
Atopic disease and exhaled nitric oxide in an unselected population of young adults

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Background: Several studies have reported elevated levels of fractional exhaled nitric oxide (FeNO) in atopic patients, particularly in asthmatic patients, suggesting that FeNO is a marker of bronchial inflammation. However, the independent influence of different atopic entities (eczema, allergic rhinitis, and asthma) on FeNO has never been studied in the general population.

Objective: To study the influence of a questionnaire-based diagnosis of atopic diseases and IgE and lung function measurements on FeNO levels.

Methods: This study was part of a follow-up on otitis media of a birth cohort of 1,328 children born in Nijmegen, the Netherlands, between September 1, 1982, and August 31, 1983. Within the birth cohort, the incidence of asthma, allergic rhinitis, and eczema was determined, and off-line FeNO, spirometry, and IgE measurements were performed at the age of 21 years.

Results: FeNO measurements were successfully performed in 361 participants. Median FeNO levels were significantly higher in those with vs without eczema (23.6 vs 18.0 ppb; $P < .0001$), those with vs without allergic rhinitis (20.7 vs 17.8 ppb; $P = .0001$), and those with vs without atopic asthma (23.3 vs 18.1 ppb; $P = .02$) but not in those with vs without asthma (20.8 vs 18.3 ppb; $P = .24$). Eczema, allergic rhinitis, smoking, sex, and atopic sensitization appeared to be independently associated with log FeNO in this population sample, whereas (atopic) asthma was not. No effect on FeNO levels was observed for lung function parameters.

Conclusion: Eczema, allergic rhinitis, and atopic status were all independently associated with elevated FeNO levels, whereas (atopic) asthma was not. This finding implies that future studies into the role of FeNO in asthma should consider the influence of atopic disease outside the lungs.

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INTRODUCTION

Although concentrations of fractional exhaled nitric oxide (FeNO) are highly variable in healthy populations, levels outside the normal range are associated with respiratory disease. Patients with asthma show increased levels of FeNO compared with healthy controls^{1–4} and so do patients with allergic rhinitis,^{4,5} chronic obstructive pulmonary disease,^{6,7} bronchiectasis,⁸ pulmonary sarcoidosis,⁹ active fibrosing alveolitis,¹⁰ and acute lung allograft rejection.¹¹ In contrast, low levels of FeNO have been reported in patients with primary ciliary dyskinesia and cystic fibrosis.^{12,13}

Most patients with asthma are sensitized to common aeroallergens. Besides airway hyperresponsiveness, many have symptoms of eczema or allergic rhinitis, conditions characterized by local activation of macrophages and eosin-

ophils. FeNO has been suggested to reflect allergic inflammatory activity of the airways, with FeNO levels depending on the degree of atopy.²

Although numerous studies have been conducted on patients with atopic asthma and atopic sensitization, the independent effects of different atopic entities (eczema, allergic rhinitis, and asthma) on FeNO levels have not been studied in an unselected population of adults so far. Several studies have described determinants of FeNO values in community-based samples of children.^{2,5,14–16} Determinants of FeNO have been described for young adults as well,¹⁷ but eczema was not taken into account in this study. We therefore studied the influence of atopic disease, IgE levels, and lung function on FeNO levels in a large birth cohort of young adults.

METHODS

Study participants

This study was part of a follow-up on otitis media of a birth cohort of 1,328 children born in Nijmegen, the Netherlands, between September 1, 1982, and August 31, 1983.¹⁸ In 2004, the home addresses of 1,055 of the original cohort members could be traced, and 693 individuals completed a questionnaire.¹⁹ These individuals were invited to undergo measurement of their FeNO, IgE, and lung function. All participants gave written informed consent, and the study was approved

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by the Medical Ethics Committee of the University Medical Centre Utrecht.

FeNO measurement

FeNO measurements were performed with standardized off-line bag collection with a dynamic flow restrictor.^{20,21} Study participants were instructed to inhale through a nitric oxide filter to remove ambient nitric oxide. Subsequently, they were asked to exhale through the dynamic flow restrictor, with a target mouth pressure of 15 cm H₂O. This approach resulted in a flow limitation of 50 mL/s, in accordance with recommendations of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) for FeNO measurement.²² Contamination of FeNO with nasal nitric oxide was prevented by exhalation against a positive mouth pressure to ensure closure of the soft palate. After 5 seconds of exhalation of dead space air, a 150-mL Mylar bag was filled. After a resting period of at least 30 seconds, the second bag was filled, according to the same procedure.

Within 8 hours, the collected air samples were analyzed using the NIOX system (Aerocrine AB, Stockholm, Sweden). FeNO values were expressed as parts per billion. Two air samples were taken from each bag. Data were reported as mean values from the collected samples. Calibration was performed routinely.

Determinants

Data on eczema, allergic rhinitis, and asthma were collected using the core questionnaire of the International Study of Asthma and Allergies in Childhood.²³ Eczema was defined as an itchy rash that had been coming and going for at least 6 months and affected particular skin sites (eg, folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes) in the last 12 months. Allergic rhinitis was defined as the presence of a runny or blocked nose in the last 12 months without having a cold or flu, accompanied by itchy-watery eyes and/or some interference with daily activities by this nose problem. Asthma was defined as the occurrence of wheezing in the last 12 months and/or a physician's diagnosis of asthma in that same period and/or current use of asthma medication. Atopic asthma was defined as asthma plus allergic sensitization (ie, positive Phadiatop result). Parental atopic disease was defined as the occurrence of asthma, allergic rhinitis, or eczema in at least 1 parent.

Data on other possible determinants of FeNO values were also collected, such as height, body weight, current use of asthma medication and inhaled corticosteroids (ICS), smoking behavior in the last year, recent colds, and lung function parameters. A recent cold was defined as a cold or flu in the last 4 weeks. Spirometry was performed using the Lilly pneumotachometer system (Viasys healthcare, Masterscreen, Hochberg, Germany). Three technically acceptable maximal flow volume curves were obtained. Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁) were derived from the best curve according to ATS criteria.²⁴

Study participants were informed not to use a short-acting bronchodilator 6 hours and/or a long-acting bronchodilator 36 hours before spirometry.

Serum samples were taken and stored at -20°C until analysis. Total IgE and specific IgE levels were determined by a solid-phase immunometric assay on an ImmunoCAP 250 system of Pharmacia (Pharmacia Diagnostics AB, Uppsala, Sweden).

Specific IgE levels against a mixture of 10 common aeroallergens, such as birch, timothy, cat, dog, horse, and *Dermatophagoides pteronyssinus*, were determined by Phadiatop (Pharmacia Diagnostics AB), which measures IgE antibodies in serum. Results were expressed as positive (atopic) or negative (nonatopic).

Statistical analysis

FeNO values were log¹⁰ transformed, because they were skewed (Kolmogorov-Smirnov $P < .0001$), and geometric means (95% confidence intervals) and medians (interquartile ranges) were calculated. Mann-Whitney tests were used to study the association between FeNO and sex, eczema, allergic rhinitis, asthma, atopic asthma, parental atopic disease, asthma medication, ICS, smoking, recent cold, height, body mass index (BMI), FEV₁, FVC, FEV₁/FVC ratio, IgE level, and Phadiatop result. Variables that showed a significant difference between median FeNO values (defined as $P < .05$) and therefore appeared to have an effect on FeNO were included in a multivariate linear regression model to study the independent effect of these variables on FeNO levels.

RESULTS

A total of 406 cohort members agreed to participate in this study. FeNO could not be measured reliably in 26 individuals because of technical problems. In another 19 individuals measurements were considered invalid and therefore excluded. Reasons were inability to exhale at a constant flow rate or a significant difference between FeNO values in the 2 samples according to the ATS/ERS criteria.²² Participant characteristics are given in Table 1.

According to our definitions, 13% of the participants met the criteria for asthma, 8% for atopic asthma, 34% for allergic rhinitis, and 14% for eczema. FeNO values were skewed (Fig 1) but log-normally distributed (Fig 2). The mean log FeNO level was 1.28 (SD, 0.20), and the geometric mean FeNO value was 19.1 ppb (95% confidence interval, 7.7–47.0 ppb).

Median FeNO levels were significantly higher in participants with eczema than in those without (23.6 vs 18.0 ppb; $P < .0001$), those with vs without allergic rhinitis (20.7 vs 17.8 ppb; $P = .001$), those with vs without atopic asthma (23.3 vs 18.1 ppb; $P = .02$), men vs women (20.4 vs 17.4 ppb; $P < .0001$), those with elevated vs nonelevated IgE levels (19.9 vs 16.7 ppb; $P = .001$), and those with positive vs negative Phadiatop results (21.6 vs 17.3 ppb; $P < .0001$). In patients with eczema, median FeNO levels did not differ between those with positive vs negative Phadiatop results (25.9 vs 22.0 ppb; $P = .27$).

Table 1. Characteristics of the Study Participants

Characteristics	No. (%) of participants ^a		
	All participants (N = 361)	Male (n = 137)	Female (n = 224)
Eczema	52 (14)	17 (13)	35 (16)
Allergic rhinitis	122 (34)	44 (32)	78 (35)
Asthma	48 (13)	14 (10)	34 (15)
Atopic asthma	29 (8)	13 (10)	16 (7)
Current asthma medication	22 (6)	7 (5)	15 (7)
Current use of ICS	7 (2)	3 (2)	4 (2)
Parental atopic disease	174 (48)	64 (47)	110 (49)
Eczema and allergic rhinitis	20 (6)	8 (6)	20 (9)
Eczema and asthma	14 (4)	3 (2)	11 (5)
Asthma and allergic rhinitis	36 (10)	10 (7)	26 (12)
Eczema, allergic rhinitis, and asthma	13 (4)	3 (2)	10 (4)
Recent cold	79 (22)	25 (18)	54 (24)
Smoking in last 12 months	145 (40)	50 (37)	95 (43)
Positive Phadiatop result	144 (41)	74 (54)	70 (33)
Height, mean (SD), cm	173.6 (9.5)	182.2 (6.5)	168.3 (6.9)
Body mass index, mean (SD) kg/m ²	23.7 (4.1)	23.7 (3.5)	23.7 (4.4)
FEV ₁ , mean (SD), % predicted	115.8 (14.3)	116.2 (14.1)	115.5 (14.5)
FVC, mean (SD), % predicted	113.0 (13.2)	115.0 (11.9)	111.8 (13.8)
FEV ₁ /FVC ratio, mean (SD), %	86.5 (7.7)	84.1 (7.3)	88.0 (5.9)

Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhalation corticosteroids.

^a Data are presented as number (percentage) of patients unless otherwise indicated.

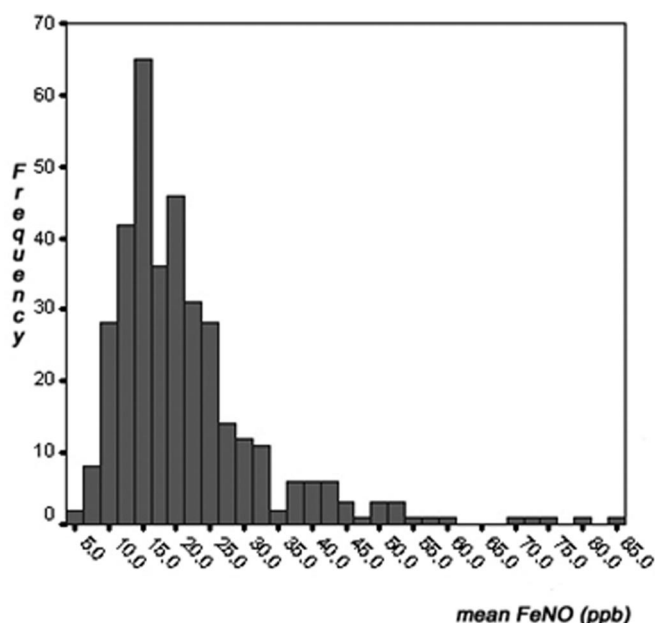


Figure 1. Skewed distribution of mean exhaled nitric oxide (FeNO) values of the study population.

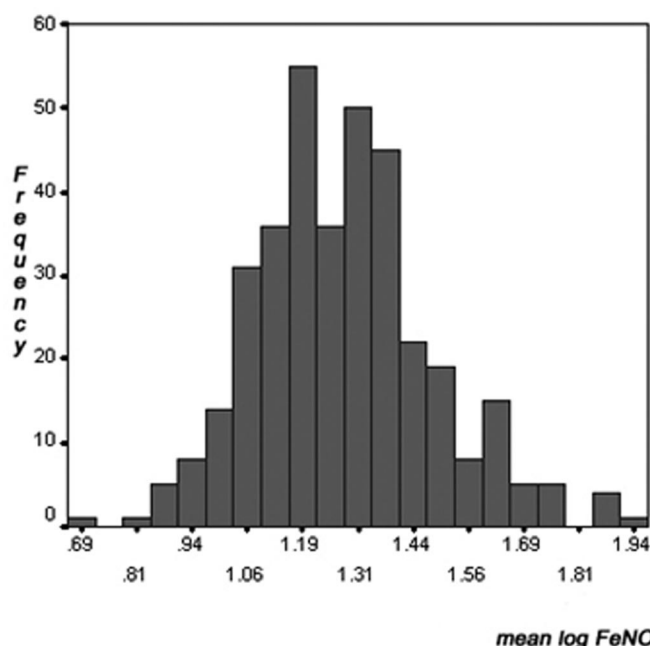


Figure 2. Normal distribution of log mean exhaled nitric oxide (FeNO) values.

Lower FeNO levels were observed in smokers vs non-smokers (16.9 vs 20.0 ppb; $P < .0001$) and individuals with a height less than 173 cm vs more than 173 cm (18.1 vs 19.3 ppb; $P = .03$) (Table 2). FeNO levels were not associated

with asthma, BMI, asthma medication, FEV₁, FVC, FEV₁/FVC ratio, or recent colds.

In the multivariate linear regression model, allergic rhinitis, eczema, sex, smoking cigarettes, IgE level, and Phadiatop

Table 2. Exhaled Nitric Oxide in Different Subgroups^a

Subgroups	Geometric mean FeNO (95% CI), ppb	Median FeNO (IQR), ppb	P value
Sex			
Men	20.9 (8.5–51.2)	20.4 (14.9–29.2)	<.0001 ^b
Women	17.5 (7.9–38.4)	17.4 (13.5–23.4)	
Eczema			
Yes	23.3 (9.4–57.6)	23.6 (16.2–34.1)	<.0001 ^b
No	18.0 (9.4–44.5)	18.0 (13.9–23.7)	
Allergic rhinitis			
Yes	20.6 (8.2–51.9)	20.7 (15.1–31.3)	.001 ^b
No	17.9 (8.1–39.6)	17.8 (13.9–23.5)	
Smoking			
Yes	16.8 (7.4–38.1)	16.9 (12.4–22.5)	<.0001 ^b
No	20.2 (8.7–46.6)	20.0 (14.9–26.2)	
Height			
<173 cm	17.7 (8.1–39.0)	18.1 (13.2–23.6)	.03 ^b
≥173 cm	19.8 (8.1–48.4)	19.3 (14.6–26.2)	
Asthma			
Yes	20.2 (8.1–50.4)	20.8 (13.7–27.8)	.24
No	18.5 (8.0–42.8)	18.3 (14.0–23.9)	
Atopic asthma			
Yes	22.8 (8.2–63.6)	23.3 (15.3–31.0)	.02 ^b
No	18.4 (8.0–42.1)	18.1 (14.0–23.9)	
Atopic parents			
Yes	19.5 (8.3–45.6)	20.0 (14.9–26.1)	.02 ^b
No	18.1 (7.6–42.2)	17.7 (13.5–23.7)	
Asthma medication			
Yes	21.5 (8.2–56.4)	21.3 (16.9–28.3)	.11
No	18.6 (8.0–43.1)	18.2 (14.0–25.1)	
Recent cold			
Yes	18.9 (8.0–44.6)	18.6 (14.1–24.8)	.72
No	18.7 (8.0–43.7)	18.7 (14.0–24.9)	
Body mass index			
<23	19.0 (8.1–44.5)	19.0 (14.0–24.1)	.63
≥23	18.4 (7.8–43.0)	18.3 (14.0–25.1)	
FEV ₁ , % predicted			
<116	19.4 (7.7–49.0)	19.0 (14.1–27.0)	.16
≥116	18.1 (8.4–38.9)	18.5 (13.8–23.7)	
FVC, % predicted			
<112	18.5 (7.8–44.0)	18.7 (14.0–24.9)	.62
≥112	19.1 (8.4–43.8)	19.1 (14.4–25.0)	
FEV ₁ /FVC ratio			
<87	20.5 (7.6–55.3)	19.5 (13.9–27.0)	.06
≥87	18.2 (7.7–43.7)	17.9 (14.0–23.7)	
IgE level			
<56	17.4 (8.3–36.6)	16.7 (13.9–22.6)	.001 ^b
≥56	20.3 (8.0–51.4)	19.9 (14.7–29.0)	
Phadiatop result			
Positive	21.5 (8.0–54.9)	21.6 (14.7–30.9)	<.0001 ^b
Negative	17.4 (8.3–36.2)	17.3 (13.9–22.0)	

Abbreviations: CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range.

^a Comparisons between median values in the groups were made using the Mann-Whitney test. Height, body mass index, FEV₁, FVC, FEV₁/FVC ratio, and IgE level were dichotomized using the median value as the cutoff point.

^b Variables that showed a significant difference between median FeNO values (defined as a $P < .05$) were included in a multivariate linear regression model to study the independent effect of these variables on FeNO.

result appeared to be independently associated with altered log FeNO levels (Table 3), whereas atopic asthma, current use of asthma medication, and height were not.

DISCUSSION

In this study, atopy, eczema, and allergic rhinitis were all independently associated with FeNO levels, whereas (atopic) asthma was not. Only a few study participants used asthma medication and/or ICS; neither of these determinants were found to alter FeNO values.

Sex, height, and current smoking appeared to influence FeNO values. However, after adjusting height for sex in the multivariate model, no significant relationship was observed, suggesting that higher FeNO levels in men are responsible for the relationship between FeNO and height. Also, Tsang et al²⁵ found higher FeNO levels in men and a correlation between FeNO and height. Higher FeNO levels in men could be due to a relatively larger lung volume when compared with women of similar height. We performed an additional analysis that showed no correlation between the absolute FVC (in liters) and FeNO levels (data not shown). The effect of current smoking on FeNO is not new; reduced FeNO levels in smokers were previously reported.²⁶

Eczema appeared to be an independent determinant of elevated FeNO levels in young adults. In the literature, only data on the relationship between eczema and FeNO in children are available and outcomes are contradicting.^{5,15,16,27,28} Higher FeNO levels in nonasthmatic children with eczema were recently described by Dinakar et al.²⁷ Franklin et al²⁸ found that children with physician-diagnosed eczema had elevated levels of FeNO. However, Buchvald et al⁵ did not find elevated FeNO levels among children with eczema, and Nordvall et al¹⁶ reported a positive association between FeNO levels and eczema in a general population cohort of children aged 13 to 14 years, but in a multivariate model this relationship was no longer significant.

Allergic rhinitis was another independent determinant of elevated FeNO levels in this study. Association of allergic rhinitis and higher FeNO values has previously been reported for atopic, nonasthmatic children and atopic adults.^{2,4,5,14,16}

Interestingly, unlike other studies,¹⁻⁴ we did not find an association between asthma and elevated FeNO levels. FeNO levels were elevated in atopic, asthmatic patients, but after adjustment for other factors this effect disappeared.

Most studies on the relationship between asthma and FeNO did not investigate coexistence of atopic entities such as eczema and allergic rhinitis. The independent influence of these entities on FeNO levels in our unselected population, regardless of atopy, suggests that the elevated FeNO levels in patients with atopic asthma as reported by others might be secondary to concomitant eczema or allergic rhinitis.

Salome et al¹⁷ found that FeNO levels in young adults were associated with asthma, defined as bronchial hyperresponsiveness and recent symptoms. Franklin et al¹⁴ also found that FeNO was associated with atopy, airway responsiveness, and blood eosinophil count in asthmatic children but not with physician-diagnosed asthma. Some therefore suggest that an elevated FeNO level reflects allergic inflammatory activity of the airways, depending on the degree of atopy,² whereas others suggest that an elevated FeNO level is a marker of eosinophilic inflammation, irrespective of atopy.²⁹

The overlapping mechanisms of asthma and allergic rhinitis, the so-called one airway, one disease concept, supports this hypothesis. Inflammatory mediators such as histamine and especially leukotrienes play an important role in inflammation of both the upper and lower airways.³⁰ However, it is not clear how eczema would fit in this concept. Nitric oxide is synthesized from L-arginine by the enzyme nitric oxide synthase in many cells of the body.³¹ It is a free radical gas that is fairly stable, but in tissues it is too short-lived to act at greater distances. An increased level of FeNO may be caused by induction of inducible nitric oxide synthase expression in residential cells but also by attraction of nitric oxide-producing inflammatory cells, such as mast cells,³² eosinophils,³³ macrophages, and neutrophils.³⁴ Increased FeNO levels in patients with allergic rhinitis and eczema might be due to endobronchial release of nitric oxide as a manifestation of allergen-induced inflammation in the nose or the skin.

To appreciate the results of our study, several limitations should be mentioned. First, a substantial number of participants in our study were tested in the pollen season. This approach may have influenced the results, since FeNO levels appear to increase after allergen challenge in sensitized individuals.⁴ Furthermore, since we asked for symptoms in the last 12 months we could not control for increased FeNO values because of recent exposure to allergens or decreased FeNO values because of relative remission of asthma symptoms when FeNO measurements were performed.

Second, anti-inflammatory drugs such as ICS are reported to lower elevated FeNO levels in atopic, asthmatic patients.³⁵ We cannot rule out some influence of the use of ICS on FeNO levels in 7 of our asthmatic study members. Although recent use of asthma medication and/or ICS was not associated with decreased FeNO levels, current use of ICS may have decreased the average FeNO level in the group of asthmatic

Table 3. Final Multivariate Linear Regression Model With Log Fractional Exhaled Nitric Oxide as the Dependent Variable and Allergic Rhinitis, Eczema, Smoking, Sex, IgE Level, and Phadiatop Result as Independent Variables

Variable	β	SE	P value
Allergic rhinitis	-.053	.022	.02
Eczema	-.067	.029	.02
Smoking	.078	.020	<.001
Sex	-.076	.021	<.001
IgE level	.00007	.00001	.001
Phadiatop result	-.04	.022	.06

participants, resulting in a nonsignificant association between FeNO and asthma.

Third, some participants had some extremely high FeNO values, which might have influenced our results. A sensitivity analysis (data not shown), in which we excluded outliers with FeNO levels greater than 55 ppb, did not influence our results.

Fourth, the individuals who participated in the follow-up ($n = 406$) might not be representative of the entire birth cohort ($N = 1,328$). Furthermore, we noticed an increased number of participating females, for which we have no explanation.

The major strength of our study is that we had the unique opportunity to study FeNO values in a large group of young adults from the general population. In conclusion, eczema, allergic rhinitis, and atopy were independently associated with elevated FeNO levels, whereas (atopic) asthma was not. The results shed new light on the value of FeNO in the diagnosis of atopic disease. Future studies on the role of FeNO in asthma should take account of the confounding effects of nonpulmonary atopic features on FeNO values.

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