

Exhaled nitric oxide, lung function, and exacerbations in wheezy infants and toddlers

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Background: There are limited data assessing the relationship between fraction of exhaled nitric oxide and lung function or exacerbations in infants with recurrent wheezing.

Objectives: In a longitudinal pilot study of children less than 2 years old, we assessed whether baseline fraction of exhaled nitric oxide was associated with lung function, bronchodilator responsiveness, changes in lung function, or subsequent exacerbations of wheezing.

Methods: Forced expiratory flows and volumes using the raised-volume rapid thoracic compression method were measured in 44 infants and toddlers (mean age, 15.7 months) with recurrent wheezing. Single-breath exhaled nitric oxide (SB-eNO) was measured at 50 mL/s. Lung function was again measured 6 months after enrollment.

Results: At enrollment, forced expiratory volume in 0.5 seconds (FEV_{0.5}), forced expiratory flow at 25% to 75% of expiration (FEF₂₅₋₇₅), and forced expiratory flow at 75% of expiration (FEF₇₅) z scores for the cohort were significantly less than zero. There was no correlation between enrollment SB-eNO levels and enrollment lung function measures. SB-eNO levels were higher in infants with bronchodilator responsiveness (46.1 vs 23.6 ppb, $P < .001$) and was associated with a decrease in FEV_{0.5} ($r = -0.54$, $P = .001$), FEF₂₅₋₇₅ ($r = -0.6$, $P < .001$), and FEF₇₅ ($r = -0.55$, $P = .001$) over 6 months. A 10-ppb increase in SB-eNO level was associated with a 0.4-point z score decrease in FEV_{0.5}, a 0.4-point z score decrease in FEF₂₅₋₇₅, and a 0.42-point z score decrease in FEF₇₅. SB-eNO level was superior to lung function and bronchodilator responsiveness in predicting subsequent wheezing treated with systemic steroids.

Conclusions: SB-eNO level might predict changes in lung function and risk of future wheezing and holds promise as a biomarker to predict asthma in wheezy infants and toddlers. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Exhaled nitric oxide, fraction of exhaled nitric oxide, recurrent wheezing, infants, pulmonary function, raised-volume rapid thoracic compression

Eighty percent of children with persistent asthma are symptomatic by age 6 years, with more than half being symptomatic by age 3 years.¹ There is evidence that children with an early onset of persistent asthma have diminished lung function by age 6 years,² suggesting that early intervention might be important to alter the natural course of asthma. However, children younger than 3 years with asthma coexist with a larger group of children with recurrent transient wheezing. Episodes of recurrent wheezing affect 20% to 30% of infants and toddlers yet resolve in at least 50% of these children by age 6 years.² Clinicians generally cannot distinguish young children with transient wheezing from those with early persistent asthma. Biomarkers to predict asthma in wheezy infants and toddlers are needed to assist in the timely diagnosis and treatment of asthma. National Heart, Lung, and Blood Institute guidelines recommend daily inhaled corticosteroid therapy for children younger than 5 years who have had more than 3 episodes of wheezing and epidemiologic risk factors for asthma,³ yet many parents and clinicians are reluctant to chronically treat young children with inhaled steroids if the diagnosis of asthma is in doubt.

The fraction of exhaled nitric oxide (F_{ENO}) is a proposed biomarker of airway inflammation in patients with asthma.⁴ F_{ENO} has repeatedly been shown to be increased in adults and children with allergic asthma⁵ but has been minimally studied in infants and toddlers. In adults and school-aged children with atopic asthma, F_{ENO} is correlated with sputum and bronchoalveolar eosinophil counts, peak flow variability, and bronchial reactivity.⁶⁻¹³ F_{ENO} decreases after treatment with systemic or inhaled corticosteroids in adults and school-aged children with asthma^{14,15} and has been shown to have a superior diagnostic accuracy for asthma than conventional diagnostic approaches, including spirometry.¹⁶⁻¹⁹ In adults F_{ENO} measurement was shown to be a superior predictor of steroid responsiveness than spirometry, bronchodilator responsiveness, methacholine challenge, or adenosine challenge.²⁰

A flow-regulated single-breath exhaled nitric oxide (SB-eNO) measurement technique during infant lung function testing using the raised-volume rapid thoracoabdominal compression (RVRTC) technique has been described.^{21,22} However, the relationship between F_{ENO} and lung function has been minimally studied in infants and toddlers, and there are no reports of longitudinal data assessing the association between F_{ENO} and subsequent changes in lung function in this age group. We recruited a cohort of infants and toddlers with recurrent episodes of wheezing

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Abbreviations used

CV:	Coefficient of variation
FEF ₂₅₋₇₅ :	Forced expiratory flow 25% to 75% of expiration
FEF ₇₅ :	Forced expiratory flow at 75% of expiration
F _{ENO} :	Fraction of exhaled nitric oxide
FEV _{0.5} :	Forced expiratory volume in 0.5 seconds
FVC:	Forced vital capacity
IPL:	InSpire Infant Pulmonary Lab
ROC:	Receiver operating characteristic
RVRTC:	Raised-volume rapid thoracic compression
SB-eNO:	Single-breath exhaled nitric oxide

to prospectively evaluate associations between SB-eNO level and lung function, as well as exacerbations of wheezing over time in a longitudinal pilot study. We hypothesized that SB-eNO levels would be associated with measures of lung function, bronchodilator responsiveness, and subsequent exacerbations of wheezing. Some of these results have been reported in abstract form.²³

METHODS**Subjects**

Children 6 to 24 months of age with 3 or more episodes of physician-diagnosed wheezing treated with bronchodilators or corticosteroids were recruited for a single-center longitudinal study. Subjects with birth at less than 36 weeks' gestation, congenital heart disease, dysphagia, severe gastroesophageal reflux, or upper airway obstruction were excluded. Systemic or inhaled corticosteroid treatment was not permitted for 3 weeks before baseline lung function and F_{ENO} measurement. Thereafter, corticosteroid treatment was at the discretion of the subject's primary care or emergency department provider, who did not have access to F_{ENO} data. Written consent was obtained from parents of subjects. The study was approved by the Seattle Children's Hospital Institutional Review Board.

Study visits

Study visits occurred a minimum of 3 weeks after resolution of an upper or lower respiratory tract infection or acute exacerbation of wheezing. At enrollment, medical history was reviewed, length and weight were measured with a calibrated stadiometer and digital scale, SB-eNO levels were measured, and lung function testing was performed. Between enrollment and follow-up visits, parents completed monthly symptom and medication diaries that recorded wheezing episodes lasting 3 or more days treated with at least a bronchodilator, use of systemic (oral or intravenous) corticosteroids, and use of inhaled corticosteroids. Diaries were returned to study personnel on a monthly basis. Interval medical history and medication use were reviewed, and lung function tests were repeated at a follow-up visit 6 months after enrollment.

Lung function measurements

Forced expiratory volumes and flows (forced vital capacity [FVC], forced expiratory volume in 0.5 seconds [FEV_{0.5}], forced expiratory flow 25% to 75% of expiration [FEF₂₅₋₇₅], and forced expiratory flow at 75% of expiration [FEF₇₅]) were measured by using the RVRTC technique according to American Thoracic Society/European Respiratory Society guidelines for raised-volume forced expirations in infants²⁴ by using the InSpire Infant Pulmonary Lab (IPL; nSpire Health, Inc, Longmont, Colo). Additional details on the methods for obtaining these measurements are provided in the Methods section of this article's Online Repository at www.jacionline.org. Lung function tests were performed at least 3 weeks after resolution of acute exacerbations of wheezing or respiratory tract illnesses. Lung function parameters were analyzed as *z* scores calculated from published normative data.²⁵ Measurement of forced expiratory flows and volumes were repeated 10 minutes after administration of 4 puffs of albuterol. Bronchodilator responsiveness was defined as

12% or greater improvement in FEV_{0.5} or 25% or greater improvement in FEF₂₅₋₇₅.²⁶

Exhaled nitric oxide measurements

At least 3 SB-eNO measurements were performed for each subject immediately before lung function testing by using a method modified from previous descriptions.^{21,22} The subject's lungs were inflated to 30 cm H₂O 2 to 3 times to induce a respiratory pause, and then a thoracoabdominal compression was performed by using a jacket pressure of 25 cm H₂O. Exhaled gas was collected with a full facemask. Sliding valves triggered after the last lung inflation diverted the IPL expiratory flow through a mass flow controller (Aerocrine, Solna, Sweden) that maintained flow at 50 mL/s (± 5 mL/s) when expiratory pressure was between 8 and 30 cm H₂O (see Fig E1 in this article's Online Repository at www.jacionline.org) and bias flow away from the IPL circuit. Nitric oxide was measured with a Sievers NOA 280 chemiluminescence analyzer (GE Analytical Instruments, Boulder, Colo). Additional details on the methods for SB-eNO measurements are provided in the Methods section of this article's Online Repository.

Statistical analysis

Forty subjects were estimated to provide 80% power to identify SB-eNO level as a significant predictor of FEV_{0.5} if the SB-eNO level accounted for 15% or greater of the total variance in FEV_{0.5} (ie, $r^2 = 0.15$). SB-eNO and lung function data were determined to be normally distributed by using the D'Agostino and Pearson and Kolmogorov-Smirnov tests.

The percentage coefficient of variation (CV%) was calculated for SB-eNO measurements from each subject. The mean intraindividual CV% and intraclass correlation coefficient for SB-eNO measurements were calculated. The Pearson coefficient was used to estimate correlations between enrollment SB-eNO and lung function measures. Multiple variable linear regression was used to assess associations between enrollment SB-eNO level, baseline subjects' characteristics, and changes in lung function during follow-up. A receiver operating characteristic (ROC) analysis was performed to determine the enrollment SB-eNO level that best predicted a decrease in FEV_{0.5}.

Multiple variable logistic regression was used to assess associations between enrollment SB-eNO level and exacerbations of wheezing (defined as an acute episode of wheezing lasting ≥ 3 days treated with albuterol and systemic corticosteroids for ≥ 3 days) during the follow-up period, adjusting for enrollment FEV_{0.5}, bronchodilator responsiveness, enrollment age, sex, family history of asthma, eczema, sustained inhaled corticosteroid treatment, and tobacco smoke exposure. ROC analyses were performed to determine the enrollment SB-eNO, FEV_{0.5}, and FEF₂₅₋₇₅ values that best predicted systemic corticosteroid treatment for exacerbations of wheezing during the follow-up period. By using the best cutoff points for SB-eNO level and FEV_{0.5}, the sensitivity, specificity, positive predictive value, and negative predictive value of enrollment SB-eNO level, FEV_{0.5}, bronchodilator responsiveness, and the combination of SB-eNO level with FEV_{0.5} or bronchodilator responsiveness were calculated. Data analyses were conducted with GraphPad Prism 4.0 software (GraphPad Software, Inc, La Jolla, Calif) and SPSS for Windows version 11.5 software (SPSS, Inc, Chicago, Ill). Additional details regarding statistical analysis are provided in the Methods section of this article's Online Repository.

RESULTS

Forty-seven infants and toddlers were recruited with a mean age of 15.6 months (SD, ± 5.2 months) at enrollment. Technically acceptable RVRTC flow-volume curves were obtained from 44 subjects at enrollment. Baseline characteristics of the cohort are presented in Table I. The mean FEV_{0.5}, FEF₂₅₋₇₅, and FEF₇₅ *z* scores for the cohort at enrollment were significantly less than zero (a *z* score equal to zero represents the mean value of published normative data). Four subjects were lost to follow-up, and 8 subjects who completed technically acceptable lung function measurements at enrollment were unable to do so at

TABLE I. Baseline characteristics of subjects completing lung function testing

	All subjects completing enrollment lung function testing	Subjects completing enrollment and follow-up lung function testing	Subjects completing only enrollment lung function testing
Subjects (n)	44	32	12
Sex (male)	29 (66%)	22 (69%)	7 (58%)
Race (white)	32 (73%)	26 (81%)	6 (50%)
Mean age at enrollment (mo)*	15.7 ± 5.2	15.2 ± 4.9	15.5 ± 5.4
Eczema (physician diagnosed and steroid treated)	16 (36%)	10 (31%)	6 (50%)
Family history of asthma (first-degree relative)	23 (52%)	14 (44%)	9 (75%)
Environmental tobacco smoke exposure	12 (27%)	9 (28%)	3 (25%)
Prior treatment with systemic steroids for wheezing	33 (75%)	25 (78%)	8 (67%)
History of ED visit for wheezing	33 (75%)	24 (75%)	9 (75%)
History of hospitalization for wheezing	16 (36%)	10 (31%)	6 (50%)
History of wheezing apart from viral infections	16 (36%)	12 (38%)	4 (33%)
Enrollment SB-eNO (ppb)*	28.7 ± 16.1	27.4 ± 15.3	31.2 ± 19.5
Enrollment FVC (z score)†	−0.04 (−0.42 to 0.35)	−0.01 (−0.51 to 0.49)	−0.24 (−0.65 to 0.17)
Enrollment FEV _{0.5} (z score)†	−0.42 (−0.78 to −0.07)	−0.41 (−0.80 to −0.04)	−0.21 (−0.46 to −0.05)
Enrollment FEF ₂₅₋₇₅ (z score)†	−0.67 (−1.10 to −0.25)	−0.82 (−1.33 to −0.30)	−0.45 (−1.15 to 0.27)
Enrollment FEF ₇₅ (z score)†	−0.72 (−1.14 to −0.31)	−0.84 (−1.31 to −0.43)	−0.56 (−1.22 to −0.12)

ED, Emergency department.

*Data are presented as means ± SDs.

†Data are presented as means ± 95% CIs.

TABLE II. Associations between enrollment SB-eNO levels and changes in lung function (n = 32 subjects)

	Δ FEV _{0.5} z score		Δ FEF ₂₅₋₇₅ z score		Δ FEF ₇₅ z score	
	β Coefficient	P value	β Coefficient	P value	β Coefficient	P value
Enrollment SB-eNO (10-ppb increments)	−0.40	.01	−0.40	.01	−0.42	.003
Eczema (yes/no; physician diagnosed and steroid treated)	−0.34	.3	−0.73	.1	−0.3	.4
Sustained ICS use during follow-up (yes/no)	0.84	.1	1.3	.03	1.1	.04
Sex	−0.08	.8	−0.11	.8	0.07	.9
Age at enrollment (1-mo increments)	−0.03	.4	−0.06	.2	−0.04	1.0
FH asthma (yes/no)	0.24	.5	0.32	.5	−0.04	.9
Tobacco smoke exposure (yes/no)	−0.56	.2	−0.62	.2	−0.47	.3

The β coefficient represents change in lung function z score associated with each variable or increment of a variable (eg, 10-ppb increments of SB-eNO). Boldfacing denotes a statistically significant association.

FH, Family history; ICS, inhaled corticosteroids.

the 6-month follow-up visit (see Fig E2 in this article's Online Repository at www.jacionline.org). There were no significant differences in baseline characteristics between the subjects completing lung function testing at both visits and the subjects who did not (Table I). Enrollment of subjects was evenly distributed across seasons (see Fig E3 in this article's Online Repository at www.jacionline.org). Eight of the 32 subjects completing lung function testing at both visits used inhaled corticosteroids on a sustained basis during the follow-up period.

Enrollment visit SB-eNO levels were measured in 45 subjects, including all 44 infants with enrollment lung function measurements. The mean intraindividual CV% of SB-eNO level for the cohort was 6.2% (range, 0.9% to 13.1%), and the intraclass correlation coefficient was 0.92. The mean enrollment SB-eNO level for the cohort was 28.7 ppb (range, 3-76 ppb). There was a small but significant association between increasing age and enrollment SB-eNO level (see Table E1 in this article's Online Repository at www.jacionline.org). A history of eczema was also associated with enrollment SB-eNO level. There was no association between SB-eNO levels and sex, family history of asthma, environmental tobacco smoke exposure, wheezing apart

from viral respiratory tract infections, hospitalization for wheezing, or oral steroid treatment.

There was no correlation between enrollment SB-eNO level and enrollment FVC ($r = -0.07$, $P = .7$), FEV_{0.5} ($r = 0.05$, $P = .7$), FEF₂₅₋₇₅ ($r = 0.2$, $P = .2$), or FEF₇₅ ($r = 0.2$, $P = .1$) before or after adjustment for sex, eczema, family history of asthma, and tobacco smoke exposure by using multiple variable linear regression analyses.

Enrollment SB-eNO levels were significantly higher in subjects whose lung function subsequently decreased between enrollment and the 6-month follow-up visit (see Fig E4 in this article's Online Repository at www.jacionline.org). There were significant correlations between enrollment SB-eNO levels and decreases in FEV_{0.5} ($r = -0.54$, $P = .001$), FEF₂₅₋₇₅ ($r = -0.6$, $P < .001$), and FEF₇₅ ($r = -0.55$, $P = .001$; see Fig E5 in this article's Online Repository at www.jacionline.org). After adjustment for age at enrollment, sex, family history of asthma, history of eczema, sustained use of inhaled corticosteroids during follow-up, and environmental tobacco smoke exposure, each additional 10-ppb increment in enrollment SB-eNO level (eg, 0-10, 11-20, and 21-30 ppb) was associated with a

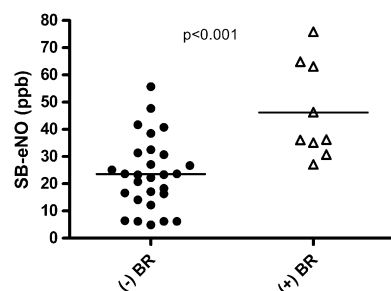


FIG 1. Relationship between SB-eNO levels and bronchodilator responsiveness (BR). BR is defined as a 12% or greater improvement in FEV_{0.5} or a 25% or greater improvement in FEF₂₅₋₇₅ after administration of albuterol (open triangles).

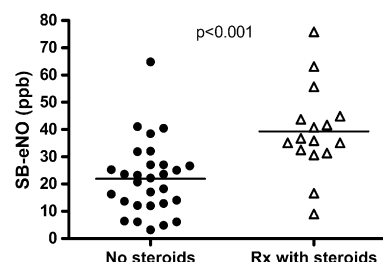


FIG 2. Enrollment SB-eNO levels in subjects treated with systemic (oral or intravenous) steroids for an acute episode of wheezing during the follow-up period (open triangles) versus SB-eNO levels in subjects not treated with systemic steroids (solid circles).

0.4-point z score decrease in FEV_{0.5}, a 0.4-point z score decrease in FEF₂₅₋₇₅, and a 0.42-point z score decrease in FEF₇₅ (Table II). Sustained use of inhaled corticosteroids was associated with an increase in forced expiratory flows. An ROC analysis showed that an enrollment SB-eNO level of greater than 29 ppb predicted a decrease in FEV_{0.5} during follow-up with a sensitivity of 67% and a specificity of 86%, with an area under the curve of 0.89 (95% CI, 0.77-1.0; see Fig E6 in this article's Online Repository at www.jacionline.org).

Thirty-seven subjects completed forced expiratory volume and flow measurements after administration of albuterol at either enrollment or follow-up visits. SB-eNO levels were significantly higher in subjects with bronchodilator responsiveness than in subjects without bronchodilator responsiveness (46.1 vs 23.6 ppb, $P < .001$, Fig 1).

During the follow-up period, 16 subjects experienced an exacerbation of wheezing for which a primary care or emergency department provider treated them with systemic corticosteroids for 3 or more days. Enrollment SB-eNO levels were significantly higher in subjects who were subsequently treated with systemic steroids for an acute episode of wheezing than in those who were not (39.3 vs 22 ppb, $P < .001$, Fig 2). The odds of an exacerbation of wheezing treated with systemic steroids during the follow-up period increased more than 3-fold (odds ratio, 3.3; 95% CI, 1.2-10.1) for every 10-ppb increase in enrollment SB-eNO level (see Table E2 in this article's Online Repository at www.jacionline.org). An ROC analysis showed that an enrollment SB-eNO level of greater than 29 ppb predicted treatment with systemic corticosteroids for wheezing exacerbations during follow-up with a sensitivity of 88%, a specificity of 82%, and an area under the curve of 0.83 (95% CI, 0.69-0.96; see Fig E7). The sensitivity, specificity, positive predictive value, and negative predictive value of enrollment SB-eNO levels for wheezing exacerbations during follow-up was superior to enrollment FEV_{0.5}, enrollment FEF₂₅₋₇₅, or bronchodilator responsiveness (Table III). The predictive value of a combination of enrollment SB-eNO level with enrollment lung function measures or bronchodilator responsiveness was not superior to enrollment SB-eNO level alone.

After excluding subjects who used inhaled corticosteroids during the study ($n = 8$), there was a significant direct correlation between enrollment and follow-up SB-eNO levels ($r = 0.5$, $P = .01$, see Fig E8 in this article's Online Repository at www.jacionline.org), with slightly higher SB-eNO levels at the follow-up visit (mean, 29.3 vs 22.5 ppb; $P = .01$; see Fig E9 in this article's Online Repository at www.jacionline.org). There was no correlation between intraindividual changes in SB-eNO

levels and changes in z scores for FEV_{0.5} or forced expiratory flows between enrollment and follow-up visits (see Fig E10 in this article's Online Repository at www.jacionline.org).

DISCUSSION

In a cohort of infants and toddlers with a history of recurrent episodes of wheezing, we found that enrollment SB-eNO levels were not associated with baseline forced expiratory volumes or flows. However, enrollment SB-eNO levels were significantly higher in subjects with a subsequent decrease in lung function than in subjects with an increase in lung function over 6 months of follow-up. SB-eNO levels were significantly higher in subjects with bronchodilator responsiveness. A higher enrollment SB-eNO level was associated with an increased risk of treatment with systemic corticosteroids for acute exacerbations of wheezing during the 6-month follow-up period. Furthermore, enrollment SB-eNO level was superior to enrollment lung function and bronchodilator responsiveness as a predictor of wheezing exacerbations during follow-up. Finally, we have shown that SB-eNO measurements during RVRTC maneuvers are feasible and highly reproducible using an automated technique to control expiratory and standardized criteria to determine SB-eNO plateau acceptability.

Other groups have reported SB-eNO measurements in infants and toddlers obtained during RVRTC maneuvers against an expiratory resistance.^{22,27} Wildhaber et al²² demonstrated, by measuring nasal end-tidal CO₂, that this technique results in closure of the velum, thereby minimizing contamination of SB-eNO measurements by nasal nitric oxide. Studies by both Wildhaber et al²² and Franklin et al²⁷ found that SB-eNO levels were higher in wheezy infants than in healthy infants. Our findings are consistent with those of Wildhaber et al,²² who reported no association between SB-eNO levels and FEV_{0.5} in a cross-sectional study of wheezy infants, as well as with results from studies of older children in which no association between F_{ENO} and lung function was found at a single time point.^{28,29} SB-eNO levels in our subjects were similar to results reported from wheezy infants by Wildhaber et al²² using an expiratory flow of 50 mL/s and Franklin et al²⁷ using an expiratory flow of 40 mL/s. To our knowledge, our study is the first to perform SB-eNO measurements using an automated method to regulate expiratory flow, apply rigorous *a priori* criteria to the determination of the SB-eNO plateau during single-breath measurements, and obtain longitudinal lung function measurements in a cohort of infants and toddlers with recurrent physician-diagnosed wheezing.

TABLE III. Sensitivity, specificity, PPV, and NPV of enrollment SB-eNO level, FEV_{0.5}, FEF₂₅₋₇₅, and BR for wheezing exacerbations treated with systemic corticosteroids during follow-up

	Cutoff	Sensitivity	Specificity	PPV	NPV
SB-eNO level	>29 ppb	88%	82%	70%	92%
Enrollment FEV _{0.5}	<i>z</i> Score < −0.53	31%	59%	29%	61%
Enrollment FEF ₂₅₋₇₅	<i>z</i> Score < −1.0	31%	58%	29%	60%
BR	≥12% Improvement in FEV _{0.5} or ≥25% improvement in FEF ₂₅₋₇₅	33%	78%	44%	69%
SB-eNO level and FEV _{0.5}	>29 ppb and <i>z</i> score < −0.53	25%	89%	57%	68%
SB-eNO level and FEF ₂₅₋₇₅	>29 ppb and <i>z</i> score < −1.0	38%	93%	75%	73%
SB-eNO level >29 ppb and BR	>29 ppb and BR	33%	91%	67%	72%

BR, Bronchodilator responsiveness; NPV, negative predictive value; PPV, positive predictive value.

Follow-up of this cohort into school age will be essential to determine whether SB-eNO level predicts which children will have asthma and which will experience resolution of wheezing. It is also possible that the intraindividual change in SB-eNO levels between infancy and school age might be more predictive of asthma than a single measurement. Findings from the Tucson Children's Respiratory Study² suggest that infants with transient wheezing have diminished lung function shortly after birth that might predispose them to wheeze with viral infections yet experience improvement in lung function by age 6 years, whereas infants who continue to wheeze into the school-age years have normal lung function in early infancy yet experience a decrease in lung function by age 6 years. It is possible that in our cohort a low SB-eNO level might identify a transient wheezing phenotype, whereas an increased SB-eNO level, which in older children and adults is associated with lower airway eosinophilic inflammation, might identify infants and toddlers at risk for atopic asthma. In adults and school-aged children F_{ENO} measurement has been shown to be a superior adjunctive diagnostic test for asthma than conventional objective diagnostic strategies, including spirometry, peak flow measurement, exercise challenge, or methacholine challenge.¹⁶⁻¹⁹ The lower age limit at which this applies has yet to be determined.

Infants and toddlers with recurrent wheezing are a heterogeneous group. The majority of school-aged asthmatic subjects experience their initial symptoms during the first several years of life, typically during viral respiratory tract infections. However, the majority of wheezy infants and toddlers do not have asthma but rather wheeze for a variety of anatomic reasons, pathophysiologic reasons, or both, including small airway caliber, postviral airway inflammation and injury, tracheomalacia, and dysphagia. Episodic wheezing resolves in most of these children by school age, leading to the epidemiologic characterization of this phenomenon as transient wheezing. Discriminating between infants with transient wheezing and infants with asthma is a major challenge for clinicians. A predictive biomarker for asthma does not exist for infants or toddlers, greatly complicating efforts to prevent thousands of asthma exacerbations, emergency department visits, and hospitalizations in young children each year.³⁰ Castro-Rodriguez et al³¹ proposed an index of epidemiologic risk factors to assist in predicting asthma in wheezy infants and toddlers; however, this index has not been assessed in a prospective manner, and when applied to children in the cohort from which it was developed, it had a positive predictive value for asthma at age 8 years of only 29%. Our data suggest that SB-eNO measurement might hold promise as an adjunctive diagnostic test for asthma in wheezy infants and toddlers. We found that SB-eNO levels were a superior predictor of subsequent

exacerbations of wheezing than measurement of forced expiratory flows and volumes or bronchodilator responsiveness.

Furthermore, the combination of lung function measurements and SB-eNO measurement was not superior to SB-eNO measurement alone as a predictor of subsequent exacerbations of wheezing. Our results are consistent with the recent finding by Beigelman et al³² in a subgroup of preschool-aged children with recurrent wheezing from the Acute Intervention Management Strategies clinical trial of the Childhood Asthma Research and Education Network that F_{ENO} was associated with subsequent respiratory tract illnesses. Diagnosing asthma at an early age with an objective test, such as F_{ENO} measurement, could greatly facilitate more targeted use of asthma therapies, prevent undertreatment and overtreatment with corticosteroids, and reduce preventable emergency department visits and hospitalization for asthma exacerbations.

There are several limitations to this study. First, change in lung function was estimated from only 2 measurements separated by 6 months. Infant lung function testing determined with the RVRTC method is time consuming, highly specialized, and requires sedation, limiting the feasibility of more frequent assessment. We were careful to only obtain measurements when subjects were at their baseline without acute respiratory tract infections or exacerbations within 3 weeks of testing. Multiple variable linear regression estimated decreases of 0.4 points in *z* scores for FEV_{0.5}, FEF₂₅₋₇₅, and FEF₇₅ for each 10-ppb incremental increase in enrollment SB-eNO level. Data from large longitudinal pediatric cohort studies suggest that decreases in lung function in asthmatic subjects occur early in childhood, with little decrease from age 6 years onward.³³⁻³⁵ Although our results clearly demonstrate an association between F_{ENO} and change in lung function, we do not believe that the magnitude of decrease we observed over 6 months of follow-up can be extrapolated to predict a sustained rate of decrease over many years.

Another limitation is that only 73% of subjects completed acceptable lung function testing at both time points. Of the 87 attempts to perform RVRTC lung function measurements at both visits, 76 (87%) were successful. Although a higher percentage of subjects completing follow-up measurements would be preferable, the failure rate of sedation with chloral hydrate increases with age.³⁶ Chloral hydrate is used to sedate infants for lung function testing by nearly all centers using the RVRTC method, and our overall success rate is comparable with those of other centers.^{37,38} Although lung function measurements in our subjects were expressed and analyzed as *z* scores based on published normative data,²⁵ our study lacks a within-center healthy control group. US regulations and ethical guidelines for research involving children require that subjects not be exposed to procedures involving

greater than minimal risk without the prospect of direct benefit to individual subjects.³⁹ The interpretation of these guidelines by the majority of pediatric institutional review boards in the United States precludes the use of infant pulmonary function testing in healthy infants given the need for sedation without the prospect of direct benefit to normal subjects.

Finally, an important limitation of this pilot study is that we did not perform skin or radioallergosorbent testing of subjects to food or inhalant allergens at enrollment. Kulig et al⁴⁰ demonstrated in a birth cohort that development of allergic sensitization to inhalant allergens occurs mostly beyond age 2 years; however, ideally, we would have objective data characterizing the sensitization status of our subjects at enrollment. Future studies evaluating the predictive value of F_{ENO} must include prospective serial measures of allergic sensitization. We chose the very general enrollment criterion of 3 or more episodes of physician-diagnosed and treated wheezing episodes because we believed that assessing the predictive value of SB-eNO level for changes in lung function and future wheezing in such a population was a relevant clinical question commonly faced by pediatric practitioners, as well as academic researchers. Until this and other cohorts of wheezy infants and toddlers can be followed into the school years, it is important not to overinterpret our results by concluding that SB-eNO levels predict asthma in this population.

To be consistent with American Thoracic Society recommendations for F_{ENO} measurements in older children and adults,⁴¹ we chose an expiratory flow of 50 mL/s to obtain SB-eNO measurements. However, 50 mL/s might not be an ideal flow to measure F_{ENO} in infants and toddlers given their small airway caliber. A positive association between age and F_{ENO} has been reported in several large studies of healthy school-aged children.⁴²⁻⁴⁴ Possible mechanisms for the age dependency of F_{ENO} include increased lung volume and airway surface area, changes in nitric oxide diffusion coefficients, changes in nitric oxide synthase expression with age, or changes in S-nitrosoglutathione kinetics.⁴⁵ In addition, a fixed flow of 50 mL/s represents a higher relative flow in young subjects with smaller airways compared with that seen in older subjects with larger airways, resulting in lower F_{ENO} because of the flow dependence of F_{ENO} . Future studies in this age group should also assess F_{ENO} at several lower flows.

In conclusion, we have shown that baseline SB-eNO measurements in a cohort of infants and toddlers with a history of recurrent episodes of physician-diagnosed wheezing did not correlate with baseline forced expiratory volumes or flows. However, higher enrollment SB-eNO levels were associated with a decrease in lung function and an increased risk of acute exacerbations of wheezing treated with systemic corticosteroids during the follow-up period. Furthermore, we found that SB-eNO levels were higher in subjects with bronchodilator responsiveness. Although follow-up into the school-aged years with a larger sample size will be essential to definitely evaluate its predictive value for asthma in wheezy infants and toddlers, these data suggest that SB-eNO measurement in this population is a promising objective biomarker that might prove very useful clinically but might also increase our understanding of the evolution of asthma in young children.

Clinical Implications: In wheezy infants SB-eNO levels are associated with subsequent changes in lung function over time, bronchodilator responsiveness, and subsequent exacerbations of wheezing.

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METHODS

Lung function measurements

Forced expiratory volumes and flows (FVC, FEV_{0.5}, FEF₂₅₋₇₅, and FEF₇₅) were measured with the RVRTC technique according to American Thoracic Society/European Respiratory Society guidelines for increased volume forced expirations in infants^{E1} with the IPL. Testing was performed while subjects were sedated after receiving chloral hydrate (100 mg/kg). Bronchodilators were not administered to subjects within 12 hours of testing. A lung inflation pressure of 30 cm H₂O was used for RVRTC maneuvers. Jacket pressure was sequentially increased until no further increase in forced expiratory flows and volumes was obtained. At least 2 technically satisfactory and reproducible (within 10%) maximal RVRTC expiratory flow-volume curves were collected. Lung function tests were performed at least 3 weeks after resolution of acute exacerbations of wheezing or respiratory illnesses. Lung function parameters were analyzed as *z* scores calculated from published normative data.^{E2} Measurement of forced expiratory flows and volumes were repeated 10 minutes after administration of 4 puffs of albuterol. Bronchodilator responsiveness was defined as 12% or greater improvement in FEV_{0.5} or 25% or greater improvement in FEF₂₅₋₇₅.^{E3}

Exhaled nitric oxide measurements

At least 3 SB-eNO measurements were performed in each subject immediately before lung function testing using a method modified from previous descriptions.^{E4,E5} The subject's lungs were inflated to 30 cm H₂O 2 to 3 times to induce a respiratory pause, and then a thoracoabdominal compression was performed with a jacket pressure of 25 cm H₂O. Exhaled gas was collected with a full facemask. Sliding valves triggered after the last lung inflation diverted the IPL expiratory flow through a mass flow controller (Aerocrine) that maintained flow at 50 mL/s ($\pm$ 5 mL/s) when expiratory pressure was between 8 and 30 cm H₂O (Fig E1) and bias flow away from the IPL circuit. Nitric oxide was measured with a Sievers NOA 280 chemiluminescence analyzer (GE Analytical Instruments). Nitric oxide was sampled from the expiratory circuit at a flow of 300 mL/min. An SB-eNO plateau was determined if the total exhalation duration was 3 seconds or longer with flow of 50 mL/s ($\pm$ 5 mL/s) and the F_{ENO} plateau duration was 1 or more seconds with no point within the plateau deviating by $\pm$ 5 ppb or 10% of the plateau mean (Fig E1). SB-eNO levels for each subject were calculated as the mean of at least 3 acceptable plateau values. Ambient nitric oxide was scrubbed continuously from the IPL bias flow (to <3 ppb) with a Sievers filter.

Statistical analysis

Forty subjects were estimated to provide 80% power to identify SB-eNO level as a significant predictor of FEV_{0.5} if the SB-eNO level accounted for 15% or more of the total variance in FEV_{0.5} (ie, r^2 = 0.15). SB-eNO and lung function data were determined to be normally distributed by using the D'Agostino and Pearson and Kolmogorov-Smirnov tests.

The CV% was calculated for SB-eNO measurements from each subject. The mean intraindividual CV% and intraclass correlation coefficient for SB-eNO measurements were calculated. The Pearson coefficient was used to

estimate correlations between enrollment SB-eNO and lung function measures. Multiple variable linear regression was used to assess associations between enrollment SB-eNO level and the following variables assessed at enrollment: sex, enrollment age, environmental tobacco smoke exposure (parental report), history of physician-diagnosed eczema treated with corticosteroids, family history (first-degree relative) of physician-diagnosed asthma, wheezing apart from viral respiratory tract infections, hospitalization for wheezing, and treatment with oral steroids.

Paired *t* tests were used to assess changes in SB-eNO levels between enrollment and follow-up visits. Unpaired *t* tests were used to compare enrollment SB-eNO levels of those subjects with an increase in lung function *z* scores (FEV_{0.5}, FEF₂₅₋₇₅, or FEF₇₅) with SB-eNO levels of those subjects with a decrease in lung function *z* scores between enrollment and follow-up visits and to compare enrollment SB-eNO levels between subjects with or without bronchodilator responsiveness or systemic corticosteroid treatment during follow-up. Multiple variable linear regression was used to assess associations between enrollment SB-eNO levels and changes in lung function, adjusting for enrollment age, sex, family history of asthma, eczema (physician-diagnosed and corticosteroid treated), sustained ($\geq$ 3 months during follow-up) inhaled corticosteroid treatment, and tobacco smoke exposure. An ROC analysis was performed to determine the enrollment SB-eNO level that best predicted a decrease in FEV_{0.5}. Multiple variable logistic regression was used to assess associations between enrollment SB-eNO levels and exacerbations of wheezing (defined as an acute episode of wheezing lasting $\geq$ 3 days treated with albuterol and systemic corticosteroids for $\geq$ 3 days) during the follow-up period, adjusting for enrollment FEV_{0.5}, bronchodilator responsiveness, enrollment age, sex, family history of asthma, eczema, sustained inhaled corticosteroid treatment, and tobacco smoke exposure. ROC analyses were performed to determine the enrollment SB-eNO, FEV_{0.5}, and FEF₂₅₋₇₅ values that best predicted systemic corticosteroid treatment for exacerbations of wheezing during the follow-up period. By using the best cutoff points for SB-eNO level and FEV_{0.5}, the sensitivity, specificity, positive predictive value, and negative predictive value of enrollment SB-eNO level, FEV_{0.5}, bronchodilator responsiveness, and the combination of SB-eNO with FEV_{0.5} or bronchodilator responsiveness were calculated. Data analyses were conducted with GraphPad Prism 4.0 and SPSS for Windows version 11.5 software.

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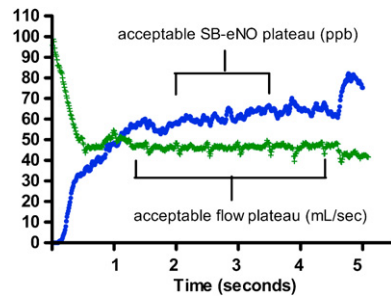


FIG E1. Superimposed SB-eNO and expiratory flow tracings from a single-breath expiratory maneuver. The *blue tracing* illustrates an acceptable SB-eNO plateau, and the *green tracing* illustrates an acceptable expiratory flow plateau.

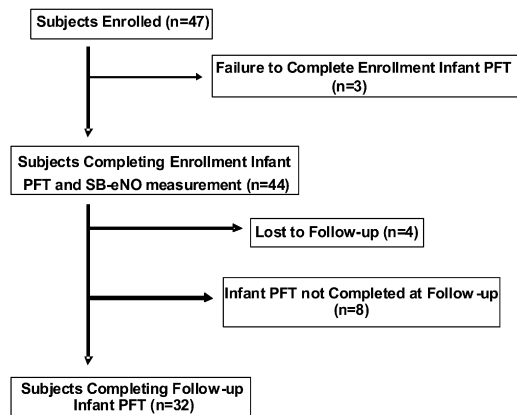


FIG E2. Flowchart of the patients in the study. Of the 8 subjects who did not complete infant pulmonary function testing (*PFT*) at visit 2, 4 could not be adequately sedated, and PFT results from 4 subjects did not meet American Thoracic Society/European Respiratory Society guidelines^{E2} for acceptable infant spirometry because of glottic closures.

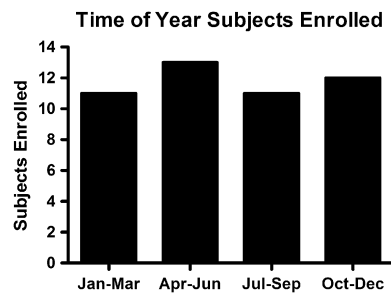


FIG E3. Plot of number of subjects enrolled by time of year in 3-month intervals based on seasons.

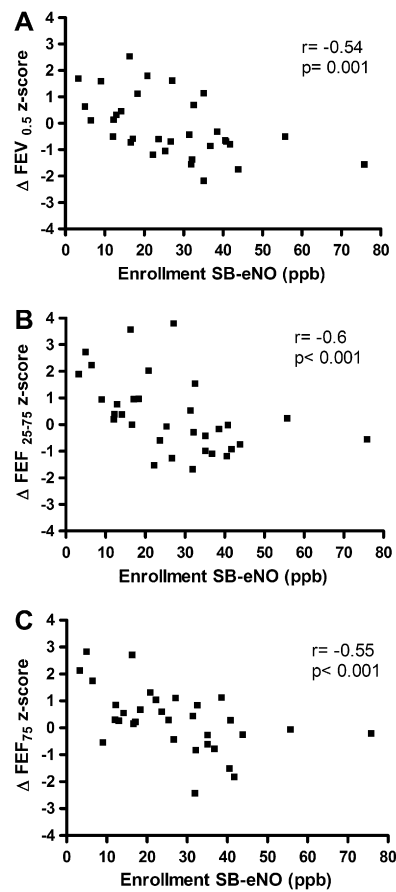


FIG E4. Enrollment SB-eNO levels in subjects based on subsequent change in $FEV_{0.5}$ (A), FEF_{25-75} (B), and FEF_{75} (C) z scores.

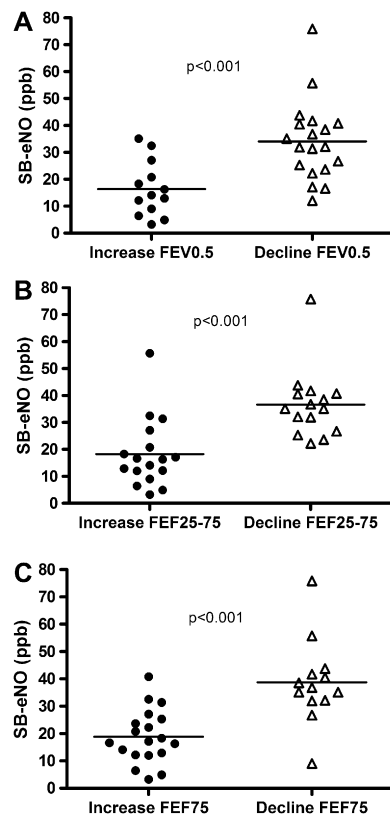


FIG E5. Correlation between enrollment SB-eNO level and change in FEV_{0.5} (A), FEF₂₅₋₇₅ (B), and FEF₇₅ (C) z scores during the 6-month follow-up period.

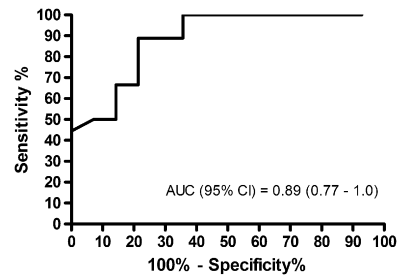


FIG E6. Area under the ROC curve (*AUC*) for enrollment SB-eNO level as a predictor for a decrease in $FEV_{0.5}$ during the study follow-up period.

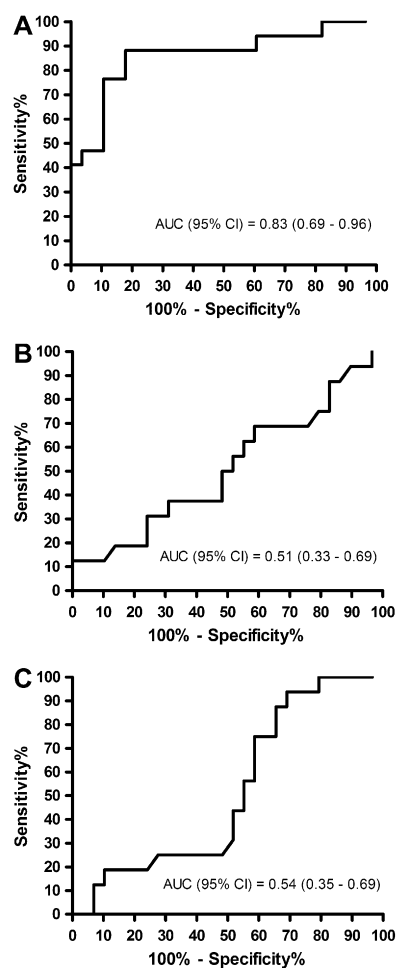


FIG E7. Area under the ROC curves (*AUC*) obtained for enrollment SB-eNO level (**A**), enrollment FEV_{0.5} (**B**), and enrollment FEF₂₅₋₇₅ (**C**) as predictors of wheezing exacerbations treated with systemic corticosteroids during follow-up.

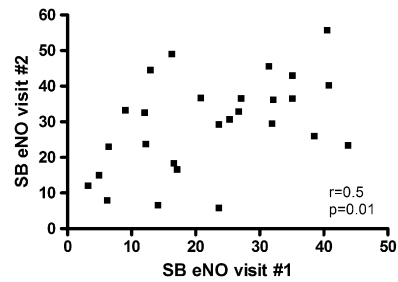


FIG E8. Correlation between SB-eNO levels at enrollment (visit 1) and the 6-month follow-up visits (visit 2), excluding subjects who used inhaled corticosteroids.

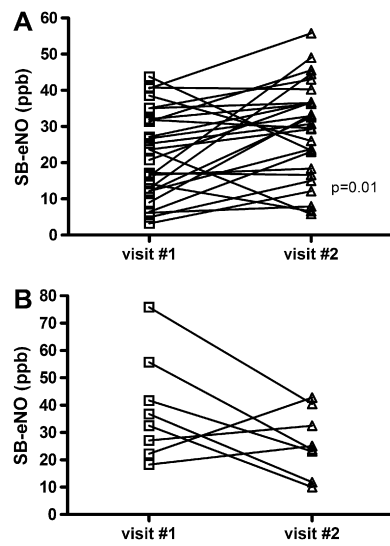


FIG E9. Change in SB-eNO levels between enrollment and follow-up visits in subjects who did not use inhaled corticosteroids (**A**) and those who did use inhaled corticosteroids (**B**) during the follow-up period.

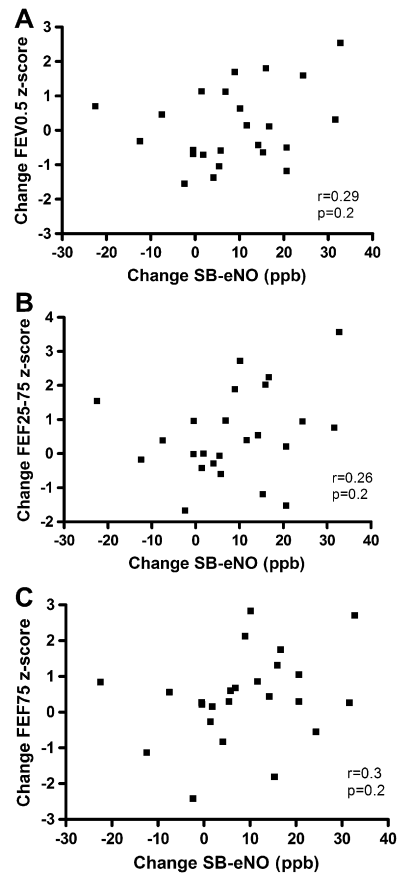


FIG E10. Correlation between change in SB-eNO levels and change in FEV_{0.5} (A), FEF₂₅₋₇₅ (B), and FEF₇₅ (C) z scores during the 6-month follow-up period. Subjects using inhaled corticosteroids during the follow-up period were excluded from analysis.

TABLE E1. Associations between baseline characteristics and enrollment SB-eNO level (n = 44 subjects)

	β Coefficient, difference in SB-eNO levels (1-ppb increments)	<i>P</i> value
Age at enrollment (1-mo increments)	1.5	.002
Sex	0.7	.9
Family history of asthma (yes/no)	−5.7	.3
Eczema (yes/no; physician diagnosed and steroid treated)	11.4	.03
Tobacco smoke exposure (yes/no)	−2.2	.7
History of wheezing apart from viral respiratory tract infections (yes/no)	7.6	.2
Hospitalization for wheezing (yes/no)	−4.6	.5
Treatment with oral steroids for wheezing (yes/no)	2.3	.7

Boldfacing denotes a statistically significant association.

TABLE E2. Adjusted odds ratios of wheezing exacerbations treated with systemic corticosteroids during follow-up (n= 44 subjects)

	OR	95% CI	P value
Enrollment SB-eNO (10-ppb increments)	3.3	1.2-10.1	.03
Enrollment FEV _{0.5} (0.5-point z score increments)	1.5	0.8-2.9	.2
Bronchodilator responsive (yes/no)	1.8	0.1-32	.7
Sustained ICS use during follow-up (yes/no)	2.4	0.2-38	.5
Eczema (yes/no; physician diagnosed and steroid treated)	2.5	0.2-29	.5
Sex	0.2	0.01-3.7	.3
Age at enrollment (1-mo increments)	1.1	0.9-1.5	.3
Family history of asthma (yes/no)	0.3	0.02-4.7	.4
Tobacco smoke exposure (yes/no)	0.7	0.1-6.8	.8

ICS, Inhaled corticosteroids; OR, odds ratio. Boldfacing denotes a statistically significant association.