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Original article

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Title page

Contributions of asthma, rhinitis, and IgE to exhaled nitric oxide in adolescents

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Abstract

Fractional exhaled nitric oxide (FeNO) is an indicator of allergic airway inflammation. However, it is unknown how asthma, allergic rhinitis (AR) and allergic sensitization relate to FeNO, particularly among adolescents and in overlapping conditions. We sought to determine the associations between asthma, AR, and aeroallergen IgE and FeNO in adolescents.

We measured FeNO among 929 adolescents (11-16 years) in Project Viva, an unselected prebirth cohort in Massachusetts. We defined asthma as ever asthma physician diagnosis plus wheezing in the past year or taking asthma medications in the past month; AR as a physician diagnosis of hay fever or AR; and aeroallergen IgE as any IgE >0.35 IU/mL among 592 participants who provided blood samples. We examined associations of asthma, AR, and IgE with percent difference in FeNO in linear regression models adjusted for sex, race/ethnicity, age and height; maternal education and smoking during pregnancy; and household/neighborhood demographics.

Asthma (14%) was associated with 97% higher FeNO (95%CI 70, 128%), AR (21%) with 45% higher FeNO (95%CI 28, 65%), and aeroallergen IgE (58%) with 102% higher FeNO (95%CI 80, 126%) compared to those without each condition, respectively. In the absence of asthma or AR, aeroallergen IgE was associated with 75% higher FeNO (95%CI 52, 101), while asthma and AR were not associated with FeNO in the absence of IgE.

The link between asthma and AR with FeNO is limited to those with IgE-mediated phenotypes. FeNO may be elevated in those with allergic sensitization alone, even in the absence of asthma or AR.

Key words

Exhaled nitric oxide, adolescents, asthma, allergic rhinitis, airway inflammation, IgE, allergen, environment

Abbreviations

FeNO = fractional exhaled nitric oxide

AR = allergic rhinitis

IgE = immunoglobulin E

ATS = American Thoracic Society

Ppb = parts per billion

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While asthma, allergic rhinitis (AR) and allergic sensitization are associated with higher fractional exhaled nitric oxide (FeNO), asthma and AR in the absence of aeroallergen IgE are not associated with FeNO. When elevated in asthma or AR, FeNO suggests allergic sensitization.

Introduction

Fractional exhaled nitric oxide (FeNO) is an indicator of allergic airway inflammation and can aid in the diagnosis of allergic asthma [1]. The influence of concurrent allergic rhinitis (AR) and allergic sensitization to environmental allergens on FeNO measurements is not well-known in adolescents. How each of these—asthma, AR, and aeroallergen IgE—relate to FeNO is important not only for interpretation of FeNO in clinical use but also for a greater understanding of the factors that lead to elevated FeNO. We sought to understand the associations of asthma, AR, and aeroallergen IgE with FeNO in an adolescent population.

Asthma and AR are common and contribute to significant morbidity, with poorer quality of life, missed days of school, healthcare costs, and emergency room visits for asthma exacerbations. resulting from difficulty sleeping, fatigue, and changes in mood and cognition [2]. AR and asthma share epidemiologic overlap. AR occurs in over 75% of patients with asthma, and asthma is seen up to 40% of those with AR [3]. In those with both AR and asthma, AR may present first and is a risk factor for subsequent development of asthma [4–7].

Asthma and AR are each known to be associated with elevated FeNO in both adults and children. Nitric oxide (NO) is an intercellular messenger known to mediate a variety of processes, including immune function and inflammation [8]. In the lung, NO acts as a signaling molecule in bronchial and vascular dilatation, ciliary kinesis, and neurotransmission in the non-adrenergic and non-cholinergic systems [9]. In allergic inflammation, Th2 cells, ILC2 cells, mast cells, and eosinophils produce cytokines like IL-4 and IL-13, with downstream effects including the activation of inducible NO

synthase (iNOS) and thus elevated NO levels. Therefore, FeNO is considered a marker of type 2 airway inflammation, which occurs with allergic rhinitis in the upper airway or allergic asthma in the lower airway [9–13]. Elevation of FeNO may indicate not only allergic asthma or AR but also elevations in circulating IgE against environmental allergens. In previous work in this same Project Viva cohort, an unselected pre-birth cohort in Massachusetts, we have found differences in the nasal epigenome in association with asthma, AR, aeroallergen IgE and FeNO that are annotated with genes implicated in type 2 inflammatory responses [14]. Currently, the American Thoracic Society (ATS) recommends FeNO in the diagnosis of eosinophilic airway inflammation, and suggests that it may be used to support the diagnosis of asthma where the diagnosis is less clear [1]. FeNO, however, may also be elevated in allergic rhinitis or allergic sensitization to environmental allergens [15, 16]. Therefore, we sought to elucidate the contributors to FeNO and supplement our understanding of its diagnostic utility. In this study, we examined the relationships of asthma, AR, and aeroallergen IgE with FeNO in an adolescent population in Project Viva, a cohort not selected on the basis of asthma or allergy.

Materials and Methods

Study design and participants

Between 1999 and 2002 we recruited women in early pregnancy into Project Viva from eight obstetric offices of Atrius Harvard Vanguard Medical Associates, a multi-specialty group practice in eastern Massachusetts. Exclusion criteria included multiple gestation, inability to answer questions in English, and gestational age ≥ 22 weeks at recruitment. Details of recruitment and retention are available elsewhere [17]. Of the 2,128 infants, we included in this analysis those participants with FeNO measurements at an in-person visit in early adolescence, which totaled 929. Compared with the 929 included participants, the 1199 excluded participants were less likely to have college-educated mothers (59% vs. 71%) and more likely to have mothers who smoked during pregnancy (15% vs. 9%). Maternal age at enrollment, gestational age at delivery, and sex and race/ethnicity, however, were similar. Those 1199 were excluded due to either missing FeNO or no early adolescent visit. The median adolescent age was 12.9 years with a range of 11.9-16.6; there were no participants older than this due to cohort inception dates.

Asthma and allergic rhinitis

We defined current asthma as a maternal report of ever asthma diagnosis plus wheeze symptoms in the past 12 months or use of asthma medications in the past month, reported on an early teen questionnaire in keeping with the Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [18]. We used as a comparison group those with no asthma diagnosis, no wheezing and no use of asthma medications or wheezing in the past 12 months. Participants were defined as having allergic rhinitis

(AR) if they reported ever receiving a physician diagnosis of hay fever or AR. Both current asthma and AR were reported by parental questionnaire at the early teen follow up visit.

Aeroallergen IgE

Trained research phlebotomists collected blood from participants at the early teen visit, which we centrifuged and stored at -80C. We measured plasma IgE against Dermatophagoides farina (dust mite), cat or dog dander, Aspergillus fumigatus (mold), Alternaria alternata (plant fungi), common ragweed, oak, ryegrass, or silver birch. Allergen extract-specific IgE antibodies were measured by ImmunoCap (Thermo Fisher Scientific/Phadia, Kalamazoo, Michigan)—a widely used in vitro sandwich immunoassay, previously described [19, 20]. We defined aeroallergen IgE as having IgE against any of these outdoor or indoor allergens > 0.35 IU/mL. We defined "perennial IgE" as having IgE > 0.35 IU/mL against any of the following: dermatophagoides farina (dust mite), cat or dog dander, Aspergillus fumigatus (mold), Alternaria alternata (plant fungi). We defined "seasonal IgE" as having any IgE > 0.35 IU/mL against any of the following: common ragweed, oak, ryegrass, or silver birch.

Measurement of FeNO

Exhaled NO levels were measured twice for each participant with a portable electrochemical device (NIOX MINO; Aerocrine AB); this has been validated by chemiluminescence technology, with an accuracy of ± 5 parts per billion (ppb) [21]. Prior to each measurement, participants breathed in through an NO scrubbing filter and exhaled into room air twice. This was done in keeping with prior studies using FeNO; the ambient air NO was not measured [22–24]. On the third breath, participants inhaled

through the filter and exhaled into the FeNO analyzer. The last three seconds of the exhalation were utilized for FeNO measurement; this ensures lower rather than upper airway measurement. Nose clips were not used.

Statistical analysis

Potential confounders were selected a priori, based on known or suspected associations with asthma or allergy. Model 1 adjusted for sex and age and height at the early teen visit. Model 2 additionally adjusted for race/ethnicity; maternal education and smoking during pregnancy; median value of owner-occupied housing and education (% with a bachelor degree) based on year 2000 census tract of home residence at time of mid-childhood visit; and household income and any smokers at home at the early teen visit. We additionally adjusted for BMI in Model 3.

We averaged the two FeNO measurements and included the log-transformed value as a continuous outcome in linear regression models. We present effect estimates as % change (95% CI) in FeNO, calculated as (exponentiated (beta)–1)*100. In secondary analyses, we examined FeNO in categories (<20, 20-≤35, and >35 ppb) based on American Thoracic Society guidelines [1] using multinomial logistic regression models. We examined the associations of asthma, AR, and aeroallergen IgE with FeNO using separate linear and logistic regression models. We repeated linear regression models for the associations of seasonal and perennial IgE with FeNO.

To examine associations of overlapping conditions of asthma, AR and aeroallergen IgE, we derived an 8-category exposure based on the combination of these three exposures. We ran linear regression models with this 8-category exposure and used no aeroallergen IgE, no AR, no asthma as the reference category. We

performed all analyses using SAS 9.4 (SAS Institute. Cary, NC).

Results

Study population

The characteristics of the 929 study participants included in any analysis and their mothers are shown in Table 1. Reported smoking in the home was rare (12%) and mean reported annual household income was $109,000. Children were predominantly white (64%) and their average age was 13 years. Mean (SD) FeNO was 26 parts per billion, ppb (SD 27) and 19% of study participants had exhaled NO levels above the upper limit of normal for this age group (35 ppb) [1].

Overall, 115/797 (14%) reported asthma while 179/869 (21%) reported AR. Aeroallergen IgE >0.35 IU/mL was detected in 345/592 (58%) of those providing blood samples with IgE results. Mean (SD) FeNO in those with asthma was 48.4 ppb (43.0) compared to 21.8 (20.4) ppb in those without asthma. Mean (SD) FeNO in those with AR was 35.7 ppb (32.8) compared to 23.5 ppb (24.9) without AR. Participants with aeroallergen IgE had mean (SD) FeNO 36.2 ppb (34.2) compared to 14.8 ppb (9.5) in those without IgE.

Associations of each condition with FeNO are shown in Table 2, where model 1 is parsimoniously adjusted and model 2 is fully adjusted, with similar results. Asthma was common in those with FeNO > 35 ppb; 35% of teens with FeNO higher than 35 ppb had asthma versus 7% among participants with FeNO less than 20 ppb and 18% among participants with FeNO 20-≤ 35 ppb. Of the 3 conditions, the presence of aeroallergen IgE and asthma diagnosis each had a similar association with FeNO, while the magnitude of the association was half as great for AR. Specifically, those with asthma had a 97% higher FeNO (95% CI 70, 128%) compared to those without asthma;

those with AR had a 45% higher FeNO (95% CI 28, 65%) compared to those without AR; and those with aeroallergen IgE had 102% higher FeNO (95% CI 80, 126%) compared to those without aeroallergen IgE. Perennial IgE was associated with 119% higher FeNO (95% CI 97, 144) while seasonal IgE was associated with 71% higher FeNO (95% CI 52, 93). Similar results were seen in logistic regression analyses for the clinical thresholds of FeNO. Asthma was associated with 8 times higher odds (95% CI 5, 14) of FeNO > 35 ppb, AR was associated with 3 times higher odds (95% CI 2, 5) of FeNO > 35 ppb and aeroallergen IgE was associated with 28 times higher odds (95% CI 12, 63) of FeNO >35 ppb compared to those without each condition (Table 2).

We found similar associations when stratified by sex (supplement E2). Since steroids can affect FeNO, we ran a sensitivity analysis, excluding participants who used oral or inhaled corticosteroids within 72 hours of FeNO testing (N=22) and found similar associations between asthma, AR, and aeroallergen IgE with higher FeNO. Finally, we adjusted for BMI and found similar associations with asthma, AR, and aeroallergen IgE with FeNO in both linear and logistic regression models (supplement E3).

Overlapping associations of allergy, asthma, and aeroallergen IgE with FeNO

To examine associations of overlapping conditions of asthma, AR and aeroallergen IgE, we derived an 8-category exposure based on the combination of these three exposures among N=476 with non-missing values for the 3 exposures; no asthma, no AR and no aeroallergen IgE was the reference category) (Figure 1; Supplement E1). We found that, in the absence of asthma or AR, aeroallergen IgE alone (n=173, 36%) was associated with 75% higher FeNO (95% CI 52, 101%). Asthma in the absence of AR and aeroallergen IgE (n=10, 2%), AR in the absence of

asthma and aeroallergen IgE (n=14, 3%), and asthma and AR in the absence of aeroallergen IgE (n=6, 1%) were uncommon and not associated with FeNO. The presence of all three exposures—asthma, AR, and aeroallergen IgE (n=32, 7%)—was associated with 272% higher FeNO (95% CI 189, 379%) compared to having none of these (Figure 1).

Discussion

In this study of adolescents, we found, as expected, that asthma and allergy were each associated with higher FeNO. However, this association was absent among those without detectable aeroallergen IgE. Additionally, aeroallergen IgE was associated with elevated FeNO even in the absence of asthma or AR.

The association between asthma and elevated FeNO among adolescents in this study is consistent with the well-established connection between allergic asthma and airway inflammation as measured by FeNO. Exhaled NO greater than 35 ppb in children strongly supports the diagnosis of asthma and can be used as a diagnostic tool [1]. FeNO is a marker of an allergic asthma phenotype, characterized by elevations in type 2 cytokines IL-4, IL-5, and IL-13 and eosinophils in sputum, serum, and bronchial biopsy [25–27].

However, we found that this association between asthma and increased FeNO was seen only when aeroallergen IgE was detected. In the absence of aeroallergen IgE, the association between asthma and FeNO was lost. Our results suggest that asthma is only associated with FeNO in the presence of allergic sensitization, and that FeNO is more likely to indicate the presence of aeroallergen IgE. We found that FeNO of > 35 ppb had a positive predictive value of only 35% for the diagnosis of asthma, compared to a positive predictive value of 94% for the presence of aeroallergen IgE. Our findings contrast with a study of 1,156 children in France, which found that non-atopic asthmatics had higher FeNO than non-atopic children without asthma (average FeNO 13.4 ppb in non-atopic children with asthma vs 10.6 ppb in non-atopic children without asthma) [28]. However, our findings are consistent with other birth cohorts, including

one from the Netherlands, which observed higher FeNO only among young adults with atopic asthma, defined as asthma plus allergic sensitization with positive serum IgE against 10 common aeroallergens, but not non-atopic asthma [29]. The Isle of Wight birth cohort in England similarly found that atopy (measured by skin prick testing) was associated with higher FeNO, while the level of FeNO did not differ between non-atopic teens with and without asthma [30]. The authors concluded, as we do, that FeNO is a biomarker for atopy rather than asthma.

Our study also confirmed the association between AR and higher FeNO among adolescents, which has been well established among adults and children [31–35]. Similar to our findings in asthma, we found that this association between AR and FeNO was observed only in the presence of aeroallergen IgE. Other studies have also found that those with atopy and rhinitis have higher FeNO than those with rhinitis but no atopy [28, 36, 37]. This same large cohort of 1,156 children in France found that non-asthmatic atopic participants (as determined by skin prick test to common allergens) with rhinitis had significantly higher FeNO than non-atopic participants with rhinitis (20.7 $\pm$ 13 versus 12.5 $\pm$ 6.4 ppb) [28]. Likewise, in an adult population examining atopic participants identified by skin prick testing, Gratziou, et al. found that FeNO was significant higher in atopic rhinitis than in those with non-atopic rhinitis (13.3 $\pm$ 1.3 versus 5.8 $\pm$ 1.2 ppb) [37].

One explanation of our findings is that asthma in the setting of low aeroallergen IgE is mediated by a non-allergic inflammatory pathway. This distinct non-allergic asthma phenotype represents a subset of asthmatics that may have more severe and more difficult to control asthma [38, 39]. Rather than an allergic, type 2-mediated

pathways, these non-atopic asthmatics may have neutrophilic airway inflammation [40]. This may be determined in part by genetic factors, and those with allergic and non-allergic asthma have distinct HLA haplotypes [41]. Environmental exposures such as particulate air pollution or childhood viral infections may also contribute to non-allergic asthma, in a process mediated through neutrophilic inflammation [42]. A similar neutrophilic process may be occurring in our participants with asthma or AR and no significant serum IgE.

We found that aeroallergen IgE is associated with elevated FeNO even in the absence of asthma or AR. A few studies have similarly found that atopy is associated with FeNO regardless of symptoms. For example, both Choi, et al. and Franklin, et al. examined children with asthma and atopy and found that atopy increases FeNO regardless of asthma diagnosis [36, 43]. This has been similarly observed among Pacific Islander adults, where positive skin prick testing to house dust mite was associated with higher exhaled and nasal NO even in the absence of asthma symptoms [44]. In a study comparing asthmatic and healthy children, Barreto, et al. found that even in those without respiratory symptoms, atopy (defined by skin prick testing) and peripheral eosinophil count were associated with significantly higher FeNO compared to those without atopy or eosinophilia [45]. In a population of school children, van Amsterdam, et al. similarly found that allergic sensitization was associated with higher FeNO even in those without wheeze, although the association was significantly augmented in those with wheeze [46].

Our findings suggest that IgE is an important factor in the elevated FeNO observed in asthma and AR in adolescents. While the exact mechanism by which IgE

may influence FeNO is not entirely clear, the pathophysiology by which each are elevated in asthma and AR has been previously explored. IgE may increase FeNO along with inflammatory cytokines inducing iNOS. In immediate hypersensitivity reactions, IgE cross-links to the FcεR1 receptor on mast cells, resulting in mast cell degranulation, inflammatory cytokine release, and activation of inflammatory cells at sites sensitive to the inciting allergy. Local IgE-mediated mechanisms may explain the localized reactions in skin, nasal turbinates, airways, and gut in eczema, rhinitis, asthma, and food allergy respectively. In allergic asthma and AR specifically, the inflammatory cytokines that occur as a result of IgE-FcεR1 cross-linking may induce iNO synthase, resulting in the increased FeNO observed in AR and asthma [47, 48]. The upregulated eosinophils in type 2 inflammation of asthma and AR may also exert direct oxidative damage and promote the continued release of inflammatory cytokines that activate inducible NO synthase (iNOS), increasing NO in exhaled air [28, 45, 49].

Our study is one of the largest studies conducted in a well-characterized adolescent age group that examines the associations of asthma, allergy, and IgE with airway inflammation as measured by FeNO. However, we acknowledge limitations to this study. Specifically, this is a cross sectional study evaluating FeNO, asthma and AR at a single time point in adolescence. Asthma and allergic rhinitis vary widely in symptoms, severity, and control, and so associations may change depending on different time points in the disease trajectory. Additionally, steroid use may reduce FeNO although we had similar findings when excluding participants with any steroid use within 72 hours of testing. In addition, there were very few participants who had asthma or AR but no aeroallergen IgE. This may represent the fact that the majority of those

with asthma or AR in this study had an allergic asthma phenotype. There were, however, a large portion of our participants (36%) who did not have asthma or AR but who did have aeroallergen IgE. Finally, we did not include those with previous or non-active asthma for those without wheeze in the last 12 months. Similarly, we did not include IgE positivity to non-tested allergens such as food-specific IgE. Future work may include these other allergens. We would also explore the comparison of FeNO with blood eosinophils as a more widely available biomarker than FeNO.

Our study confirms the expected pattern of FeNO elevation among adolescents with asthma and AR and implicates IgE-mediated pathways in the association between asthma, AR, and FeNO. Our findings suggest that the presence of aeroallergen IgE was critical to the FeNO elevations observed in those with asthma or AR. Without aeroallergen IgE, that relationship was lost. Aeroallergen IgE can be considered a marker for elevated FeNO, since FeNO was detected whenever aeroallergen IgE was present, regardless of the presence of clinical disease. Furthermore, even if FeNO is elevated, it does not necessarily indicate asthma or AR, and patients with asthma and no aeroallergen IgE may not have an elevated FeNO. This study therefore places FeNO into perspective as a marker predominantly for sensitization rather than for clinical disease, and raises higher the need to identify alternative markers for airway inflammation in those children and adolescents with non-IgE-mediated asthma or AR.

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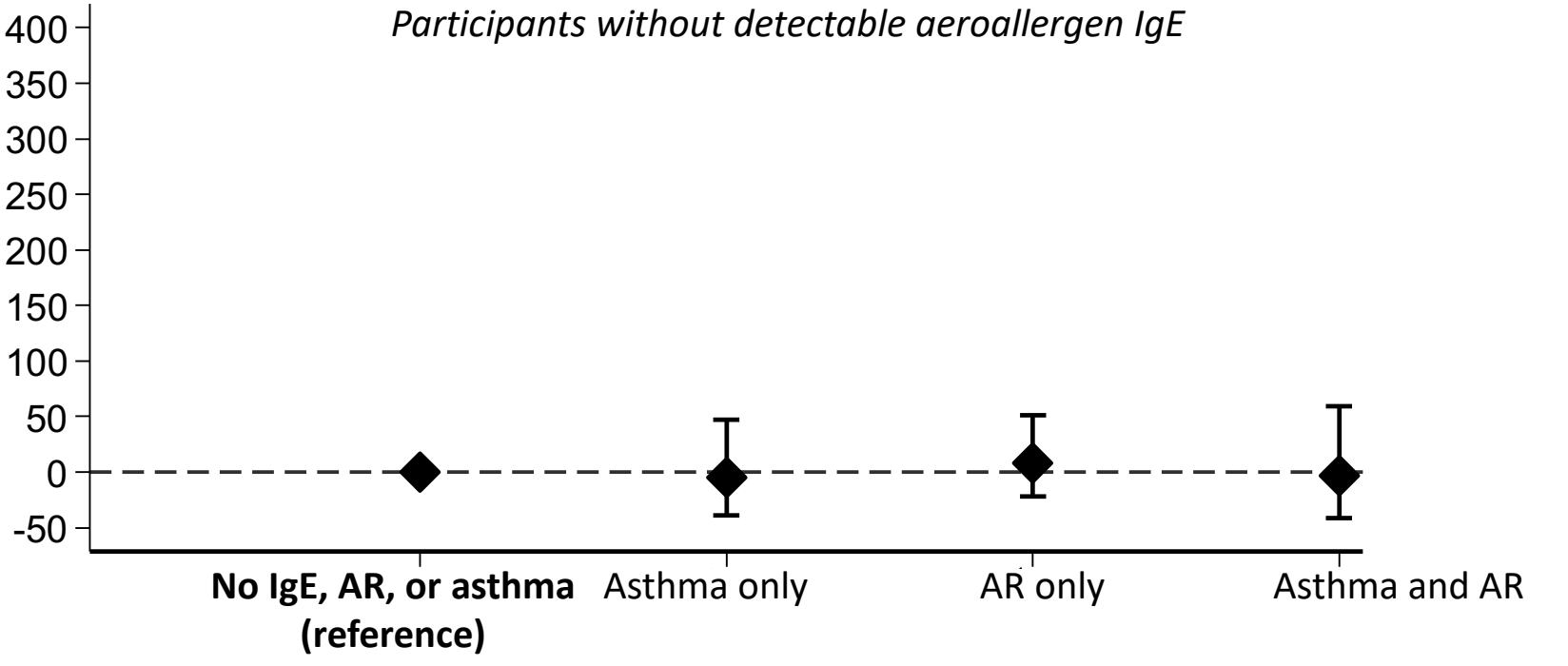
Fig 1. Percent difference in FeNO relative to reference group without aeroallergen IgE, asthma, or AR diagnosis



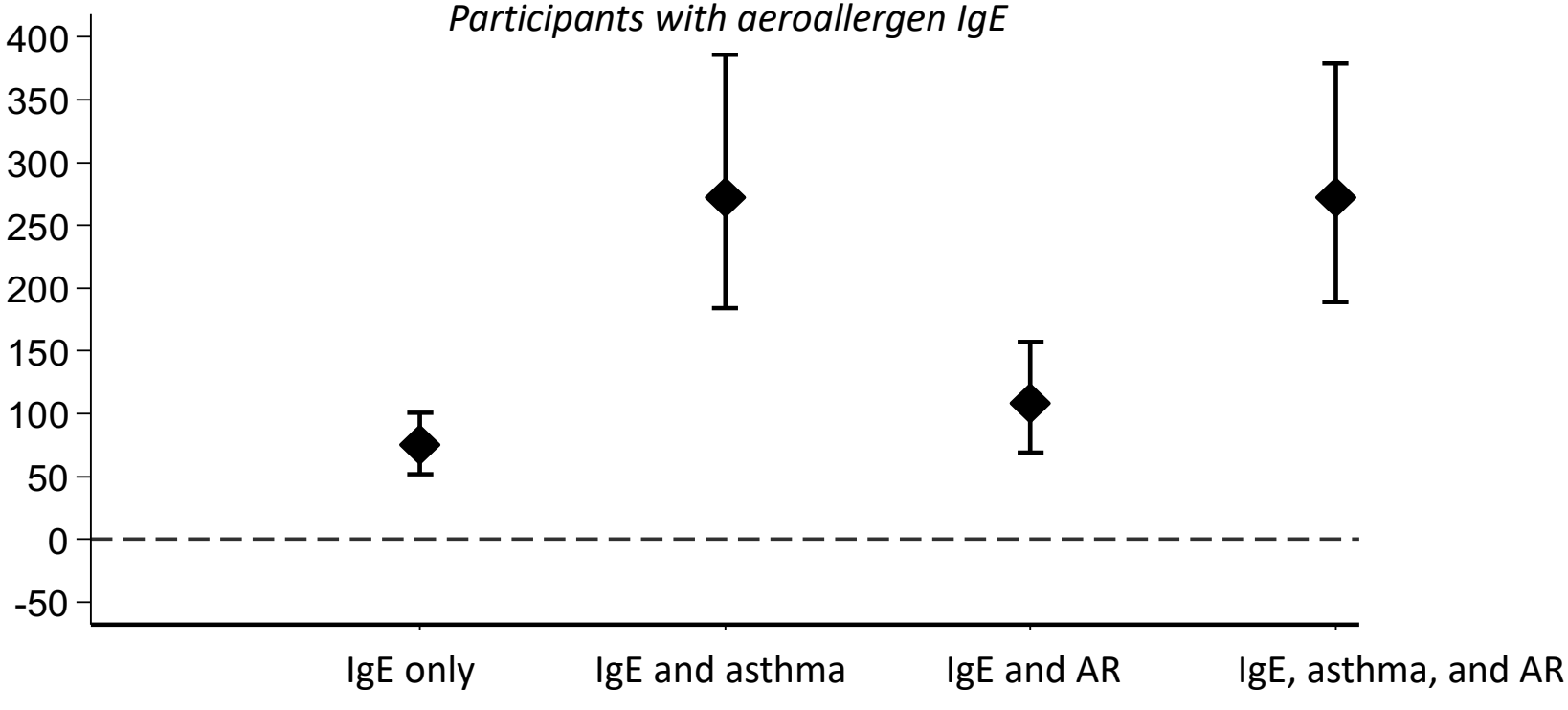


Table 1. Participant characteristics (N=929)

Characteristic	n=929 Mean (SD) or n (%)
Child	
Sex, n (%)	
. Male	466 (50)
. Female	463 (50)
Race/ethnicity, n (%)	
. Black	154 (17)
. Hispanic	40 (4)
. Asian	28 (3)
. White	593 (64)
. Other or >1 race/ethnicity	113 (12)
Early teen visit	
Age, years mean (SD)	13 (1)
Age at visit, n (%)	
. 11.9 to <13 years, n (%)	505 (54.4%)
. 13.0 to < 15.0 years, n (%)	374 (40.3%)
. 15.0 to 16.6 years, n (%)	50 (5.4%)
Height, cm, mean (SD)	160 (9)
BMI percentile category, %	
. < 5th	30 (3)
. 5-<85th	634 (68)
. >85th	262 (28)
Exhaled Nitric Oxide, ppb, mean (SD)	26 (27)
Exhaled Nitric Oxide, n (%)	
. <20 ppb	553 (60)
. 20-≤35 ppb	196 (21)
. >35 ppb	180 (19)
Current asthma, n (%)	
. No	682 (86)
. Yes	115 (14)
Ever allergic rhinitis, n (%)	
. No	690 (79)
. Yes	179 (21)
Any aeroallergen IgE >0.35 kU/L, n (%)	
. No	247 (42)
. Yes	345 (58)
Annual household income at early teen visit, $1000s, mean (SD)	109 (44)
Any smokers at home at early teen visit, n (%)	114 (12)
Mother/family	
Pregnancy smoking status, n (%)	
. Never	653 (71)
. Former	188 (20)

. During pregnancy	85 (9)
College graduate, n (%)	
. No	264 (29)
. Yes	662 (71)
Median value owner-occupied housing, $1000s*, mean (SD)	263 (149)
Percent ≥ bachelor's degree* mean (SD)	42 (20)
*Based on census tract, mid-childhood	

Table 2: Associations of asthma, allergic rhinitis, and aeroallergen IgE with FeNO using linear and multinomial logistic regression models

	Model 1			Model 2		
	Linear regression % difference in FeNO (95% CI)	Logistic regression* OR (95% CI)		Linear regression % difference in FeNO (95% CI)	Logistic regression* OR (95% CI)	
		20-≤35 ppb	>35 ppb		20-≤35 ppb	>35 ppb
Asthma vs no asthma**	97 (73, 124)	3 (2, 5)	8 (5, 13)	97 (70, 128)	3 (1, 5)	8 (5, 14)
AR vs. no AR†	48 (32, 66)	2 (1, 3)	3 (2, 5)	45 (28, 65)	2 (1, 3)	3 (2, 5)
Aeroallergen IgE vs. no aeroallergen IgE‡	99 (79, 121)	4 (3, 7)	26 (12, 59)	102 (80, 126)	4 (3, 7)	28 (12, 63)

*<20 ppb as the reference group

**Current asthma defined as ever having an asthma diagnosis plus wheezing in the past year or taking asthma medications in the past month; “no asthma” defined as no asthma diagnosis, no wheezing and no use of asthma medications or wheezing in the past 12 months.

† AR defined as ever having a diagnosis of hay fever or AR; “no AR” with never diagnosis of hay fever or AR

‡Aeroallergen IgE defined as having any aeroallergen IgE > 0.35 IU/mL; “no aeroallergen IgE” with IgE ≤ 0.35 IU/mL

Model 1. Adjusted for sex and current age and height.

Model 2. Model 1 additionally adjusted for race/ethnicity; maternal education and smoking during pregnancy; median value owner-occupied housing and percent ≥ bachelor’s degree (census tract, mid-childhood); and household income and any smokers at home at early teen visit.

Supplemental Table 1: Associations of overlapping aeroallergen IgE, allergic rhinitis, and asthma with FeNO*

Conditions	N (%)	Model 1 % difference in average eNO (95% CI)	Model 2 % difference in average eNO (95% CI)
No IgE, AR, or asthma	168 (35%)	0 (ref)	0 (ref)
Asthma alone	10 (2%)	1 (-31, 48)	-5 (-39, 47)
AR alone	14 (3%)	7 (-23, 48)	8 (-22, 51)
Asthma and AR	6 (1%)	-3 (-40, 58)	-3 (-41, 59)
IgE alone	173 (36%)	71 (50, 94)	75 (52, 101)
IgE and asthma	26 (5%)	283 (199, 390)	272 (184, 386)
IgE and AR	47 (10%)	109 (72, 154)	108 (69, 157)
IgE, AR, and asthma	32 (7%)	228 (161, 310)	272 (189, 379)

*Linear regression models with log-transformed FeNO as the outcome. Effect estimates presented as % difference, calculated as (exponentiated (beta)-1)*100.
Model 1. Adjusted for sex and current age and height.
Model 2. Model 1 additionally adjusted for race/ethnicity; maternal education and smoking during pregnancy; median value owner-occupied housing and percent ≥ bachelor's degree (census tract, mid-childhood); and household income and any smokers at home at early teen visit.

Supplement E2: Associations of asthma, allergic rhinitis, and aeroallergen IgE with FeNO using linear regression models, stratified by sex

	% difference in FeNO	
Female	Model 1	Model 2
Asthma vs no asthma*	98 (66, 136)	99 (64, 143)
AR vs. no AR†	42 (21, 67)	47 (24, 76)
Aeroallergen IgE vs. no aeroallergen IgE‡	93 (67, 124)	89 (61, 122)
Male	Model 1	Model 2
Asthma vs no asthma*	96 (61, 138)	91 (53, 139)
AR vs. no AR†	54 (30, 82)	45 (21, 75)
Aeroallergen IgE vs. no aeroallergen IgE‡	104 (76, 137)	111 (80, 148)

*Current asthma defined as ever having an asthma diagnosis plus wheezing in the past year or taking asthma medications in the past month; "no asthma" defined as no asthma diagnosis, no wheezing and no use of asthma medications or wheezing in the past 12 months.

† AR defined as ever having a diagnosis of hay fever or AR; "no AR" with never diagnosis of hay fever or AR

‡Aeroallergen IgE defined as having any aeroallergen IgE > 0.35 IU/mL; "no aeroallergen IgE" with IgE ≤ 0.35 IU/mL

Model 1. Adjusted for current age and height.

Model 2. Model 1 additionally adjusted for race/ethnicity; maternal education and smoking during pregnancy; median value owner-occupied housing and percent ≥ bachelor's degree (census tract, mid-childhood); and household income and any smokers at home at early teen visit.

Supplement E3: Associations of asthma, allergic rhinitis, and aeroallergen IgE with FeNO using linear and multinomial logistic regression models

Exposure	Model 1			Model 2			Model 3		
	Linear regression	Logistic regression*		Linear regression	Logistic regression*		Linear regression	Logistic regression*	
	% difference in FeNO (95% CI)	OR (95% CI)		% difference in FeNO (95% CI)	OR (95% CI)		% difference in FeNO (95% CI)	OR (95% CI)	
		20-≤35 ppb	>35 ppb		20-≤35 ppb	>35 ppb		20-≤35 ppb	>35 ppb
Asthma vs no asthma**	97 (73, 124)	3 (2, 5)	8 (5, 13)	97 (70, 128)	3 (1, 5)	8 (5, 14)	99 (72, 130)	3 (1, 5)	8 (5, 14)
AR vs. no AR†	48 (32, 66)	2 (1, 3)	3 (2, 5)	45 (28, 65)	2 (1, 3)	3 (2, 5)	46 (28, 66)	2 (1, 3)	3 (2, 5)
Aeroallergen IgE vs. no aeroallergen IgE‡	99 (79, 121)	4 (3, 7)	26 (12, 59)	102 (80, 126)	4 (3, 7)	28 (12, 63)	102 (81, 126)	4 (3, 7)	28 (12, 64)

*<20 ppb as the reference group

**Current asthma defined as ever having an asthma diagnosis plus wheezing in the past year or taking asthma medications in the past month; “no asthma” defined as no asthma diagnosis, no wheezing and no use of asthma medications or wheezing in the past 12 months.

† AR defined as ever having a diagnosis of hay fever or AR; “no AR” with never diagnosis of hay fever or AR

‡Aeroallergen IgE defined as having any aeroallergen IgE > 0.35 IU/mL; “no aeroallergen IgE” with IgE ≤ 0.35 IU/mL

Model 1. Adjusted for sex and current age and height.

Model 2. Model 1 additionally adjusted for race/ethnicity; maternal education and smoking during pregnancy; median value owner-occupied housing and percent ≥ bachelor’s degree (census tract, mid-childhood); and household income and any smokers at home at early teen visit.

Model 3. Model 2 additionally adjusted for BMI at early teen visit