and polyamines, which lead to collagen and mucus formation and cell proliferation, respectively. Epithelial and smooth muscle cells from smokers have been found to have higher levels of arginase and ornithine decarboxylase (a downstream enzyme of the arginase pathway) than those of non-smokers.[30]

Between 2002 and 2006, smoking prevalence decreased among adults in NYC.[31] The prevalence of domestic ETS exposure in our cohort decreased over the same time. FeNO levels were significantly higher among children exposed to ETS at baseline than among those not exposed, even after adjustment for current smoke exposure and other potential confounders. To our knowledge, we are the first to report a positive association between previous ETS exposure and FeNO in a prospective study. Although the mechanism is not clear, it may reflect long term up-regulation of the arginase pathway, leading to chronic inflammation and airway remodeling that could be associated with increased FeNO in the absence of current ETS exposure.[30, 32–35] However, persistent tobacco smoke exposure leads to chronic neutrophilic inflammation, while FeNO is thought to be more associated with eosinophilic inflammation.[9, 36] In our study, the detrimental effect of ETS was also supported by the significantly lower FEV_1 and $FEF_{25–75}$ among the children exposed to tobacco smoke earlier as compared to those who were never exposed. Of course, smoking parents of children who developed respiratory symptoms (after the age 4 survey) may have been more likely to stop smoking than parents of other children, and those children who had respiratory symptoms at age 7 would have been more likely than other children to have elevated FeNO. However, adjustment for a report that the child ever had asthma in the multivariable model did not alter the association, suggesting that parental behavior change does not account for the association.

A clear limitation of our study was its small sample size. The FeNO analyses used a convenience sample subset of the original age 4 cohort, which had a greater proportion of children of Mexican ethnicity and fewer children of Puerto Rican ethnicity and African-American race than the baseline sample. We have reported previously that asthma was less prevalent among children of Mexican background than among others.[18] However, the FeNO group and the group of children that did not participate in FeNO measurement did not differ with respect to frequencies of respiratory symptoms, ETS exposure, and prevalence of atopy at age 4. In addition, we did not start administering our questionnaire about medications taken on the day of FeNO measurement until after first 31 (final analyses) children had completed their participation. Although some of those children could have taken medications that lowered their FeNO, only 2/90 children on whom data was collected had taken any relevant medication. Another limitation of our study was that ETS exposure was assessed by questionnaire. Further, the lack of a measure of FeNO at age 4 to prospectively evaluate the association between ETS exposure and change in FeNO over time was a weakness. In our study we only assessed allergic sensitization to four indoor allergens. Given the demonstrated association between elevated FeNO with increasing degree of allergic sensitization (e.g., IgE level or number of allergen specific sensitizations) and

outdoor allergen sensitization, it would have been preferable to have been able to classify atopic children according to a wider set of inhalant allergens.[28, 37]

Due to our study's small sample size, we were unable to perform a cut-point analysis, which could have provided clinically meaningful FeNO values above which children in this population would be more likely to wheeze. In our sample, we did observe that a majority (63%) of the children with FeNO above 30 ppb had wheezed in the past 12 months; however, there were only 8 children in this category. We are currently collecting FeNO on a much larger population of inner-city children, and we hope that we will be able to evaluate clinically relevant cut points in that sample.

In conclusion our findings demonstrate the feasibility and usefulness of conducting offline FeNO measurements in inner-city cohort studies of asthma and the importance of evaluating the influence of current and previous tobacco smoke exposure on FeNO measurements. Our findings also suggest that ETS exposure may elevate FeNO and decrease lung function in children over a period of few years; however, these findings should be confirmed in a larger study and other populations.

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Abbreviation list

ATS	American Thoracic Society
BMI	Body mass index
ETS	Environmental tobacco smoke
$FEF_{25–75\%}$	Forced expiratory flow 25–75%
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
FeNO	Fractional exhaled nitric oxide
IgE	Immunoglobulin E
iNOS	inducible nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase

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Table 1

Demographics of the original study cohort stratified by those children who had FeNO measured at age 7 and whether they were included in the final analyses

	Children with FeNO not tested age 7 (n=831)	Children with FeNO tested age 7 (n=144)	Children included in analyses[a] (n=89)[b]
Age at enrollment (mean [CI])	4.05 [4.01–4.09]	3.93 [3.84–4.01]*	3.91 [3.79–4.03]
Age at eNO measurement(mean [CI])	-	6.87 [6.71–7.03]	6.87 [6.65–7.07]
Boys (%)	50	50	46
Race/Ethnicity			
Dominican (%)	23	29	34
Mexican (%)	25	42**	43
Puerto Rican (%)	17	6.9**	6.7
Other Hispanic/Mixed (%)	16	17	14
African-American (%)	20	4.2**	3.4
Other (%)	1.2	0.7	0
Current wheeze age 4 visit (%)	27	28	27
Current wheeze at eNO age (%)	-	27	21
ED visit wheeze initial visit (%)	16	17	15
ED visit at eNO visit (%)	-	15	14
Seroatopy age 4[c](%)	32	40	33
Seroatopy age 7[d](%)	-	47	47
Any smoker in home age 4 (%)	24	29	29
Any smoker in home at age 7 (%)	-	11	15

*P<0.05,

**P<0.01 for difference from children without FeNO collected.

[a]Children were included in the analyses if they had valid FeNO and lung function tests, serum IgE results and questionnaire information on wheeze at age 7 years.

[b]There were no statistically significant (P<0.05) differences in any of the demographics represented between the children with FeNO tested, but not included in the analyses (n=55) and the children included in the analyses (n=89).

[c]Seroatopy was defined as having measurable specific IgE (≥0.35 IU/ml) to at least one of any of the four allergens tested (*Dermatophagoides farinae*, German cockroach, mouse or cat). By study design, only half of the original cohort subjects at age 4 were eligible for serum IgE measurement. The sample size for the prevalence figures in the table were 354, 52 and 86 for the children without FeNO, with FeNO and included in analyses, respectively.

[d] There were 135 children who had both FeNO and IgE measured at age 7.

Table 2

FeNO concentrations and lung function outcomes by subject characteristics and ETS exposure (n=89)

				Lung function percent predicted		
		Frequency (%)	**FeNO (ppb)[a]**	**FVC (%)[b]**	**FEV_1(%)**	**$FEF_{25–75}$(%)**
Sex	Boy	54	12.6 [10.6–13.7]	89.9 [85.7–94.1]	94.2 [89.0–99.5]	101 [92.7–109]
	Girl	46	11.4 [9.4–13.8]	89.5 [84.7–94.2]	92.0 [86.2–97.8]	97.5 [089–1.06]
Ethnicity[c]	Mexican	43	11.1 [9.1–13.5]	98.5*** [94.4–103]	105*** [100–110]	114*** [107–122]
	Dominican	34	12.4 [9.8–15.6]	80.9*** [76.6–85.3]	81.2*** [76.4–86.1]	84.7*** [76.1–92.9]
	Other Hispanic	20	13.4 [10.5–18.1]	88.4 [81.7–95.3]	91.6 [82.5–101]	96.0 [82.2–110]
Body Mass Index	<95%	44	12.2 [9.6–15.5]	90.5 [84.7–96.3]	95.7 [88.4–102]	103 [93.1–112]
	≥95%	56	11.7 [10.0–13.6]	89.9 [86.1–93.7]	92.1 [87.4–96.9]	99.3 [90.3–108]
Seroatopy[d]	No	53	10.1 [8.7–12.0]	91.6 [87.2–95.9]	95.6 [90.2–101]	99.5 [92.8–106]
	Yes	47	14.6** [12.0–17.8]	87.6 [83.1–92.1]	90.5 [85.0–96.1]	99.5 [89.3–110]
Environmental tobacco smoke exposure	None	67	12.0 [10.4–13.8]	91.9 [88.3–95.6]	96.6 [92.1–101]	104 [97.1–111]
	Age 4 only	17	18.5* [13.3–25.7]	84.1 [76.7–91.5]	82.7** [73.9–91.5]	81.6** [66.3–97.0]
	Age 7 only	3.3	6.1* [4.3–8.7]	94.8 [71.7–118]	101 [76.4–125]	108 [81.8–135]
	Age 4 and 7	12	8.3 [6.0–11.5]	83.7 [73.9–93.6]	87.3 [75.5–99.1]	96.9 [80.9–113]
Respiratory symptoms in the past 12months						
Wheeze	No	79	11.0 [9.6–12.5]	91.3 [87.8–95.0]	95.9 [91.4–100]	103 [96.9–110]
	Yes	21	17.0** [12.2–23.8]	83.7 [78.0–89.4]	83.4** [77.5–89.2]	84.9* [73.2–96.6]
Awakened at night by cough	No	67	10.6 [9.3–12.2]	92.2 [88.2–96.2]	97.6 [92.7–103]	106 [99.0–113]
	Yes	33	15.6** [12.0–20.2]	84.5* [80.0–89.0]	84.1** [79.2–88.9]	86.0** [76.7–95.4]
Acute medical visit for breathing problems	No	86	11.5 [10.1–13.1]	90.8 [87.4–94.1]	95.0 [90.9–99.2]	103 [96.7–109]
	Yes	14	17.0* [10.9–26.5]	83.0 [73.9–92.2]	82.3 [72.4–92.2]	77.4** [65.1–89.7]

*p<0.05,

**p<0.01,

***p<0.001 For the ethnicity comparisons “other Hispanic” was the reference group.

[a]FeNO geometric mean concentrations with 95% CI are reported.

[b]Lung function outcomes as percent predicted arithmetic means with 95% CI are reported.

[c]The 3 African-American children are not represented in the ethnicity means.

[d]Seroatopy at age 7 was defined as specific IgE ≥0.35 IU/ml to at least one of the allergens tested (*Dermatophagoides farinae*, German cockroach, mouse, and cat)

Table 3

Logistic regression analysis of FeNO, FEV_1 and seroatopy as predictors of current respiratory symptoms (n=89)

	Current wheeze		Awakened at night by cough		Acute medical visit for difficulty breathing	
	OR[a]	[95% CI]	OR	[95% CI]	OR	[95% CI]
FeNO (log-transformed)	4.0*	[1.3–12.8]	3.4*	[1.2–9.8]	3.1	[0.96–10.1]
FEV1 % predicted	0.013*	[0.002–0.65]	0.010*	[0.003–0.33]	0.049	[0.001–4.81]
Seroatopy[b]	0.95	[0.91–1.02]	2.3	[0.67–8.0]	2.2	[0.38–13.6]

*P<0.05

[a]Odds ratio for logistic regression model including FeNO, FEV1, seroatopy, sex, maternal asthma, presence of a smoker in the home, and ambient NO.

[b]Seroatopy was defined as specific IgE ≥0.35 IU/ml to at least one of the allergens tested (*Dermatophagoides farinae*, German cockroach, mouse, and cat)

Table 4

Linear regression models predicting FeNO at age 7 years[a]

	Model 1		Model 2	
	β	P	β	P
Previous domestic ETS (age 4)	0.36	0.015	0.34	0.032
Current domestic ETS (age 7)	−0.74	<0.001	−0.69	0.001
Total IgE (log-transformed)	0.17	<0.001	-	
Seroatopy[b]	-		0.34	0.017

[a] Linear regression models with log-transformed FeNO as the dependent variable and the independent variables sex, maternal asthma, previous domestic environmental tobacco smoke (ETS), current domestic ETS, ambient NO and either log-transformed total IgE (model 1) or seroatopy (model 2).

[b] Seroatopy was defined as specific IgE ≥0.35 IU/ml to at least one of the allergens tested (*Dermatophagoides farinae*, German cockroach, mouse, and cat)