

Allergen exposure modifies the relation of sensitization to fraction of exhaled nitric oxide levels in children at risk for allergy and asthma

Joanne E. Sordillo, ScD,^{a,b} Tara Webb, BS,^a Doris Kwan, BS,^a Jimmy Kamel, BA,^a Elaine Hoffman, PhD,^c Donald K. Milton, MD, DrPH,^d and Diane R. Gold, MD, MPH^{a,b} Boston, Mass, and College Park, Md

Background: Studies on airway inflammation, measured as fraction of exhaled nitric oxide (FENO), have focused on its relation to control of asthma, but the contribution of allergen exposure to the increase in FENO levels is unknown.

Objective: We evaluated (1) whether FENO levels were increased in children with allergic sensitization or asthma; (2) whether specific allergen exposure increased FENO levels in sensitized, but not unsensitized, children; and (3) whether sedentary behavior increased FENO levels independent of allergen exposures.

Methods: At age 12 years, in a birth cohort of children with a parental history of allergy or asthma, we measured bed dust allergen (dust mite, cat, and cockroach) by means of ELISA, specific allergic sensitization primarily based on specific IgE levels, and respiratory disease (current asthma, rhinitis, and wheeze) and hours of television viewing/video game playing by means of questionnaire. Children performed spirometric maneuvers before and after bronchodilator responses and had FENO levels measured by using electrochemical detection methods (NIOX MINO).

Results: FENO levels were increased in children with current asthma (32.2 ppb), wheeze (27.0 ppb), or rhinitis (23.2 ppb) compared with subjects without these respective symptoms/diagnoses (16.4-16.6 ppb, $P < .005$ for all comparisons). Allergic sensitization to indoor allergens (cat, dog, and dust mite) predicted higher FENO levels and explained one third of the variability in FENO levels. FENO levels were highest in children both sensitized and exposed to dust mite. Greater than 10 hours of weekday television viewing was associated with a 0.64-log increase in FENO levels after controlling for indoor allergen exposure, body mass index, and allergic sensitization.

Conclusion: Allergen exposures and sedentary behavior (television viewing/video game playing) might increase airway

inflammation, which was measured as the FENO. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Asthma, dust mite, cat, allergens, exhaled nitric oxide, allergic sensitization, home environment

The fraction of exhaled nitric oxide (FENO) has been used in epidemiologic studies of allergic disease and in clinical practice as a supplemental tool for monitoring exacerbations and treatment efficacy in asthmatic patients.¹⁻³ FENO serves as a biomarker of airway inflammation that often correlates with the presence of sputum eosinophil counts.^{4,5} The increase in FENO levels observed in allergic disease is most likely driven by an upregulation of inducible nitric oxide synthase by cytokines released during airway inflammation.^{6,7} In addition to established reports of increased FENO levels in asthmatic patients, studies have shown increased FENO levels in subjects with allergic rhinitis and asymptomatic allergic sensitization.^{1,8-11}

Over the past decade, the majority of studies on FENO have sought to relate this inflammatory biomarker with clinical diagnoses or symptom status in allergic disease. Although environmental factors might play a significant role in airway inflammation, few investigators have examined their effect on FENO levels. Most studies accounting for the environmental contribution to this inflammatory biomarker are conducted solely in asthmatic subjects. A cross-sectional analysis in an asthmatic cohort showed an interaction between environmental allergen exposure and allergic sensitization, with the highest production of FENO in sensitized asthmatic subjects exposed to increased home allergen levels.¹² Allergen challenge studies in asthmatic subjects demonstrate increases in FENO levels within the short-term period (1-7 days) after administration of inhaled allergen.^{10,13}

In other literature lifestyle factors, such as sedentary behavior (ie, watching television), have been associated with asthma and wheezing^{14,15} but have not been evaluated as potential predictors of increased FENO levels. Sedentary behavior might reflect a constellation of lifestyle choices capable of promoting airway inflammation and asthma, including decreased physical activity, increased caloric intake, and low fruit and vegetable consumption.

We have previously demonstrated the relationship between early-life allergen exposure and the onset of asthma and atopy in our birth cohort of children at high risk for allergies and asthma.^{16,17} In the present work we show the effect of current allergen exposure on airway inflammation in sensitized versus unsensitized subjects (both with and without asthma or rhinitis). We

From ^athe Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston; ^bHarvard Medical School, Boston; ^cthe Department of Biostatistics, Harvard School of Public Health, Boston; and ^dthe University of Maryland, College Park.

Supported by National Institutes of Health grants AI35786 and ES07036.

Disclosure of potential conflict of interest: D. K. Milton and D. R. Gold have received research support from the National Institutes of Health. The rest of the authors have declared that they have no conflict of interest.

Received for publication June 17, 2010; revised December 23, 2010; accepted for publication January 31, 2011.

Reprint requests: Joanne E. Sordillo, ScD, 181 Longwood Ave, Boston, MA 02115.

E-mail: rejoa@channing.harvard.edu.

0091-6749/\$36.00

© 2011 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2011.01.066

Abbreviations used

FENO: Fraction of exhaled nitric oxide
ICS: Inhaled corticosteroid

also investigate the relationship between sedentary behavior (television watching/video game playing) and FENO levels.

METHODS**Study cohort**

Study participants were recruited between September 1994 and August 1996. The screening and recruitment of families have been described in detail elsewhere.¹⁸ In brief, eligibility criteria included residence in the Boston metropolitan area, maternal age of 18 years or greater, and a history of hay fever, asthma, or allergies in at least 1 of the child's parents. Families were not screened if the newborn was hospitalized in the intensive care unit, if his or her gestational age was less than 36 weeks, or if he or she had a congenital anomaly. After written informed consent was obtained from the child's primary caretaker, a series of home visits were made (at age 2-3 months, age 7 years, and age 12 years), and a questionnaire regarding demographics, home characteristics, environmental exposures, tobacco use, and health outcomes was administered by trained research assistants at each home visit. Over the course of follow-up, a telephone questionnaire (modified from the American Thoracic Society's Division of Lung Diseases questionnaire) was administered by trained research assistants to the child's primary caretaker every 6 months. Of the 505 children enrolled in the study, 430 (85%) were followed until age 12 years. Of the children followed until age 12 years, 283 had FENO measurements, and 250 had both FENO and allergen exposure assessment at age 12 years. The study was approved by the Institutional Review Board of Brigham and Women's Hospital.

Home visit and dust sample collection

Index children in the cohort ranged from 11 to 13.9 years (average, 11.8 years) at this home visit to assess current allergen exposure. Methods used for collection of dust samples at the home visit and for measurement of dust mite, cat, and cockroach allergen have been described previously.¹⁸ Briefly, to collect bed dust, we used a Eureka Mighty-Mite vacuum cleaner (Model 3621; Eureka Co, Bloomington, Ind) modified to hold 19 × 90-mm cellulose extraction thimbles. All layers of the bedding were vacuumed for a total of 10 minutes. Allergen concentrations (in micrograms per gram of dust) for Der f 1, Der p 1, Fel d 1, and Bla g 2 were quantified by means of ELISA. Consistent with previous analyses in our cohort, concentrations of dust mite allergen were grouped in categories potentially relevant to respiratory disease outcomes.¹⁶ For dust mite, we used the highest concentration of Der f 1 or Der p 1 in the child's bed to categorize allergen levels as 10 µg/g or greater or less than 10 µg/g. For cat allergen, levels were categorized as 8 µg/g or greater or less than 8 µg/g. Cockroach allergen in bed dust was defined as greater than or less than detectable levels. In addition to the current allergen exposure measurements listed above (average age, 11.8 years), prior allergen exposures (Der f 1, Der p 1, Fel d 1, and Bla g 2) were also assessed at a mean age of 7 years by using the same methodology and exposure cut points.

Assessment of allergic sensitization

At a mean age of 12 years (range, 11.2-13.5 years), levels of IgE specific to common allergens were measured in 189 children, and allergy skin testing was performed in an additional 19 children with FENO measurement. Serum samples were assayed for IgE to common indoor (cat dander, dog dander, cockroach [*Blattella germanica*], dust mite [*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*], and murine epithelial extract) and outdoor (ragweed, mixed trees, *Aspergillus* species, *Alternaria* species, mixed grasses, *Cladosporium* species, and *Penicillium* species) allergens by using the UniCAP 250 system. Levels of IgE to specific allergens were considered positive

at 0.35 IU/mL or greater. Allergy skin testing was performed on the volar aspect of the lower arms for the allergens listed above. A subset of children (n = 36/244 [15%]) were missing allergic sensitization assessment at age 12 years, even though data on allergen exposures, respiratory symptoms, and exhaled nitric oxide levels were complete. For these subjects, allergic sensitization data from an earlier time point (mean age, 7.4 years) were used in the analysis. In children with data at both time points, sensitization at age 7.4 years was associated with sensitization at age 12 years ($\chi^2 = 39.7$, $P < .0001$). Seventy-four percent had the same sensitization status at both time points. Of the children who were unsensitized at age 7.4 years, 13% later had positive test results for allergic sensitization at age 12 years.

Assessment of television watching/video game playing

Data on hours of watching television or playing video games were gathered every 6 months by means of telephone questionnaire. We used data from the questionnaire closest in time to FENO measurement to assess hours of weekday and weekend time spent watching television or playing video games. Respondents classified television watching/video game playing by choosing a category that best represented their child's exposure (none, 1-5 hours, 6-10 hours, 11-15 hours, or 16-20 hours). Total hours of exposure during the week (weekday) and total exposure over the weekend were assessed separately. Data on physical activity were also gathered for comparison with time spent watching television/playing video games. Children were asked the following: "How many of the past 7 days did you participate in physical activity for at least 20 minutes that made you sweat/breathe hard?" The response to this question was used to characterize the number of "physically active" days per week for each subject.

Definition of respiratory symptom outcomes

Health outcome data were gathered every 6 months by means of telephone questionnaire. The disease and symptom data closest to the time of FENO measurement were used in the cross-sectional analysis. Outcomes included (1) current asthma, which was defined as a doctor's diagnosis of asthma with any wheeze in the past 12 months, including wheeze after exposure to cold air or exercise; (2) any wheeze (≥ 1 wheezing events in the last year); (3) dry cough at night without cold or flu; and (4) current rhinitis, which was defined as a doctor's diagnosis of hay fever with runny nose apart from cold in the past 12 months.

Spirometry

Standard spirometry was performed in accordance with American Thoracic Society criteria.¹⁹ Albuterol (180 µg, 2 puffs with a spacer) was administered to determine bronchodilator response. The subjects were asked to wait 10 minutes. Spirometry was then repeated. A positive bronchodilator response was defined as a 12% or greater increase in predicted FEV₁ after the administration of albuterol. Children were asked to refrain from both long-acting bronchodilator use 12 hours before assessment and short-acting bronchodilator use 4 hours before assessment.

FENO measurement

FENO levels were evaluated with a portable electrochemical device (NIOX MINO; Aerocrine AB, Stockholm, Sweden), which is in agreement with published procedures for FENO measurement.²⁰ This method has been validated by using chemiluminescence technology, with an accuracy of ± 5 parts per billion (NIOX MINO, Aerocrine AB). Before FENO measurement, subjects breathed in through a nitric oxide scrubbing filter and exhaled out into the room air twice before inhaling a third time through the filter and exhaling into the FENO analyzer. Exhaled FENO measurements were conducted without a nose clip at a flow rate of 50 mL/s. (The last 3 seconds of the exhalation was used for FENO measurement to ensure quantification of lower rather than upper airway FENO.) In accordance with American Thoracic Society guidelines, we did not use nose clips because blocking the nasal passages can cause exhaled

nitric oxide to accumulate in the nose and potentially leak into the exhaled air stream through the posterior nasopharynx.²⁰ This procedure was performed 3 times, so that each subject had a total of 3 FENO measurements. The median of these 3 measurements was used in data analysis.

Data analysis

In children followed up to age 12 years, χ^2 analyses were used to determine whether demographic characteristics were different in those with versus without FENO measurements. FENO levels were log transformed (ln) to approximate normality. A multiple regression model containing dichotomous variables for individual indoor and outdoor allergies was constructed to investigate the association of specific allergic sensitization with FENO. Allergic sensitization variables that were significant in univariate analyses were included in the multiple regression model. (Variance inflation factors were examined to ensure that multicollinearity was not an issue when adding multiple allergic sensitization variables to the same model.) Mean FENO levels (least squares means) in those with adverse respiratory health outcomes (current asthma, any wheeze, dry cough at night, and current rhinitis) were examined in models controlling for sex and maternal asthma. Inhaled corticosteroid (ICS) use was examined as a potential confounder of the association between asthma symptoms and FENO levels. For the entire group with allergy testing, as well as for the subgroup with diagnosed asthma or hay fever, we also compared least squares means for FENO levels in those sensitized versus not sensitized. To determine whether the relationship between allergic sensitization and FENO levels was modified by allergen exposure, we entered an interaction term, as well as main effects of specific allergy and allergen exposure level, into models for FENO. Sex, race, season, and age at FENO measurement were considered as potential confounders. A *P* value of .05 or less was considered significant, whereas *P* values of .10 or less were taken to indicate borderline significance.

The statistical software package SAS 9.1.3 (SAS Institute, Inc, Cary, NC) was used to perform statistical analyses.

RESULTS

FENO measurement

The median level of FENO was 16.0 ppb (range, 2.0-169 ppb) in all subjects with FENO measurements (Table I). On average, children were 11.7 years of age at the time of FENO quantification (age range, 11-13.9 years). Of the 430 children followed to age 12 years by questionnaire, 277 (64%) had FENO measurements. Children with FENO measurements were more likely to be white and less likely to be Hispanic. Distribution of sex and prevalence of maternal asthma were similar for those with versus without FENO measurements at age 12 years (Table I).

Symptoms and FENO levels

Of the children with FENO measurement and health data determined by means of questionnaire at age 12 years (*n* = 277), 16% had current asthma (*n* = 44), 22% wheezed in the past year (*n* = 60), and 14% had dry cough at night without a cold. Eighty-one percent of the current asthmatic subjects had allergic sensitization. Current asthma was associated with a 2-fold increase in FENO levels (*P* < .0001), from 16.5 ppb (nonasthmatic subjects) to 32.2 ppb (subjects with current asthma, Table II). Children with any wheeze in the past year also had increased FENO levels (16.4 ppb in no wheeze vs 27.0 for any wheeze). Current rhinitis was associated with a similar increase in FENO levels (*P* < .01). Dry cough at night was not associated with FENO levels. Eight percent of the subjects with FENO measurement reported use of ICSs. Use of ICSs did not confound the relationship between asthma symptoms and FENO levels (see Table E1 in this article's Online Repository at www.jacionline.org). Sensitized children also

TABLE I. Characteristics of subjects tested for FENO

Characteristic	Children followed up to age 12 y with FENO measurement	Children followed up to age 12 y without FENO measurement
Sex		
Male	160 (58)	76 (48)
Female	117 (42)	81 (52)
Race		
White	233 (84)	116 (74)*
Black	23 (8)	14 (9)
Hispanic	6 (3)	12 (7)*
Asian (other)	15 (5)	15 (10)
Maternal asthma	79 (29)	48 (31)
Age at FENO measurement (y)	Mean, 11.7; range, 11-13.9	NA
FENO (ppb)	Median, 16; IQR, 11.0-32.0	NA

Values are presented as numbers (percentages).

IQR, Interquartile range; NA, not applicable.

**P* < .05 for comparison with children followed to age 12 years with FENO measurement.

TABLE II. Current asthma, rhinitis, respiratory symptoms, and atopy and FENO levels*

	No.	Disease status	FENO (ppb [LS mean])	<i>P</i> value
Current asthma (all)	233	No	16.5	<.0001
	44	Yes	32.2	
Current asthma (nonatopic)	7	Yes	15.0	.01†
Current asthma (atopic)	29	Yes	36.7	
Any wheeze	217	No	16.4	.0008
	60	Yes	27.0	
Dry cough at night	235	No	18.0	.34
	40	Yes	20.4	
Current rhinitis (all)	185	No	16.6	.005
	56	Yes	23.2	
Current rhinitis (nonatopic)	9	Yes	13.3	.02†
Current rhinitis (atopic)	39	Yes	26.5	
Allergic sensitization‡	116	No	12.6	<.0001
	130	Yes	24.5	
Allergic sensitization (in those without diagnosis of asthma or rhinitis)§	73	No	11.9	<.0001
	43	Yes	22.4	

LS, Least squares.

*Models controlled for sex and maternal history of asthma.

†For comparison of atopic versus nonatopic subjects with disease diagnosis.

‡Sensitization to indoor allergens (cat, dog, dust mite, cockroach, and mouse), outdoor allergens (pollen, ragweed, and grass), or mold.

§All children ever given a diagnosis of rhinitis or asthma have been removed from this comparison.

produced higher FENO levels (24.5 ppb) compared with children who were unsensitized (12.6 ppb, *P* < .001). The association of FENO levels with any allergic sensitization remained significant, even after children with rhinitis (*n* = 48) or prior diagnoses of asthma (*n* = 84) were removed from the analysis (Table II). Children with a prior asthma diagnosis who had positive test results for allergic sensitization (67%) had higher FENO levels (26.1 ppb) than unsensitized children with a diagnosis of asthma (14.2 ppb, *P* < .0001). Sensitized children with current asthma also produced higher FENO levels than subjects with current asthma who were unsensitized (Table II).

TABLE III. Allergic sensitization and FENO levels

Allergic sensitization	No.	Univariate models		Multiple regression model*			
		FENO (ppb [LS mean])	P value	FENO (ppb [LS Mean])	P value	Partial R ² value	Model R ² value
Dust mite	Negative (151)	13.5	<.0001	22.8	<.0001	19.1%	31.9%
	Positive (99)	27.0		36.6			
Cat	Negative (180)	14.3	<.0001	22.6	<.0001	8.7%	
	Positive (70)	31.4		37.0			
Dog	Negative (237)	16.9	<.0001	22.3	.01	2.1%	
	Positive (13)	43.9		37.5			
Cockroach	Negative (237)	17.3	.014	27.9	.7	0.05%	
	Positive (13)	29.6		30.0			
Mouse	Negative (241)	17.4	.02	29.0	.98	0.06%	
	Positive (6)	37.6		28.8			
Tree mix	Negative (174)	16.6	.03	31.1	.2	0.5%	
	Positive (73)	20.9		26.9			
Ragweed	Negative (220)	17.1	.02	28.3	.77	0.06%	
	Positive (30)	24.0		29.6			
<i>Alternaria</i> species	Negative (219)	17.1	.03	27.8	.43	0.8%	
	Positive (28)	23.9		31.0			
<i>Penicillium</i> species	Negative (236)	17.7	.60	—	—	—	
	Positive (11)	20.1		—			
Grass	Negative (206)	17.4	.30	—	—	—	
	Positive (41)	20.0		—			

LS, Least squares.

*Adjusted for sex and maternal history of asthma.

Allergic sensitization and FENO levels

The most prevalent indoor allergies were to dust mite (40%) and cat (28%). Allergic sensitization to other indoor allergens was much less common, with prevalence ranging from 5% (dog and cockroach allergy) to 2.4% (mouse allergy). The most prevalent outdoor allergy was to tree pollen (29%). Few subjects were allergic to fungi (4.4% to 11%). In univariate models both indoor and outdoor allergies were significant predictors of increased FENO levels (Table III). Allergies to mites, pets (dog and cat), pests (mice), pollen (tree and ragweed), and fungi (*Alternaria* species) were all associated ($P < .05$) with FENO measurements of at least double the levels in unsensitized subjects. However, in the multiple regression model only the indoor allergies to dust mite, cat, and dog remained significant (Table III). In addition to considering specific allergic sensitization, we modeled the association between the total number of positive sensitization test results and FENO levels. FENO levels were shown to increase with each additional positive skin test result (0.13-log increase in FENO level per positive allergic sensitization test, see Table E2 in this article's Online Repository at www.jacionline.org). An increased percentage of circulating eosinophils was also associated with higher FENO levels (see Table E3 in this article's Online Repository at www.jacionline.org).

Effect of allergic sensitization modified by allergen exposure

The effect of allergic sensitization on FENO levels was modified by current allergen exposure (Table IV). Thirty-two percent of the children fell into the category of high dust mite exposure ($\geq 10 \mu\text{g/g}$ in bed dust), whereas 27% were observed to have increased cat allergen exposure. Detectable cockroach allergen was found in 6% of children's bed dust samples.

Current dust mite exposure did not increase FENO levels in children without dust mite allergy ($P = .2$). However, among children with dust mite allergy, higher exposure levels were associated with a significant increase in FENO levels ($P = .003$). This interaction remained even after children with a prior diagnosis of asthma were removed from the analysis (see Table E4 in this article's Online Repository at www.jacionline.org). Unsensitized children produced the lowest FENO levels. Subjects who were sensitized but had low exposure produced levels twice as high (24.7 ppb) as those seen in unsensitized children (12.4–14.8 ppb), whereas the sensitized and exposed subjects produced 3 times the level of FENO (38.3 ppb) compared with unsensitized subjects. Similar interactions were observed for cat allergen exposure. Increased current cat allergen exposure was associated with higher FENO levels only in sensitized subjects; however, this association was not observed in the subset without asthma or rhinitis. Exposure to cockroach allergen did not increase FENO levels in subjects with cockroach allergy. Similarly, an interaction between dog allergen exposure and sensitization to dog was not observed for FENO. Season did not confound the main effects or interaction terms in these models.

Prior allergen exposure (mean age, 7 years) was also predictive of increased FENO levels at age 12 years in allergic subjects. Prior allergen exposure levels tended to overlap with current allergen exposure. Bed dust mite levels ($\geq$ or $< 10 \mu\text{g/g}$) were consistent over time for 72% of subjects. For dust mite, the interaction observed for prior allergen exposure in bed dust was similar to the interaction observed for current allergen exposure. Prior allergen exposure (dust mite, cat, or cockroach), as measured by means of assay of bed dust at age 7 years, showed a similar interaction of sensitization and exposure with the highest FENO levels in those both sensitized and exposed to increased levels of the specific allergen (see Table E5 in this article's Online Repository at www.jacionline.org).

TABLE IV. Effect of current allergen exposure on FENO levels, as modified by allergic sensitization*

	Allergic sensitization	Exposure category†	No.	FENO (ppb [LS mean])	P value
Dust mite (Der f 1, Der p 1)	–	<10 µg/g	103	14.8	.2
		≥10 µg/g	44	12.4	
	+	<10 µg/g	64	24.7	.003
		≥10 µg/g	33	38.3	
Cat (Fel d 1)	–	<8 µg/g	123	14.9	.9
		≥8 µg/g	51	14.6	
	+	<8 µg/g	53	30.7	.06
		≥8 µg/g	15	44.7	
Dog (Can f 1)	–	<20 µg/g	158	18.5	.2
		≥20 µg/g	62	16.2	
	+	<20 µg/g	8	29.5	.5
		≥20 µg/g	4	33.7	
Cockroach (Bla g 2)	–	Nondetectable	217	17.9	.7
		Detectable	13	19.7	
	+	Nondetectable	11	33.6	.3
		Detectable	2	18.2	

LS, Least squares.

*Models controlled for sex and maternal history of asthma.

†Allergens measured in bed dust samples at mean age of 12 years.

Bronchodilator response and FENO level

Response to albuterol (12% increase in predicted FEV₁) was associated with higher FENO levels (28.0 vs 17.9 ppb for nonresponders, $P = .02$, Table V). Of those who responded to albuterol, 56% (9/16) had a prior asthma diagnosis, 37% (6/16) had wheezed in the past year, and 31% (5/16) had current active asthma. Of sensitized children with a past diagnosis of asthma, 9.8% (5/51) responded to albuterol, whereas 3.7% (1/27) of unsensitized asthmatic children were responders. Of sensitized children with prior asthma diagnosis and current symptoms, 11.1% (3/27) responded to albuterol, whereas none of the unsensitized subjects with current asthma (0/7) were responders. Neither sensitization nor allergen exposure (or their interaction) was predictive of bronchodilator response (data not shown).

Television watching/video game playing and FENO levels

Total hours of weekday television watching or video game playing was associated with increased FENO levels compared with those seen in the reference group (zero hours of television watching/video game playing), even after controlling for allergen exposure, allergic sensitization, and body mass index (Table VI). Ten percent of children did not watch television or play video games during the week, 47% were in the 1- to 5-hour exposure category, 32% were in the 6- to 10-hour exposure category, and the remaining 11% were in the greater than 10-hour exposure category. The 1- to 5-hour and 6- to 10-hour exposure categories showed similar effects when compared with the reference group and were therefore collapsed into a single 1- to 10-hour exposure category. In those with 1 to 10 hours of weekday exposure, FENO levels were associated with a 0.43-log increase in FENO levels ($P = .02$). Greater than 10 hours of weekday exposure was associated with a slightly larger increase (0.64-log increase in FENO levels vs reference category, $P = .005$). For weekend television watching/video game playing, the reference category (zero hours) had too few children ($n = 3$) to make a valid comparison with higher exposure levels. Children's physical activity levels were inversely

TABLE V. Bronchodilator response and FENO levels*

Bronchodilator response	Level	No.	LS mean	P value for difference
12% Increase in FEV ₁ after albuterol	N	218	17.9	.02
	Y	16	28.0	

LS, Least squares.

*Model adjusted for sex and maternal history of asthma.

TABLE VI. Television watching/video game playing and FENO levels (multiple regression model)*

Weekday television/video game playing	No.	Parameter estimate $\Delta \log$ FENO vs reference	P value
None	27		
1-10 h	220	0.43	.02
>10 h	30	0.64	.005

*Model controlled for dust mite allergen (>10 µg/g), Fel d 1 (>8 µg/g), and endotoxin (> median), all measured in living room dust; body mass index; sex; current asthma; and allergic sensitization.

associated with television watching/video game playing. Of children who did not watch television during the week, 87% reported at least 5 “physically active” days per week compared with 58% in the 1- to 10-hour category and 42% in the greater than 10-hour television/video game category ($P = .03$, χ^2 test).

DISCUSSION

The importance of ascertaining specific allergic sensitization when evaluating the clinical significance of allergen exposure on asthma morbidity was highlighted, first in the observational National Inner City Asthma Study^{21,22} and then in the subsequent intervention study that was targeted at allergen exposures to which asthmatic children were sensitized.²³ Our study highlights the importance of allergic sensitization when evaluating the

effects of allergen exposures on subclinical pulmonary inflammatory outcomes that might ultimately have clinical import, even in asymptomatic children. Most prior research on allergen exposure and FENO levels has been focused on asthmatic populations. To the best of our knowledge, this is the first study to investigate the independent effect of home exposures (allergens) and sedentary home behaviors (television viewing/video game playing) on FENO levels in a birth cohort at high risk for allergies and asthma.

Respiratory symptoms, bronchodilator response, allergic sensitization, and FENO levels

We reproduced several documented associations between clinical outcomes and FENO levels in our cohort. As in a number of other studies,^{2,9,11} subjects with current asthma or wheeze had increased FENO levels. Similar to prior reports,^{24,25} bronchodilator response was associated with increased FENO levels. Rhinitis was an important contributor to FENO levels in our study and others,^{9,26} suggesting that rhinitis might be linked to lower, as well as upper, airway inflammation. The association we observed between increased FENO levels in sensitized subjects without allergic disease reflects similar reports of sensitization as a predictor of airway inflammation in healthy asymptomatic children.^{1,26,27} The relationship between increased circulating eosinophils (as a percentage) and higher FENO levels demonstrated in this report provides further supportive evidence that allergic sensitization is a key factor in FENO production. Contrary to these results, other investigators have found increased FENO levels only in atopic subjects with concomitant asthma or rhinitis.^{9,28} In these studies,^{9,28} offline FENO analysis (by collecting exhaled air into Mylar bags before chemiluminescent detection) might have added noise to the measurement, potentially preventing discrimination between healthy atopic and nonatopic subjects.

In our analysis of specific allergic sensitization and FENO levels, indoor allergies to dust mites and pets (cats and dogs) explained approximately one third of the variability in FENO levels and were the only significant predictors remaining after adjustment for sensitization to other allergens, including plants (trees and ragweed) and fungi (*Alternaria* species). This result is in agreement with a cross-sectional study of Dutch schoolchildren, which demonstrated that sensitization to indoor rather than outdoor allergens was the main determinant of increased FENO levels.²⁹

Effect modification of allergic sensitization by allergen exposure

This is the first study to investigate effect modification of specific allergic sensitization by home allergen exposure in a cohort of children at high risk for allergies and asthma. Of the few existing experimental and epidemiologic studies on this type of interaction, all have been conducted in subjects with symptomatic allergic disease. In one report repeated low-dose inhalant allergen challenges over the course of 6 days were shown to double FENO production in sensitized adult asthmatic subjects, with a simultaneous increase in bronchial hyperresponsiveness to histamine over the study period.¹³ Bronchial challenge with dust mite allergen in adults with mite allergy with asthma or rhinitis has been shown to significantly increase FENO levels 24 hours after challenge.¹⁰ Asthmatic children with dust mite allergy living in a mite-free environment for 3 months showed a significant increase in FENO levels only a few days after returning to an area with

natural mite exposure.³⁰ One epidemiologic survey demonstrated increased FENO levels among sensitized asthmatic subjects exposed to indoor allergens, although the authors lacked the power to analyze specific types of allergen exposure by means of sensitization interactions (subjects exposed and sensitized to any allergen were lumped together in one group).¹² Another study in asthmatic children did not detect any interaction between sensitization and home allergen exposure.³¹

In our study the association between FENO levels and allergen exposure in dust mite-sensitized subjects remained, even after removing those with a prior asthma diagnosis from the analysis. This interaction was also observed after removing subjects with rhinitis from the analysis, although the *P* value for the interaction was borderline significant (*P* = .09). Because allergen exposures tended to remain constant over time in our cohort, it is possible that current allergen levels reflect persistent exposure from age 7 to 12 years. Therefore persistent rather than transient exposure to perennial allergens might be responsible for driving increased FENO levels in sensitized subjects. Although some allergen exposures (dust mite and cat) were associated with substantial increases in FENO levels, exposures to cockroach and dog allergen did not appear to modify FENO levels. Lack of significant findings for these perennial allergens might simply be due to reduced power to detect effect modification in the relatively small groups of cockroach- and dog-sensitized subjects. Although our primary focus was on home exposures to perennial allergens, we also investigated the effect of the spring season (when tree pollen allergens are increased) on FENO levels in subjects sensitized to tree allergens (data not shown). We did not detect any significant interaction or trend, suggesting that perennial allergens might play a larger role than seasonal allergens in predicting increased FENO levels in sensitized subjects.

Our findings have important implications for clinical practice. With the exception of the National Inner City Asthma trials, most asthma clinical trials are not focused on environmental intervention. Our study highlights the importance of considering modification of the home environment when planning a comprehensive intervention aimed at reducing airway inflammation in asthmatic or symptomatic allergic children. It is also possible that there are long-term clinical implications of our findings of increased FENO levels in asymptomatic children who are sensitized and exposed to high allergen levels. Our geometric mean FENO levels in non-asthmatic children who were sensitized and exposed to dust mite (33.7 ppb) were similar to levels measured in subjects with uncontrolled asthma.³² Persistent airway inflammation might lead to symptomatic disease and even to airway remodeling.^{33,34}

Television viewing/video game playing and FENO levels

Even taking into account allergic sensitization, allergen exposures, and body mass index, we demonstrated a positive association between hours of television viewing or video game playing and FENO levels in children at age 12 years. Although television viewing has been associated with increased risk of wheeze and current asthma in school-aged children,^{14,15} ours is the first report on the relationship between this sedentary behavior and a quantifiable biomarker of airway inflammation (FENO). Children who watch more television engage in less physical activity,³⁵ consume higher-calorie diets with lower amounts of fruits and vegetables,^{36,37} and have increased body fat.³⁸

Television viewing/video game playing might represent a surrogate for one or a combination of these factors related to diet or physical activity. It has been hypothesized, and there is some evidence to suggest, that decreased physical activity can alter physiologic or immune response (with potential implications for airway inflammation and asthma), whereas dietary factors (eg, reduced antioxidant intake) might also play a role in asthma. Watching television has also been associated with decreased sigh rates, which could contribute to nonspecific bronchial hyperactivity through airway smooth muscle "latching."³⁹

Limitations

Although FENO data gathered at a single time point allowed us to examine multiple associations, measurement at additional points in time would have enabled us to study changes in FENO levels with alterations in clinical symptom status or environmental exposures. Previous studies have shown higher FENO levels in atopic asthmatic versus nonatopic asthmatic subjects.²⁸ However, we were limited in our ability to assess the effect of atopy within our relatively small subgroup of subjects with current asthma. Because allergen levels tended to persist over time, we were unable to compare the effect of continual versus transient increases in exposure on FENO levels.

Conclusion

This study demonstrates the importance of environmental contributions, in the form of biological exposures (allergens) and behaviors (television viewing/video game playing), as potential determinants of FENO levels.

Key messages

- Allergen exposure increases FENO production in sensitized subjects independent of asthmatic status.
- Sedentary behaviors (television watching/video game playing) are associated with higher FENO levels.

REFERENCES

- Franklin PJ, Turner SW, Le Souef PN, Stick SM. Exhaled nitric oxide and asthma: Complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. *Thorax* 2003;58:1048-52.
- Nordvall SL, Janson C, Kalm-Stephens P, Foucard T, Toren K, Alving K. Exhaled nitric oxide in a population-based study of asthma and allergy in schoolchildren. *Allergy* 2005;60:469-75.
- Warke TJ, Mairs V, Fitch PS, McGovern V, Ennis M, Shields MD. Exhaled nitric oxide in relation to the clinical features of childhood asthma. *J Asthma* 2004;41:751-7.
- Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, et al. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. *Am J Respir Crit Care Med* 2001;164:738-43.
- Lex C, Ferreira F, Zacharasiewicz A, Nicholson AG, Haslam PL, Wilson NM, et al. Airway eosinophilia in children with severe asthma: Predictive values of noninvasive tests. *Am J Respir Crit Care Med* 2006;174:1286-91.
- Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, et al. Induction of nitric oxide synthase in asthma. *Lancet* 1993;342:1510-3.
- Byrnes CA, Dinarevic S, Shinebourne EA, Barnes PJ, Bush A. Exhaled nitric oxide measurements in normal and asthmatic children. *Pediatr Pulmonol* 1997;24:312-8.
- Jackson DJ, Virnig CM, Gangnon RE, Evans MD, Roberg KA, Anderson EL, et al. Fractional exhaled nitric oxide measurements are most closely associated with allergic sensitization in school-age children. *J Allergy Clin Immunol* 2009;124:949-53.
- Jouaville LF, Annesi-Maesano I, Nguyen LT, Bocage AS, Bedu M, Caillaud D. Interrelationships among asthma, atopy, rhinitis and exhaled nitric oxide in a population-based sample of children. *Clin Exp Allergy* 2003;33:1506-11.
- Lopuhaa CE, Koopmans JG, Jansen HM, van der Zee JS. Similar levels of nitric oxide in exhaled air in non-asthmatic rhinitis and asthma after bronchial allergen challenge. *Allergy* 2003;58:300-5.
- Cardinale F, de Benedictis FM, Muggeo V, Giordano P, Loffredo MS, Iacoviello G, et al. Exhaled nitric oxide, total serum IgE and allergic sensitization in childhood asthma and allergic rhinitis. *Pediatr Allergy Immunol* 2005;16:236-42.
- Simpson A, Custovic A, Pipis S, Adises A, Faragher B, Woodcock A. Exhaled nitric oxide, sensitization, and exposure to allergens in patients with asthma who are not taking inhaled steroids. *Am J Respir Crit Care Med* 1999;160:45-9.
- Ihre E, Gyllfors P, Gustafsson LE, Kumlin M, Dahlen B. Early rise in exhaled nitric oxide and mast cell activation in repeated low-dose allergen challenge. *Eur Respir J* 2006;27:1152-9.
- Corbo GM, Forastiere F, De Sario M, Brunetti L, Bonci E, Bugiani M, et al. Wheeze and asthma in children: Associations with body mass index, sports, television viewing, and diet. *Epidemiology* 2008;19:747-55.
- Sherriff A, Maitra A, Ness AR, Mattocks C, Riddoch C, Reilly JJ, et al. Association of duration of television viewing in early childhood with the subsequent development of asthma. *Thorax* 2009;64:321-5.
- Celedon JC, Milton DK, Ramsey CD, Litonjua AA, Ryan L, Platts-Mills TA, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol* 2007;120:144-9.
- Phipatanakul W, Celedon JC, Hoffman EB, Abdulkarim H, Ryan LM, Gold DR. Mouse allergen exposure, wheeze and atopy in the first seven years of life. *Allergy* 2008;63:1512-8.
- Gold DR, Burge HA, Carey V, Milton DK, Platts-Mills T, Weiss ST. Predictors of repeated wheeze in the first year of life: the relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. *Am J Respir Crit Care Med* 1999;160:227-36.
- Standardization of spirometry, 1994 update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152:1107-36.
- American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912-30.
- Phipatanakul W, Eggleston PA, Wright EC, Wood RA. National Cooperative Inner-City Asthma Study. Mouse allergen. II. the relationship of mouse allergen exposure to mouse sensitization and asthma morbidity in inner-city children with asthma. *J Allergy Clin Immunol* 2000;106:1075-80.
- Perry T, Matsui E, Merriman B, Duong T, Eggleston P. The prevalence of rat allergen in inner-city homes and its relationship to sensitization and asthma morbidity. *J Allergy Clin Immunol* 2003;112:346-52.
- Morgan WJ, Crain EF, Gruchalla RS, O'Connor GT, Kattan M, Evans R 3rd, et al. Results of a home-based environmental intervention among urban children with asthma. *N Engl J Med* 2004;351:1068-80.
- Covar RA, Szefer SJ, Martin RJ, Sundstrom DA, Silkoff PE, Murphy J, et al. Relations between exhaled nitric oxide and measures of disease activity among children with mild-to-moderate asthma. *J Pediatr* 2003;142:469-75.
- Puckett JL, Taylor RW, Leu SY, Guignon OL, Aledia AS, Galant SP, et al. An elevated bronchodilator response predicts large airway inflammation in mild asthma. *Pediatr Pulmonol* 2010;45:174-81.
- van Asch CJ, Bailemans WA, Rovers MM, Schilder AG, van der Ent CK. Atopic disease and exhaled nitric oxide in an unselected population of young adults. *Ann Allergy Asthma Immunol* 2008;100:59-65.
- Salome CM, Roberts AM, Brown NJ, Dermand J, Marks GB, Woolcock AJ. Exhaled nitric oxide measurements in a population sample of young adults. *Am J Respir Crit Care Med* 1999;159:911-6.
- Barreto M, Villa MP, Martella S, Ronchetti F, Darder MT, Falasca C, et al. Exhaled nitric oxide in asthmatic and non-asthmatic children: influence of type of allergen sensitization and exposure to tobacco smoke. *Pediatr Allergy Immunol* 2001;12:247-56.
- van Amsterdam JG, Janssen NA, de Meer G, Fischer PH, Nierkens S, van Loveren H, et al. The relationship between exhaled nitric oxide and allergic sensitization in a random sample of school children. *Clin Exp Allergy* 2003;33:187-91.
- Bodini A, Peroni D, Loiacono A, Costella S, Pigozzi R, Baraldi E, et al. Exhaled nitric oxide daily evaluation is effective in monitoring exposure to relevant allergens in asthmatic children. *Chest* 2007;132:1520-5.
- Spanier AJ, Hornung RW, Kahn RS, Lierl MB, Lanphear BP. Seasonal variation and environmental predictors of exhaled nitric oxide in children with asthma. *Pediatr Pulmonol* 2008;43:576-83.
- Perez de Llano LA, Gonzalez FC, Anon OC, Perea MP, Gomez RG, Villar AB, et al. Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma. *Eur Respir J* 2010;35:1200-2.

33. Olin AC, Rosengren A, Thelle DS, Lissner L, Toren K. Increased fraction of exhaled nitric oxide predicts new-onset wheeze in a general population. *Am J Respir Crit Care Med* 2010;181:324-7.
34. Davies DE. The role of the epithelium in airway remodeling in asthma. *Proc Am Thorac Soc* 2009;6:678-82.
35. Eisenmann JC, Bartee RT, Wang MQ. Physical activity, TV viewing, and weight in U.S. youth: 1999 youth risk behavior survey. *Obes Res* 2002;10:379-85.
36. Salmon J, Campbell KJ, Crawford DA. Television viewing habits associated with obesity risk factors: a survey of Melbourne schoolchildren. *Med J Aust* 2006;184:64-7.
37. Wiecha JL, Peterson KE, Ludwig DS, Kim J, Sobol A, Gortmaker SL. When children eat what they watch: Impact of television viewing on dietary intake in youth. *Arch Pediatr Adolesc Med* 2006;160:436-42.
38. Grund A, Krause H, Siewers M, Rieckert H, Muller MJ. Is TV viewing an index of physical activity and fitness in overweight and normal weight children? *Public Health Nutr* 2001;4:1245-51.
39. Hark WT, Thompson WM, McLaughlin TE, Weatley LM, Platts-Mills TA. Spontaneous sigh rates during sedentary activity: watching television vs. reading. *Ann Allergy Asthma Immunol* 2005;94:247-50.

TABLE E1. Association between asthma symptoms and F_{ENO} levels adjusted for ICS use

Model	No.	Disease status	F _{ENO} (ppb [LS mean])	P value
Current asthma (all)*	233	No	18.6	<.0001
	44	Yes	34.6	
Any wheeze*	217	No	19.9	<.0001
	60	Yes	29.9	
Dry cough at night*	235	No	18.2	.7
	40	Yes	19.2	
ICS use†	256	No	22.2	.13
	21	Yes	29.2	

LS, Least squares.

*Model adjusted for ICS use, maternal asthma, and sex.

†Model adjusted for current asthma, maternal asthma, and sex.

TABLE E2. Total number of positive skin test results and F_{ENO} levels

	Parameter estimate (increase log F _{ENO} per positive test result)*	<i>P</i> value	Partial <i>R</i> ² value
No. of positive allergic sensitization test results	0.13	<.0001	20.3

*Model adjusted for maternal asthma and sex (n = 247).

TABLE E3. Percentage of circulating eosinophils and F_{ENO} levels

	Parameter estimate (increase log F _{ENO} per percentage point increase in eosinophils)*	<i>P</i> value	Partial <i>R</i> ² value
Eosinophils in peripheral blood (%)	0.129	<.0001	20.6

*Model adjusted for maternal asthma and sex (n = 182).

TABLE E4. Effect of current allergen exposure on FENO levels, as modified by allergic sensitization status: nonasthmatic subjects (never received a diagnosed with asthma) only*

	Allergic sensitization	No.	Exposure category†	FENO (ppb [LS mean])	P value
Dust mite (Der f 1, Der p 1)	—	79	<10 µg/g	14.3	.3
		30	≥10 µg/g	12.2	
	+	33	<10 µg/g	21.1	.02
		17	≥10 µg/g	33.7	
Cat (Fel d 1)	—	91	<8 µg/g	14.5	.7
		35	≥8 µg/g	15.2	
	+	27	<8 µg/g	29.1	.9
		4	≥8 µg/g	27.8	

LS, Least squares.

*Models controlled for sex and maternal history of asthma.

†Allergens measured in bed dust samples at mean age of 12 years.

TABLE E5. Effect of prior allergen exposure on FENO levels, as modified by allergic sensitization status*

	Allergic sensitization	No.	Exposure category	FENO (ppb [LS mean])	P value
Dust mite (Der f 1, Der p 1)	—	110	<10 µg/g	13.8	.25
		28	≥10 µg/g	16.3	
	+	42	<10 µg/g	23.9	.001
		24	≥10 µg/g	42.3	
Cat (Fel d 1)	—	111	<8 µg/g	15.1	.81
		45	≥8 µg/g	15.5	
	+	36	<8 µg/g	29.7	.3
		13	≥8 µg/g	38.2	

LS, Least squares.

*Allergens measured in bed dust samples at a mean age of 7 years controlled for maternal asthma and sex.