

Original article

Exhaled nitric oxide in a population-based study of asthma and allergy in schoolchildren

Exhaled nitric oxide (NO) reflects inflammation in the lower airways and is well adapted for use in children. The aims of this study were to investigate the distribution of the fraction of expired NO (FE_{NO}) in school children and to compare FE_{NO} and spirometry in relation to the International Study of Asthma and Allergies in Childhood questionnaire. The study was performed in 959 randomly selected 13–14-year-old school children in Uppsala, Sweden. Exhaled NO was measured at an inhalation rate of 0.1 l/s ($FE_{NO0.1}$) and a spirometric test was performed and data from these measurements were related to questionnaire data. Exhaled NO was measured according to American Thoracic Society recommendations, except the use of a mouth wash and an exhalation flow rate of 0.1 l/s. The distribution of the mean $FE_{NO0.1}$ values was skewed, with a preponderance of very low levels and a widespread tail of values ranging up to 102 parts per billion (ppb). Boys exhibited significantly higher mean $FE_{NO0.1}$ values than girls, 5.2 (4.7–5.7) vs 4.4 (4.0–4.8) ppb (geometric mean and 95% CI), $P < 0.01$). Children who reported wheezing in the last year had higher $FE_{NO0.1}$ values than children that had not, 8.5 (7.1–10.2) vs 4.3 (4.0–4.6) ppb, $P < 0.001$). The same association was found to most symptoms indicating hay fever and eczema. In contrast to this, only weak or inconsistent associations were found between asthma and spirometric indices. Exhaled NO levels were found to be independently related to male gender, wheeze and rhinoconjunctivitis but not to current eczema. In conclusion, exhaled NO was closely associated with reported asthma and allergy symptoms whereas spirometric indices such as percent predicted forced expiratory volume in 1 s were not. As most asthma cases in a population are mild, the findings suggest that exhaled NO is a sensitive marker of asthma and allergy.

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Key words: asthma; breath test; children; epidemiology; nitric oxide; spirometry.

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Accepted for publication 21 June 2004

A primary objective of the International Study of Asthma and Allergies in Childhood (ISAAC) was to describe the prevalence and to some extent severity of asthma, rhinitis and eczema in children living in different areas, to enable comparisons within and between countries (1). To achieve this objective, a simple questionnaire was developed that has evolved into a widely used standard for pediatric epidemiological work. In addition to a world wide study of the prevalence of asthma, rhinitis and eczema in schoolchildren (2, 3), a large number of national studies have been undertaken using this standard questionnaire.

Apart from questionnaires, various methods of assessing bronchial hyper-reactivity have been utilized in epidemiological studies in children and young adults. In recent studies these have included challenges with hypertonic saline, methacholine, histamine, cold air and physical exercise tests. Allergy tests have also been used in epidemiological studies, primarily skin prick tests.

To date, it has not been possible to use methods that directly reflect inflammation in asthma in epidemiological research. Levels of exhaled nitric oxide (NO) have been found to be elevated in asthma (4, 5); this seems to be primarily a feature of allergic asthma (6, 7) and appears to reflect eosinophilic inflammation (8–10). The measurement of exhaled NO is one of few methods that are suitable for use in epidemiological studies in children. In the present study, we used the results of the ISAAC questionnaire to compare clinical allergological characteristics with measurements of exhaled NO and pulmonary function in schoolchildren.

Material and methods

General design

The investigation was performed in community schools in Uppsala, Sweden, from October 1998 to December 1999. The schools were

randomized prior to the investigation. For practical reasons, the investigation was started in one school located adjacent to the Children's hospital. A random order of inclusion was followed for the remainder of the schools, with the aim of including approximately 1000 school children in the study. The headmasters of 11 schools were approached and nine schools were finally included in the study. Two schools declined to participate because of the lack of a suitable examination room.

A questionnaire previously used as a part of the world wide ISAAC study (<http://isaac.auckland.ac.nz/PhaseOne/Manual/Section7/Sec7Frame.html>) was translated into Swedish, adapted for use in 13–14-year-old school children (3), and was distributed within the schools to all adolescents in the appropriate age range. The children completed the forms at school and then took them home to their parents, together with a letter of information about the study. Of 1164 pupils and parents approached, 959 (83%) agreed to participate, submitted a completed questionnaire and were finally included in the study. Following inclusion, exhaled NO and pulmonary function were measured by a study nurse who, on the same occasion, also carried out a short structured interview with the young person regarding current medication, signs of respiratory tract infection, smoking habits, etc.

Measurements of exhaled NO

Measurements of exhaled NO were performed in accordance with the recommendations for online NO measurements published by the American Thoracic Society (ATS), with the exceptions of target flow rate and a mouthwash procedure (11). We used the Aerocrine NO system (Aerocrine AB, Stockholm, Sweden), including the CLD 77 AM chemiluminescence analyser [Eco Physics AG, Dürnten, Switzerland; sensitivity, 0.1 parts per billion (ppb) NO; rise time 0–90%, <0.1 s; sample flow rate, 110 ml/s; lag time from mouthpiece, 0.7 s] for online NO measurements, and the monitoring of flow and pressure. A two-point calibration was performed before each study session using a certified calibration gas (10 parts per million NO in N₂; AGA AB, Älvsjö, Sweden). Immediately before the experiments, the mouth was rinsed for 20 s with 25 ml of 10% sodium bicarbonate (12). Within 5 min of the mouthwash, the children, comfortably seated, inhaled NO-free air from a reservoir and subsequently exhaled against a linear resistor (Hans Rudolph Inc., Kansas City, KS).

Mean oral pressure for all exhalations was 9.2 (± 0.4) cmH₂O. Three exhalations of 10 s duration were performed consecutively at a target flow rate of 0.1 l/s with the help of visual guidance from the computer screen, and an average value was calculated. Values are presented as NO concentrations (fraction of expired NO; FE_{NO}) at an exhalation flow rate of 0.1 l/s (FE_{NO0.1}). Two children were unable to perform an accepted exhaled NO experiment successfully, 23 performed only one and 218 performed only two accepted experiments.

Measurements of pulmonary function

Pulmonary function measurements were performed according to ATS criteria using a Spirolab spirometer (Medical International Research, Rome, Italy), following a routine procedure carried out by a research nurse experienced in the method. The best of three experiments was automatically chosen by the spirometer programme. Forced expiratory volume in 1 s (FEV₁), the ratio of FEV₁ to forced vital capacity (FVC) and forced expiratory flow at 25, 50 and 75% of FVC (FEF₂₅ and FEF₅₀ and FEF₇₅) were measured. Values were normalized for age, sex and body height and weight and expressed as percent, using the spirometer programme and Knudson's normal values for children (13).

Smoking

Passive smoking was defined as answering yes to the question 'Does anyone in your family smoke'. Active smoking was defined as answering yes to the question: 'Do you smoke?'

Statistical methods

All data were processed using a Microsoft Office Access database. Statistical analyses were performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA) and StatView 5.0 (SAS Institute, Cary, NC, USA). Seven cases without measurable levels of NO were arbitrarily assigned a value of 0.05 ppb to permit analysis. NO values were log-transformed prior to analyses. Geometric mean and 95% confidence limits were calculated and transformed back after that. Percent predicted values of spirometric indices were used untransformed in the statistical analyses. Student's *t*-test and ANOVA were used for comparisons between groups. The associations between FE_{NO0.1} and different explanatory variables were tested using different multiple linear regression models. The significance of the slope in the multivariate regression model was based on the *t* distribution.

Ethics

The study was approved by the Ethics Committee of the Medical Faculty of Uppsala University.

Results

The demographic characteristics and lung function values of the study population are presented in Table 1.

Exhaled NO values

The mean intraindividual coefficient of variation (CV) of the measured FE_{NO0.1} levels was 9.8% (9.0–10.6), when there were three successful experiments. The distribution of the mean FE_{NO0.1} values was skewed, with a preponderance of very low levels and a widespread tail of values ranging up to 102 ppb, but after logarithmic transformation, a symmetric distribution was achieved (Fig. 1). Boys exhibited significantly ($P < 0.01$) higher mean FE_{NO0.1} values than girls, 5.2 (4.7–5.7) vs 4.4 (4.0–4.8) ppb. No relationships were found between FE_{NO0.1} values and active or passive smoking, respectively.

Table 1. Demographic characteristics and lung function [forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC) and forced expiratory flow (FEF)] [mean $\pm$ SD or *n* (%)]

Age (years)	13.6 $\pm$ 0.4
Male gender	476 (49.7)
Passive smoking	313 (32.8)
Active smoking	10 (1.0)
FEV ₁ (% predicted)	98.0 $\pm$ 11.5
FEV ₁ /FVC (% predicted)	107 $\pm$ 6.5
FEF ₂₅ (% predicted)	88.5 $\pm$ 16.9
FEF ₅₀ (% predicted)	97.2 $\pm$ 20.8
FEF ₇₅ (% predicted)	102 $\pm$ 41.8

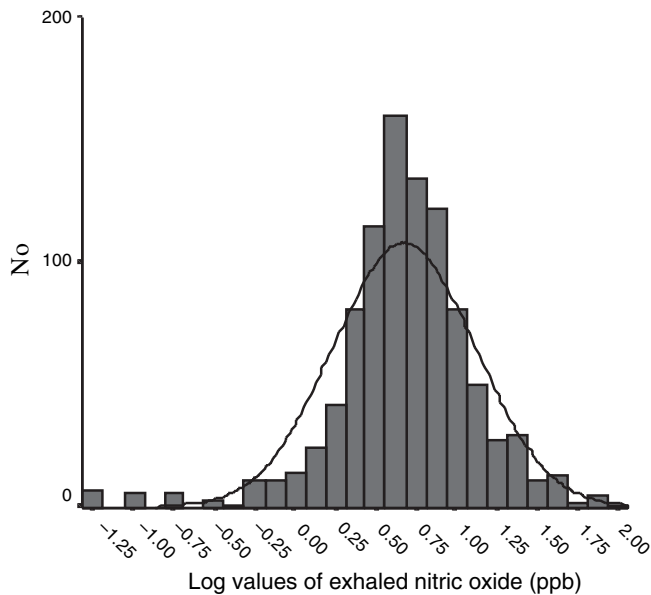


Figure 1. Histogram of levels of exhaled nitric oxide ($^{10}\log$ ppb) in population of school children. The theoretical normal distribution is given by the line.

ISAAC questionnaire prevalence data

Wheezing during the last year ('wheezing last year' group) was reported by 13.1% of the children (12.0% of boys; 14.3% of girls, $P = 0.28$) (Table 2). The corresponding values for noninfectious rhinitis and for symptoms indicative of eczema (itching rash) during the last year were 25.6% (26.3% of boys; 24.9% of girls, $P = 0.66$) and 20.5% (17.1% of boys; 24.0% of girls, $P = 0.01$), respectively (Table 3).

Exhaled NO in relation to symptoms of asthma

There were strong and significant associations between the questions regarding asthma symptoms and exhaled NO levels. $FE_{NO0.1}$ was significantly higher in children who had ever experienced wheezing ('ever-wheezing' group) compared with those who had never experienced wheezing, and in the 'wheezing last year' group compared with those who reported no wheezing in the previous year (Table 2). Likewise, children who had suffered from asthma sometime during their life ('ever asthma') had elevated $FE_{NO0.1}$ levels compared with those who had never experienced asthma. Of the 611 children who

Table 2. Exhaled nitric oxide [$FE_{NO0.1}$ = fraction of expired nitric oxide at exhalation flow rate 0.1 l/s expressed as parts per billion (ppb)] (geometric mean and 95% confidence intervals) and forced expiratory volume in 1 s (FEV_1) (mean and 95% confidence intervals) in relation to asthma questions in the ISAAC questionnaire*

Question number	ISAAC question summary	Prevalence	$FE_{NO0.1}$ (ppb)			FEV_1 (% pred)		
			Without symptom	With symptom)	P -value	Without symptom	With symptom)	P -value
A1	Ever wheezing	24.6 (21.9–27.3)	4.3 (4.0–4.6)	6.4 (5.5–7.5)	<0.001	98 (97–99)	98 (96–99)	0.51
A2	Wheezing last year	13.1 (11.0–15.3)	4.3 (4.0–4.6)	8.5 (7.1–10.2)	<0.001	98 (97–99)	97 (94–99)	0.22
A5	Wheeze-impairing speech	1.9 (1.0–2.7)	4.7 (4.4–5.0)	8.8 (5.1–15.1)	0.009	98 (97–99)	103 (97–110)	0.052
A6	Ever asthma	12.1 (10.0–14.1)	4.5 (4.2–4.8)	7.7 (6.1–9.1)	<0.001	98 (97–99)	97 (95–100)	0.39
A7	Exercise-induced wheeze last year	18.3 (15.9–20.8)	4.3 (4.0–4.6)	7.3 (6.1–8.7)	<0.001	98 (97–99)	98 (96–100)	0.73
A8	Dry cough at night	11.3 (9.3–13.3)	4.6 (4.3–5.0)	5.6 (4.5–7.0)	0.07	98 (97–99)	99 (96–101)	0.58

*For detailed ISAAC questions see 'http://isaac.auckland.ac.nz/PhaseOne/Manual/Section7/Sec7Frame.html'.

Table 3. Exhaled nitric oxide [$FE_{NO0.1}$ = fraction of expired nitric oxide at exhalation flow rate 0.1 l/s expressed as parts per billion (ppb)] (geometric mean and 95% confidence intervals) in relation to rhinitis and eczema questions in the ISAAC questionnaire*

Question number	ISAAC question summary	Prevalence	$FE_{NO0.1}$ (ppb)		
			Without symptom	With symptom	P -value
N1	Ever rhinitis without cold?	31.4 (28.5–34.4)	4.4 (4.1–4.7)	5.7 (5.0–6.5)	0.001
N2	Rhinitis last year	25.6 (22.8–28.4)	4.4 (4.0–5.0)	6.2 (5.3–7.1)	<0.001
N3	Rhinitis and conjunctivitis last year	13.4 (11.2–15.6)	4.5 (4.2–4.8)	7.1 (5.9–8.5)	<0.001
N6	Ever hay fever	18.5 (16.1–21.0)	4.5 (4.2–4.8)	6.1 (5.1–7.2)	0.003
E1	Ever itching rash	27.1 (24.3–29.9)	4.5 (4.2–4.8)	5.6 (4.9–6.3)	0.003
E2	Itching rash last year	20.5 (18.0–23.1)	4.6 (4.3–4.9)	5.5 (4.7–6.3)	0.03
E3	Typical location	16.5 (14.1–18.8)	4.6 (4.2–4.9)	5.8 (5.0–6.9)	0.005
E6	Ever eczema	46.9 (43.8–50.1)	4.9 (4.5–5.4)	4.6 (4.1–5.1)	0.28

*For detailed ISAAC questions see 'http://isaac.auckland.ac.nz/PhaseOne/Manual/Section7/Sec7Frame.html'.

Table 4. Forced expiratory flow (FEF) (mean and 95% confidence intervals) in relation to asthma questions in the ISAAC questionnaire*

Question number	ISAAC question summary	FEF ₂₅			FEF ₅₀			FEF ₇₅		
		Without symptom	With symptom	P-value	Without symptom	With symptom	P-value	Without symptom	With symptom	P-value
A1	Ever wheezing	89 (88–90)	87 (84–89)	0.04	98 (96–99)	95 (92–98)	0.10	103 (100–108)	99 (96–103)	0.21
A2	Wheezing last year	89 (88–90)	84 (81–87)	0.003	98 (96–99)	94 (90–98)	0.05	103 (100–106)	99 (94–105)	0.37
A5	Wheeze-impairing speech	88 (87–90)	93 (83–103)	0.24	97 (96–98)	107 (94–120)	0.06	103 (100–105)	112 (96–128)	0.34
A6	Ever asthma	89 (88–90)	85 (82–89)	0.04	98 (96–99)	94 (90–98)	0.07	103 (100–106)	96 (91–101)	0.08
A7	Exercise-induced wheeze last year	89 (88–90)	87 (84–89)	0.09	98 (96–99)	94 (91–97)	0.02	103 (100–106)	99 (95–103)	0.24
A8	Dry cough at night	88 (87–89)	90 (87–93)	0.31	97 (96–99)	97 (93–101)	0.79	102 (99–105)	102 (96–107)	0.90

*For detailed ISAAC questions see 'http://isaac.auckland.ac.nz/PhaseOne/Manual/Section7/Sec7Frame.html'.

reported no asthma symptoms in the ISAAC questionnaire, 23 had FE_{NO0.1} values of 20 ppb or higher, and five had FE_{NO0.1} values of 40 ppb or higher.

Only 21 of the children had used any inhaled corticosteroids during the week preceding the investigation. In these children, FE_{NO0.1} values were significantly higher than those in children not using corticosteroids, 9.7 (5.8–16.0) vs 4.7 (4.4–5.0) ppb; $P = 0.001$. Within the 'last-year wheezing' and 'ever asthma' groups, FE_{NO0.1} values did not differ in relation to inhaled corticosteroid use.

Spirometric data in relation to the symptoms of asthma

In contrast to exhaled NO, pulmonary function test results showed only weak or inconsistent associations with the answers to the ISAAC questionnaire (Tables 2 and 4). The FEV₁ values normalized for height and sex were found to be totally unrelated to symptoms, and normalized FEF values exhibited only weak associations with reported 'ever wheezing' and 'ever asthma' and a somewhat stronger association with 'wheezing last year' (Table 4).

Exhaled NO and symptoms of hay fever and eczema

Positive answers to the ISAAC questions about hay fever were associated with elevated FE_{NO0.1} levels (Table 3), but the increased levels were not as high as those seen in children experiencing asthma. Eye symptoms in conjunction with nasal symptoms exhibited the highest FE_{NO0.1} values. A positive answer to all the ISAAC eczema questions except ever having had eczema was significantly associated with higher exhaled NO levels (Table 3).

Exhaled NO, spirometry and combination of symptoms

Exhaled NO levels were significantly higher in children who had both a history of asthma and experienced wheeze in the last 12 months than in children who had either a history of asthma but no current wheeze, or current wheeze but no history of asthma, 11.8 (9.0–15.5) vs 4.7 (3.4–6.5) and 6.5 (5.3–8.1) ppb, respectively ($P < 0.001$). No corresponding difference between these groups was seen when measuring FEV₁ (Fig. 2).

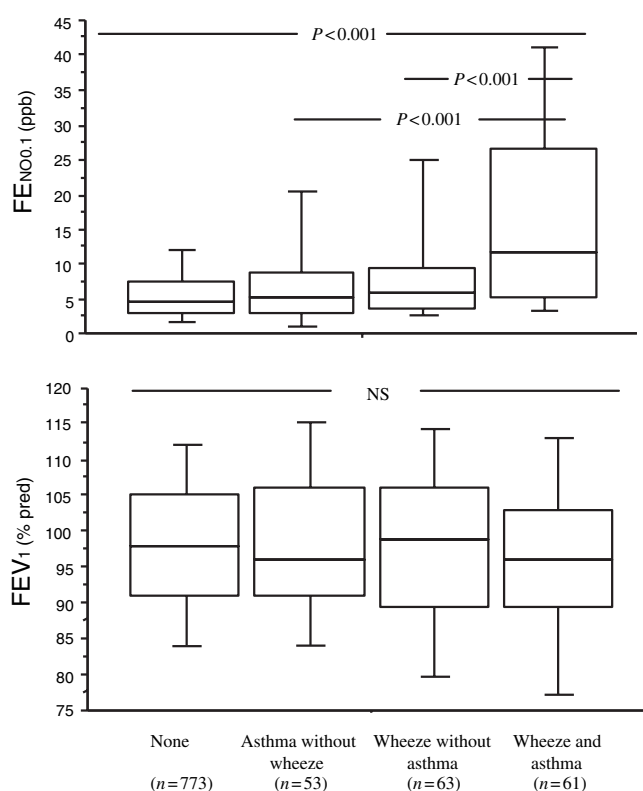


Figure 2. Levels of exhaled nitric oxide and forced expiratory volume in 1 s in the relation to ever asthma and wheeze within the last 12 months (10th, 25th, 50th, 75th and 90th percentile).

The FE_{NO0.1} levels were significantly higher in both children with asthma without rhinoconjunctivitis and wheeze, and with wheeze without rhinoconjunctivitis, than in children without both wheeze and rhinoconjunctivitis, 5.3 (4.3–6.6) and 7.0 (5.6–8.6) vs 4.2 (3.9–4.6) ppb ($P = 0.04$ and < 0.0001 , respectively). Children with the combination of wheeze and rhinoconjunctivitis had exhaled NO levels that were significantly higher than any of the three other groups, 11.9 (8.8–16.0) ppb, $P < 0.0001$ (Fig. 3). When performing multiple variable analysis, exhaled NO levels were found to be independently related to male gender, wheeze and rhinoconjunctivitis, but not to current eczema (Table 5).

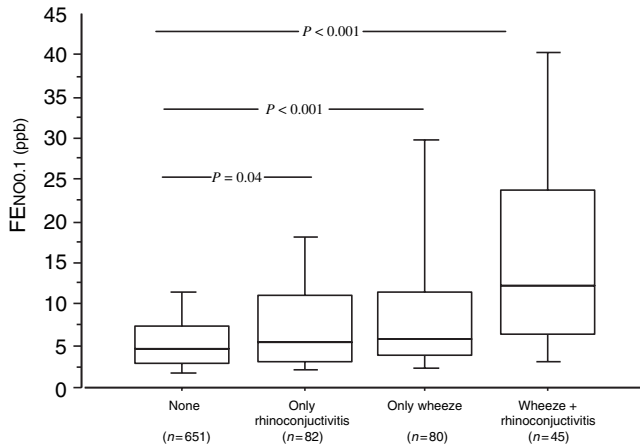


Figure 3. Levels of exhaled nitric oxide in the relation to rhinoconjunctivitis and wheeze within the last 12 months (10th, 25th, 50th, 75th and 90th percentile).

Table 5. Multiple linear regression model with log transformed fraction of expired nitric oxide at exhalation flow rate 0.1 l/s expressed as parts per billion as the dependent variable

	Coefficient (95% CI)	P-value
Male gender	0.09 (0.03, 0.10)	0.002
Wheeze in the last 12 months	0.25 (0.17, 0.34)	<0.001
Rhinoconjunctivitis in the last 12 months	0.13 (0.05, 0.22)	0.002
Rash in the last 12 months	0.04 (-0.03, 0.11)	0.26

Discussion

The results of the present population-based cross-sectional study of schoolchildren show strong relationships between exhaled NO levels and reported clinical characteristics of allergic disease, especially symptoms indicative of asthma. As exhaled NO seems to reflect inflammation in the airways (8–10), a key feature of asthma, the results are especially promising. The data are particularly interesting in view of the finding that the corresponding correlates between symptoms and spirometry were surprisingly poor. Although the study sample included almost 1000 schoolchildren, no significant correlation was found between symptoms of asthma and the most commonly used spirometric index, FEV₁. The results of the present epidemiological study would seem to suggest that a single spirometric measurement appears to add little to the evaluation of a child's asthma, in contrast to the measurement of exhaled NO. The poor achievement of spirometry is likely to be a consequence of the population-based design and the possibility that particularly mild cases of children with wheezing who may not fulfill present asthma criteria were recruited to the study. This indicates that NO measurements may be useful in the primary care setting. In hospital clinics the situation is likely to be different. The data may also be seen to

indicate that exhaled NO measurement may be particularly useful in the assessment of mild asthma.

In previous studies in Australia, asthma was defined as recent wheeze and bronchial hyper-responsiveness (14). Exhaled NO values were significantly higher among asthmatic individuals than among healthy controls, but tended only toward an increase in those that experienced wheezing but had no bronchial hyperresponsiveness. These authors also reported a number of participants who experienced bronchial hyperresponsiveness but no asthma symptoms; these patients are of interest as they may, at least in part, correspond to the children in our study who had high levels of exhaled NO but no symptoms according to the ISAAC questionnaire. These individuals may well represent 'early asthma' and will be subject to further studies.

Our results are in accordance with previous studies reporting elevated exhaled NO levels in allergic asthma (5–7). In our analysis exhaled NO levels were, however, also significantly increased in schoolchildren with current rhinoconjunctivitis that did not report wheezing in the last 12 months. This is in accordance with Henriksen *et al.* (15) who also found that patients with seasonal allergic rhinoconjunctivitis exhibit elevated exhaled NO levels even out of season, which was particularly true for those with a perennial sensitization. Like Steerenberg *et al.* (10), we found increased exhaled NO levels in children with eczema. In the present analysis the relationship between eczema and NO levels was, however, no longer significant after adjusting for wheeze and rhinoconjunctivitis. It appears not to be atopy, *per se*, that results in a high production of NO, but the combination of an allergic state plus exposure to the relevant allergen causing an allergic inflammation (16–19). The importance of cat, our major perennial allergen, which is ubiquitous in our society, can probably not be underestimated in the context (20, 21). The issue is however not addressed by the ISAAC questionnaire. In the present study we have also seen that boys have higher FE_{NO0.1} values than girls. This is in line with previous data from adults (22, 23). One explanation for this gender difference could be that males have larger lung volumes including larger respiratory dead space volumes relative body indices (24). Expiratory NO originates mainly in the dead space compartments and the size of this compartment may influence the total NO airway diffusing capacity, a factor that will influence the resulting FE_{NO0.1} value (25). Another possible explanation for the sex difference would be higher degree of atopy among males, a factor at least partly compensated for in our study by including rhinoconjunctivitis in the statistical model.

We used a research prototype for the measurement of exhaled NO with voluntary flow control, following the ATS recommendations for online measurements (11), with the exception that we used an exhalation flow rate of 0.1 instead of 0.05 l/s. Only two of the 959 children were unable to perform the procedure adequately, and the

technique was surprisingly reproducible even in this epidemiological setting. It may be suggested from the data that one successful exhalation is sufficient in an epidemiological study and in clinical practice. Dynamic flow control may make the procedure even easier and may further improve the technique. Mouth washing, which was used in the present study, may be of some importance as the oral contribution when measuring exhaled NO is diminished (12).

In summary, our results from a cross-sectional study of almost 1000 13–14-year-old schoolchildren indicate that the measurement of exhaled NO is far superior to spirometry in identifying children with asthma, particularly allergic mild asthma. The technique therefore seems suitable as an adjunct to questionnaires when

screening for asthma in large population groups. However, the importance of diagnosing mild asthma is currently unknown as the natural course of mild asthma in this age group has yet to be determined.

Acknowledgments

This study was supported by grants from the Swedish Medical Research Council (Project No. 10354), The Swedish Foundation for Health Care Sciences and Allergy Research, The Swedish Asthma and Allergy Association's Research Foundation, The Swedish Heart Lung Foundation and AstraZeneca. An Aerocrine NO System was provided by Aerocrine AB, Solna, Sweden.

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