ORIGINAL ARTICLE

Elevated Exhaled Nitric Oxide in Adolescents Is Associated With Incident Allergic Symptoms: A Prospective Cohort Study

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Abstract

Background: Fractional exhaled nitric oxide (FeNO) is a marker of type-2 inflammation in the airways. Elevated FeNO may precede the development of allergic disease. The aim of the present study was to investigate the association between elevated FeNO and the development of allergic symptoms.
Methods: A total of 959 adolescents from the general population and their parents completed a standardized questionnaire. Lung function and FeNO were assessed at baseline. Four years later, 921 of these individuals (96%) completed the same version of the baseline questionnaire.
Results: Adolescents with self-reported incident allergic symptoms to cat (n=50) or dog (n=33) had higher baseline FeNO (*P*<.001) than those without allergic symptoms to cat and dog at both time points (n=776 and n=838, respectively). Adolescents with incident allergic symptoms to pollen did not have elevated baseline FeNO. The adjusted odds ratio (aOR [95%CI]) for incident allergic symptoms to cat was 4.2 (2.2-8.0) times higher if FeNO was >75th percentile (vs <75th percentile) at baseline. This was consistent after exclusion of individuals with reported asthma, wheeze, or rhinitis at baseline (8.6 [3.0-24.1]).
Conclusion: Elevated FeNO in adolescents was associated with an increased risk of developing allergic symptoms to cat and dog allergens, but not to pollen allergens, after 4 years.

Key words: Adolescents. Breath test. Epidemiology. Hypersensitivity. Incidence. Nitric oxide.

Resumen

Introducción: La fracción de óxido nítrico exhalado (FeNO) es un marcador de inflamación de tipo 2 en las vías respiratorias y un valor de FeNO elevado puede preceder al desarrollo de enfermedad alérgica. El objetivo del presente estudio fue investigar la asociación entre FeNO elevado y el desarrollo posterior de síntomas alérgicos.
Métodos: Un total de 959 adolescentes, procedentes de población general, respondieron, junto con sus padres, a un cuestionario estandarizado, realizaron una prueba de función pulmonar y una medición de FeNO en una visita basal. Cuatro años después, 921 de estos sujetos (96%) completaron, la misma versión, en gran medida, del cuestionario de referencia.
Resultados: Los adolescentes con síntomas alérgicos incidentes autoinformados por gato (n=50) o perro (n=33) tenían mayor FeNO inicial (p <0,001) que los sujetos sin síntomas alérgicos por estos alérgenos, en cualquier momento del estudio (n=776 y n=838, respectivamente). Por el contrario, los adolescentes con síntomas alérgicos incidentes por polen no presentaban un FeNO inicial elevado. La razón de riesgo ajustada [aOR (intervalo de confianza del 95%)] para síntomas alérgicos incidentes por gato fue 4,2 (2,2, 8,0) veces mayor si el FeNO fue mayor que percentil 75 de la muestra (vs. menor del percentil 75) al inicio del estudio. Este resultado se mantuvo también después de la exclusión de los sujetos con asma, sibilancias o rinitis notificados al inicio del estudio [aOR (IC 95%) 8,6 (3,0, 24,1)].
Conclusiones: El FeNO elevado en adolescentes se relacionó con un mayor riesgo de desarrollar en los cuatro años siguientes síntomas alérgicos inducidos por gatos y perros, pero no por los alérgenos del polen.

Palabras clave: Adolescentes. Pruebas en aire exhalado. Epidemiología. Hipersensibilidad. Incidencia. Óxido nítrico.

Introduction

In the early 1990s, fractional exhaled nitric oxide (FeNO) was reported to be elevated in asthma [1]. Several studies have reported correlations between FeNO and blood and sputum eosinophils, as well as the degree of airway hyperresponsiveness [2,3]. Recent studies indicate that FeNO is more representative of type-2 inflammation, which is specifically related to the expression of interleukin (IL) 4 and IL-13 in the bronchial mucosa, than general eosinophilic inflammation [4]. FeNO is a marker of short-term changes in type-2 inflammation of the airways, but is also associated with long-term changes in IgE-antibody concentrations [5]. Furthermore, measurement of FeNO is considered a useful tool for identifying the atopic phenotype among asthmatics [4], as well as corticosteroid-sensitive inflammation in the airways [6].

Allergic sensitization is associated with increased FeNO in both the general population and asthma patients [7,8]. Moreover, exhaled NO correlates with the degree of IgE-mediated sensitization, in terms of both the number of positive skin prick test (SPT) results [9,10] and IgE antibody concentrations [11,12]. We previously reported the association between upper airway symptoms such as rhinitis, rhinoconjunctivitis, and asthma and increased exhaled NO in the present cohort at baseline [13].

Increased FeNO has been reported in both children and adults with no confirmed respiratory symptoms and is related to an increased risk of developing wheeze; thus, it may indicate subclinical inflammation or "early asthma" [13-15]. We previously reported that the adolescents with elevated NO levels in the present cohort had an increased likelihood of new-onset rhinitis within a 4-year follow-up period [16].

To our knowledge, no studies have assessed the utility of exhaled NO in predicting the incidence of allergic symptoms to airborne allergens. The aim of this study was to evaluate the predictive value of FeNO for self-reported incident allergic symptoms to cat, dog, or pollen within a 4-year time frame in a large population-based cohort of adolescents.

Materials and Methods

General Design

Baseline data were collected during 1998-1999 from 959 individuals aged 12-15 years attending 9 schools selected at random in Uppsala, Sweden. The study, *Screening Project Asthma in Schools* (SPAIS I), has previously been described in detail [13]. The pupils completed a questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC) [17,18], and lung function and FeNO were measured. Parents answered additional written questions about their children concerning hypersensitivity to cat, dog, or pollen allergens, diagnosis of asthma, asthma medication, atopic disease in childhood, family history of asthma and rhinitis, family smoking, and environmental issues. If FeNO and FEV_1 were assessed between March and September, then the measurements were considered to have been taken during the pollen season; otherwise they were not. The follow-up study, SPAIS II, with an identical ISAAC questionnaire and identical questions concerning hypersensitivity to cat, dog, and pollen allergens, diagnosis of asthma, asthma medication, environmental issues, family smoking, and an additional question concerning own smoking was performed 4 years later (2002-2003). In this case, the adolescents completed the questionnaires themselves.

Questionnaires and Definitions

At baseline, allergic symptoms were defined as the individual's hypersensitivity to cat, dog, or pollen allergens, as noticed and reported by the parents. For negative answers, there was an additional question regarding parental suspicion of hypersensitivity to cat, dog, or pollen allergens. Allergic symptoms at follow-up were defined as above but reported by the participant and only at the level of the question "*Have you noticed hypersensitivity to cat, dog, or pollen*?". Incident allergic symptoms were reported at follow-up but not at baseline.

Asthma was defined as ever having had parent- or self-reported asthma plus having received corticosteroids for treatment of asthma or having wheeze and whistling in the chest (ISAAC) or having a respiratory infection that caused wheeze and whistling in the chest during the previous year (ISAAC). Wheeze was defined as having had wheeze or whistling in the chest at any time in the previous year (ISAAC). Rhinitis was defined as having had sneezing, nasal congestion, or rhinorrhea during the previous 12 months, without having a cold (ISAAC).

Asthma and rhinitis in the family were assessed using a questionnaire, with separate questions for the mother, father, and siblings. Family smoking, the individual's own current smoking habits during follow-up, and exposure to cat and dog allergens at home were also assessed using a questionnaire.

Exhaled NO Measurements

Measurements of FeNO were performed using the Aerocrine NO system (Aerocrine AB), including the CLD 77 AM chemiluminescence analyzer (Eco Physics AG), as previously described [13] and in accordance with the recommendations of the European Respiratory Society [19]. Before measurement, each participant's mouth was washed with 25 mL of 10% sodium bicarbonate for 20 seconds. Three exhalations of 10 seconds each were performed, and an average value was calculated. A recent study has shown high reproducibility of FeNO measurements and no need for further repeated measurements during the same session [20]. FeNO was measured at 0.1 L/s. To help interpret the FeNO values in this paper, one may transform the $FeNO_{0.1}$ values to obtain a rough estimate of $FeNO_{0.05}$ by multiplying them by a coefficient of approximately 1.6 [21].

Pulmonary Function

Pulmonary function was assessed in accordance with the criteria of the American Thoracic Society using a Spirolab spirometer (Medical International Research). The lower limit of normal, Z-scores, and percentiles for FEV_1 were calculated for each individual in the study population based on the Excel macro for The Global Lung Function Initiative (GLI) [22] reference values.

Skin Prick Test

SPTs were performed in a subsample (n=374) at baseline, as previously described [9]. This subsample consisted of all volunteering participants from 2 of the schools, as well as adolescents from all the other schools who had reported asthma or symptoms suggestive of asthma or had $FeNO_{0.1}$ values ≥15 ppb or FEV_1 % predicted <80%, and attended a clinical follow-up visit within 2 months from the baseline examination at school. The most common airborne allergens in the area (cat, dog, birch pollen, and timothy pollen) were tested (Soluprick, ALK), together with *Dermatophagoides pteronyssinus*, which is uncommon in this part of Sweden. Since only 3.3% had positive SPT results for *D pteronyssinus*, these data were not included in further analyses. A positive SPT result was defined as a mean wheal diameter of at least 3 mm after 15 minutes [23].

Statistics

Statistical analyses were performed using STATA 1C 14 (StataCorp). Comparisons at the group level were performed using the *t* test for normally distributed continuous variables or χ^2 tests for categorical variables. FeNO was log-transformed to achieve a normal distribution before the *t* tests. Multiple logistic regression analyses were performed with incident allergic symptoms as dependent variables, FeNO, and relevant confounders identified as significant ($P<.05$) in the univariate analyses were independent variables. Height, which is the best determinant of FeNO in healthy schoolchildren [24], and FeNO measurement during the pollen season were additionally used as independent variables. Furthermore, a model was created where a FeNO value above arbitrary levels (50th percentile, 75th percentile, and 90th percentile) was used as an independent variable of incident allergic symptoms to cat and dog, respectively, after adjustments for confounders identified in previously described univariate analyses. A *P* value <.05 was considered statistically significant.

Ethics

The study was approved by the Ethics Committee of the Medical Faculty of Uppsala University, Sweden (registration numbers 243/1998, 499/2001). Informed consent was obtained from the children and their legal guardians.

Results

A total of 921 of the 959 participants (96%) completed the questionnaire in SPAIS II. There were 38 (4%) nonresponders, who differed only with regard to having higher FEV_1 % predicted and having reported less wheeze at baseline than those who participated in SPAIS II (Supplementary Table 1). Questions concerning smoking were only addressed to the mother, father, and older siblings in SPAIS I, although in SPAIS II there was an additional question concerning current self-reported smoking, whose frequency was found to be 8.9%.

The prevalence of wheeze, but not asthma, rhinitis, and allergic symptoms to cat, dog, and pollen allergens, increased between baseline and the end of follow-up (Table 1).

Table 1. Individuals' Characteristics at Baseline (SPAIS I) and After Follow-up (SPAIS II)[a]

	SPAIS I (n=921)	SPAIS II (n=921)	*P* Value
Male sex, %	49.5		
Age, y	13.6 (0.41)		
FeNO0.1, ppb	4.78 (4.47-5.10)		
FEV_1, % predicted	94.8 (10.8)		
Height, cm	162.4 (8.1)		
Weight, kg	52.8 (10.5)		
Asthma, %	8.7	7.9	.19
Wheeze, %	13.7	16.4	.04
Rhinitis, %	25.3	31.2	.001
Allergic symptoms to cat, %	10.3	14.7	<.001
Allergic symptoms to dog, %	5.4	7.6	.003
Allergic symptoms to pollen, %	17.5	23.7	<.001
Family members smoking, %	32.5	31.8	.59

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in 1 second; ppb, parts per billion.
[a]All results are presented as % or mean (SD) or geometric mean and 95%CI.

FeNO measured during the pollen season (March to September) was significantly higher ($P=.03$) than FeNO measured between October and February.

Incident Allergic Symptoms to Cat

Participants with self-reported incident allergic symptoms to cat had significantly higher FeNO values at baseline than those without parent- or self-reported symptoms to cat at both assessments. When individuals with parent-reported suspected hypersensitivity to cat at baseline were excluded (n=14), this result remained significant ($P<.001$). The group with self-reported incident allergic symptoms to cat had higher FEV_1 % predicted, more frequent parent- and self-reported asthma, wheeze, rhinitis, and allergic symptoms to dog and pollen allergens at baseline. Furthermore, in the incident group, fewer individuals had undergone FeNO measurements during the pollen season than individuals who never reported any allergic symptoms to cat (Table 2). During follow-up, 30% of participants with self-reported incident allergic symptoms to cat also self-reported allergic symptoms to dog and 60% to pollen. However, the number of individuals with parent- and self-reported evidence of having a cat at home decreased over the 4 years in this group (from 26% to 18%). At follow-up, but not at baseline, there was a significant difference in having a cat at home ($P=.04$), with a lower prevalence in the incident group than among individuals who never developed allergic symptoms to cat.

Incident Allergic Symptoms to Dog

Participants with self-reported incident allergic symptoms to dog had significantly higher FeNO values and more

Table 2. Individuals' Characteristics at Baseline in Relation to Incident Allergic Symptoms to Cat (SPAIS II)[a]

	No Allergic Symptoms to Cat at Baseline or After Follow-up (n=776)	Incident Allergic Symptoms to Cat (n=50)	*P* Value
Male sex, %	49.7	42	.29
Age, y	13.6 (0.41)	13.6 (0.39)	.42
$FeNO_{0.1}$, ppb	4.17 (3.90-4.46)	6.89 (4.93-9.62)	<.001
FEV_1, % predicted	94.7 (10.6)	98.0 (11.5)	.03
Height, cm	162.5 (8.1)	160.2 (6.6)	.05
Weight, kg	52.6 (10.3)	52.4 (8.8)	.89
Asthma, %	3.7	14.0	.001
Wheeze, %	8.5	20.0	.006
Rhinitis, %	18	52.0	<.001
Allergic symptoms to dog, %	0.4	8.0	<.001
Allergic symptoms to pollen, %	10.6	30.0	<.001
Exposure to cat, year 1, %	21.3	10.0	.06
Exposure to cat, SPAIS I, %	30.2	26.0	.53
Exposure to cat, SPAIS II, %	31.7	18.0	.04
FeNO measurements inside pollen season, %	36.6	22.0	.04
Ever asthma (mother), %	9.1	14.0	.26
Ever asthma (father), %	8.1	12.0	.34
Ever asthma (siblings), %	13.5	12.0	.76
Ever allergic rhinitis (mother), %	24.4	34.0	.13
Ever allergic rhinitis (father), %	22.7	22.0	.91
Ever allergic rhinitis (siblings), %	19.1	20.0	.87

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in 1 second; ppb, parts per billion.
[a]All results are presented as % or mean (SD) or geometric mean and 95% confidence interval.

Table 3. Individuals' Characteristics at Baseline in Relation to Incident Allergic Symptoms to Dog (SPAIS II)[a]

	No Allergic Symptoms to Dog at Baseline or After Follow-up (n=838)	Incident Allergic Symptoms to Dog (n=33)	*P* Value
Male sex, %	49.6	45.5	.64
Age, y	13.6 (0.41)	13.6 (0.38)	.96
$FeNO_{0.1}$, ppb	4.37 (4.10-4.67)	9.60 (7.26-12.69)	<.001
FEV_1, % predicted	94.9 (10.6)	94.7 (10.2)	.91
Height, cm	162.3 (8.1)	162.8 (8.1)	.68
Weight, kg	52.5 (10.4)	55.3 (10.0)	.14
Asthma, %	5	24.2	<.001
Wheeze, %	10.1	39.4	<.001
Rhinitis, %	21.7	60.6	<.001
Allergic symptoms to cat, %	4.7	39.4	<.001
Allergic symptoms to pollen, %	12.9	51.5	<.001
Exposure to dog, year 1, %	14.7	18.2	.58
Exposure to dog, SPAIS I, %	21.4	24.2	.69
Exposure to dog, SPAIS II, %	22.8	21.2	.83
FeNO measurements inside pollen season, %	35.7	39.4	.66
Ever asthma, (mother), %	9.4	21.2	.03
Ever asthma (father), %	8.5	18.2	.05
Ever asthma (siblings), %	13.8	27.3	.03
Ever allergic rhinitis (mother), %	25.4	45.5	.01
Ever allergic rhinitis (father), %	24.1	27.3	.68
Ever allergic rhinitis (siblings), %	19.9	27.3	.30

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in 1 second; ppb, parts per billion.
[a]All results are presented as % or mean (SD) or geometric mean and 95% confidence interval.

frequent parental- and self-reported asthma, wheeze, rhinitis, and allergic symptoms to cat and pollen at baseline than those without allergic symptoms to dog at both time points. After excluding individuals with parent-reported suspected hypersensitivity to dog at baseline (n=5), there was still a significant difference in FeNO (*P*<.001). A family history of asthma was more common in the incident group, as was rhinitis reported by the mother (Table 3). Among participants with self-reported incident allergic symptoms to dog, 76% self-reported allergic symptoms to cat and 55% to pollen in SPAIS II. There was no difference in the number of individuals with parent- and self-reported evidence of having a dog at home, at baseline or follow-up.

Incident Allergic Symptoms to Pollen

Participants who self-reported incident allergic symptoms to pollen more often had parent- and self-reported asthma, wheeze, rhinitis, allergic symptoms to cat, and allergic rhinitis

among siblings at baseline than individuals without parent- and self-reported allergic pollen symptoms at both time points. There was no significant difference regarding FeNO, for parent-and self-reported incident hypersensitivity to pollen (*P*=.08) (Supplementary Table 2) or when individuals with suspected hypersensitivity to pollen at baseline were excluded (*P*=.11). Among participants who self-reported incident allergic symptoms to pollen at SPAIS II, 29% also self-reported allergic symptoms to cat and 6% to dog.

Multivariate Analysis

Multiple logistic regression analysis revealed that elevated FeNO at baseline was independently related to self-reported incident allergic symptoms to cat (*P*<.001), after adjustments were made for possible confounders (see Material and Methods). Similarly, elevated FeNO values were independently related to self-reported incident allergic symptoms to dog (*P*<.048).

Analyses based on arbitrary FeNO cut-offs showed that having a FeNO value above the 50th, 75th, or 90th percentile was related to incident allergic symptoms to cat after the same adjustments as above. Except for FeNO >50th percentile, which only tended towards significant, this association held for individuals without parent- and self-reported asthma, wheeze, or rhinitis at baseline (18 individuals with incident self-reported allergic symptoms to cat remained in these analyses) (Table 4).

Similarly, having a FeNO above the 50th, 75th, or 90th percentile was related to self-reported incident allergic symptoms to dog after adjusting for the confounders described above. However, no significant associations were found when looking only at individuals without parent- and self-reported asthma, wheeze, or rhinitis at baseline (9 individuals with incident allergic symptoms to dog remained in these analyses) (Table 5).

Table 4. Adjusted[a] OR for Self-reported Incident Allergic Symptoms to Cat

aOR (95%CI) for Incident Allergic Symptoms to Cat	All Individuals Without Allergic Symptoms to Cat at Baseline (n = 826)	All Individuals Without Allergic Symptoms to Cat and No Asthma, Wheeze, or Rhinitis at Baseline (n=610)
$FeNO_{0.1}$ > 50th percentile[b]	3.0 (1.5-6.1)	2.9 (1.0-8.4)
$FeNO_{0.1}$ > 75th percentile[b]	4.2 (2.2-8.0)	8.6 (3.0-24.1)
$FeNO_{0.1}$ > 90th percentile[b]	4.0 (1.9-8.6)	10.9 (3.6-33.0)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; OR, odds ratio.
[a]Adjusted for FEV_1 % predicted, height, asthma, wheeze, rhinitis, allergic symptoms to dog and pollen, FeNO measurement during the pollen season, at baseline; and cat exposure at SPAIS II.
[b]In the case of incident allergic symptoms to cat, levels are 4.6 ppb for the 50th percentile, 7.3 ppb for the 75th percentile, and 12.1 ppb for the 90th percentile.

Table 5. Adjusted[a] OR for Self-reported Incident Allergic Symptoms to Dog

aOR (95%CI) for Incident Allergic Symptoms to Dog	All Individuals Without Allergic Symptoms to Dog at Baseline (n = 871)	All Individuals Without Allergic Symptoms to Dog and No Asthma, Wheeze, or Rhinitis at Baseline (n=621)
FeNO >50th percentile[b]	2.8 (1.1-7.5)	2.3 (0.5-10.2)
FeNO >75th percentile[b]	3.3 (1.5-7.6)	4.4 (1.0-20.1)
FeNO >90th percentile[b]	3.2 (1.4-7.6)	1.1 (0.1-12.6)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s.
[a]Adjusted for asthma, wheeze, rhinitis, allergic symptoms to cat and pollen, maternal asthma and rhinitis, height and FeNO measurement during the pollen season, at baseline.
[b]In the case of incident allergic symptoms to dog, levels are 4.7 ppb for the 50th percentile, 7.6 ppb for the 75th percentile, and 13 ppb for the 90th percentile.

SPT results from baseline were available for 374 participants. A positive SPT result for cat was recorded in 69 individuals, 45 of whom had parent-reported allergic symptoms to cat and 24 had not. A positive SPT result for dog at baseline was identified in 46 individuals, 25 of whom had parent-reported allergic symptoms to dog and 21 had not. In the group of participants who had developed allergic symptoms to cat in SPAIS II (n=50), 25 underwent SPT at baseline; of these, 14 had a positive SPT result and 11 had a negative SPT result for cat. Using the same logistic regression model as above for the subgroup with SPT results available (not excluding individuals with asthma, wheeze, or rhinitis at baseline) and adding adjustment for sensitization to cat, we found significant associations for FeNO >50th (aOR, 6.2 [1.2-33.3]) and > 75th percentile (aOR, 4.1 [1.2-13.9]), but not for FeNO >90th percentile. SPT results were also available for dog in 330 participants. Of the 33 individuals with self-reported incident allergic symptoms to dog, 22 underwent SPT at baseline; of these, 8 had a positive result and 14 had a negative result for dog. Adding the adjustment for sensitization to dog in a similar logistic regression model as that described above revealed no significant association with FeNO at baseline.

Discussion

The main finding of this population-based, longitudinal study of schoolchildren is that increased levels of exhaled NO predicted the onset of self-reported allergic symptoms to cat and dog within 4 years, despite the fact that the number of individuals reporting having a cat at home had decreased and remained unchanged for dogs. However, no association

between elevated FeNO at baseline and self-reported incident allergic symptoms to pollen was found in the same timeframe.

The group with self-reported incident allergic symptoms to cat or dog had elevated FeNO at baseline, and this probably signals subclinical T_H2-driven inflammation of the airways that precedes the development of such allergic symptoms. FeNO was higher in the group with self-reported incident allergic symptoms to dog than in the group with such symptoms to cat, thus indicating a higher degree of airway inflammation [9]. Less than one third of the participants who self-reported incident allergic symptoms to cat also reported allergic symptoms to dog, while more than three quarters of those with self-reported incident allergic symptoms to dog also reported allergic symptoms to cat. Thus, it seems that cat is a more common initial sensitizer than dog, and that dog-sensitized individuals are sensitized to more allergens. This may explain the higher NO values, as the level of FeNO is related to the degree of IgE-mediated sensitization [11,12], as well as exposure to allergens.

In support of the above, we previously reported, for a subgroup with SPT results in this cohort, that dog-sensitized individuals have higher levels of FeNO than cat-sensitized individuals [9]. In the case of participants with incident self-reported allergic symptoms to dog or cat, FeNO at baseline was between the levels for nonsensitized individuals and those for individuals sensitized to dog or cat. This supports the view that FeNO is a marker of subclinical airway inflammation that precedes the development of allergic symptoms and even confirmed sensitization. Other studies have shown that low-grade IgE-mediated sensitization (IgE antibody concentrations <0.35 kU_A/L) may precede symptoms [25]. Such low-grade IgE-mediated sensitization cannot be detected through SPTs.

A family history of asthma and allergy is a known risk factor for developing asthma and allergic symptoms. In our study, a family history was seen more frequently among participants who developed allergic symptoms to dogs than among those who developed allergic symptoms to cats. This may be because cat is a stronger sensitizing allergen that is not dependent on family history of atopic disease to break tolerance, whereas sensitization to dog may require a family predisposition. This reasoning is further supported by the findings described above. Moreover, in line with a previous study [26], our results showed a trend towards a lower risk of developing allergic symptoms to cat for individuals who had been exposed to cat during the first year of life. However, no such effect was found for having a dog in the home during the first year of life.

Adolescents with self-reported incident allergic symptoms to cat or dog had parent- and self-reported asthma, wheeze, and rhinitis to a larger extent at baseline; all of these conditions are related to elevated FeNO [1,16,15]. Therefore, it could be argued that the presence of these conditions was related to both elevated FeNO at baseline and self-reported incident allergic symptoms. However, we were able to confirm the main findings after adjustments for asthma, wheeze, or rhinitis at baseline. Furthermore, this association was found even after exclusion of individuals with parent- and self-reported asthma, wheeze, or rhinitis at baseline, at least for incident allergic symptoms to cat.

Elevated FeNO at baseline was not associated with self-reported incident allergic symptoms to pollen, which is in line with findings from other studies. Together with asthma, sensitization to perennial but not seasonal allergens is the most important determinant for FeNO [9,27]. The baseline FeNO measurements were performed during the school year, from the beginning of September to the end of May and included the birch pollen period, but not the grass or mugwort pollen periods. Participants whose FeNO measurements were taken between March and September had significantly higher values than those whose values were measured between October and February. These findings were adjusted for in the logistic regression models and did not affect the relationship between FeNO and incident self-reported allergic symptoms to cat or dog.

A major strength of the current longitudinal study of schoolchildren is the very high participation rate during follow-up (96%). Another strength is the use of well-validated questions from the ISAAC questionnaire and, to a large extent, the same additional questions at both time points. However, questionnaire data are limited because they depend on how individual participants interpret the questions and how they assess possible experienced symptoms. Furthermore, some additional bias might have been introduced by the fact that parents reported their children's allergic symptoms at baseline, whereas the participants themselves reported their allergic symptoms after follow-up. There is a risk of report bias, as the parents may not remember their child's past medical history and may not perceive the child's symptoms appropriately. While the adolescents were judged to be too young to answer that part of the questionnaire in SPAIS I, 4 years later, when they were aged 16-19 years, they were more appropriate responders than their parents. However, we find bias unlikely, as baseline FeNO is an objective measure and the participants did not have information on FeNO available during follow-up. Furthermore, sensitivity at baseline was increased by asking the parents if they suspected hypersensitivity in their child, thus making the omission of hypersensitivity less likely.

Another limitation of the study may be that we only had SPT results for a subpopulation. However, our study focused on allergic symptoms and not on IgE-mediated sensitization, and the SPT results were only used to validate the specificity of the questions regarding parent- and self-reported allergic symptoms. Furthermore, the available data showed a poor relationship between a positive SPT result and parent-reported ongoing allergic symptoms at baseline, as well as incident self-reported symptoms. Moreover, we could confirm that elevated FeNO was associated with incident allergic symptoms to cat, even after adjusting for a positive SPT result for cat. Given our generally cold and dry climate, *D pteronyssinus* is not a major sensitizer or inducer of allergic symptoms in this part of Sweden. Consequently, we chose not to study these allergic symptoms any further, thus preventing us from generalizing our results to other parts of Europe or the world, where mite is a major cause of allergic symptoms.

This study was performed 20 years ago with a questionnaire-based follow-up 4 years later. The data reported on the prevalence of allergic diseases differ from those reported today, although the aim of this study was mainly to evaluate

the relationship between FeNO and the development of allergic symptoms in a 4-year time frame.

We are aware that by using $FeNO_{100}$ and presenting the results using percentiles, our data are not entirely typical of clinical practice. More studies are needed to establish useful reference values for FeNO. Nevertheless, this study highlights elevated FeNO as a risk factor for the development of perennial allergies.

Conclusions

Our results showed that increased levels of exhaled NO in adolescents aged 12-15 years precede incident self-reported allergic symptoms to cat and dog within 4 years. These results were consistent for cat when individuals with any kind of respiratory symptoms at baseline were excluded. Therefore, elevated FeNO seems to indicate an increased risk of perennial allergies.

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Conflicts of Interest

KA has received research support from Aerocrine AB. The other authors declare that they have no conflicts of interest.

Previous Presentation

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