Elevated exhaled nitric oxide in adolescents relates to incident allergic symptoms: a prospective cohort study

Running title: Elevated FeNO predicts allergic symptoms

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Abstract

Background: The fraction of exhaled nitric oxide (FeNO) is a marker of type-2 inflammation in the airways and elevated FeNO may precede 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 a general population answered, together with their parents, a standardized questionnaire, performed lung function and FeNO measurements at a baseline visit. Four years later, 921 of these subjects (96%) completed a to a great extent 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 ($p < 0.001$) than subjects without allergic symptoms to cat and dog at either time point ($n = 776$ and $n = 838$, respectively). Adolescents with incident allergic symptoms to pollen did not have elevated baseline FeNO.

The adjusted odds ratio [aOR (95% confidence interval)] for incident allergic symptoms to cat was 4.2 (2.2, 8.0) times higher if FeNO was $> 75^{th}$ percentile (vs. $< 75^{th}$ percentile) at baseline. This was consistent after exclusion of subjects with reported asthma, wheeze or rhinitis at baseline [aOR (95% CI) 8.6 (3.0, 24.1)].

Conclusion: Elevated FeNO in adolescents related to an increased risk of developing allergic symptoms to cat and dog, but not pollen allergens, within four years.

Key words: Adolescents, Breath test, Epidemiology, Hypersensitivity, Incident, Nitric oxide

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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 custro 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, Incidente, Óxido nítrico

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Introduction

In the beginning of the 1990's, it was found that the fraction of exhaled nitric oxide (FeNO) was 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, specifically related to the expression of interleukin (IL)-4 and IL-13 in the bronchial mucosa, than general eosinophilic inflammation [4]. FeNO signals short-term changes in type-2 inflammation of the airways, but has also been shown to associate with long-term changes in IgE-antibody concentrations [5]. Furthermore, measurement of FeNO is valued as a useful tool to identify the atopic phenotype among asthmatics [4], as well as corticosteroid-sensitive inflammation in the airways [6].

Allergic sensitization relates to increased FeNO in both population-based studies and asthma patients [7], [8]. Moreover, exhaled NO correlates with the degree of IgE sensitization, in terms of both the number of positive skin-prick tests [9, 10] and IgE-antibody concentrations [11, 12]. We have previously reported the association of upper airway symptoms such as rhinitis and rhino-conjunctivitis, as well as asthma, with increased exhaled NO in the present cohort [13].

Increased FeNO has been reported in both children and adults without acknowledged respiratory symptoms, and is related to an increased risk of developing wheeze; thus, it may indicate subclinical inflammation or "early asthma" [13-15]. We have previously reported that adolescents in the present cohort with elevated NO levels had an increased likelihood of new-onset rhinitis within a four-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

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the predictive value of FeNO for self-reported incident allergic symptoms to cat, dog or pollen within a four-year time frame, in a large population-based cohort of adolescents.

Materials and methods

General design

Baseline data were collected 1998−1999 and included 959 subjects, aged 12−15 years, from nine randomised schools in Uppsala, Sweden. The study, *Screening Project Asthma in Schools* (SPAIS I), has previously been described in detail [13]. The pupils answered a questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC) [17], [18], performed lung function and FeNO measurements in their schools, and their parents answered additional written questions about their children concerning hypersensitivity to cat, dog or pollen, asthma diagnosis, asthma medication, atopic disease in childhood, family history of asthma and rhinitis, family smoking and environmental issues. If the measurements of FeNO and FEV1 were performed between March to September, they were judged to be inside 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 or pollen, asthma diagnosis, asthma medication, environmental issues, family smoking, and an additional question concerning own smoking was performed four years later (2002−2003), in which the adolescents completed the questionnaires themselves.

Questionnaires and definitions

At baseline, allergic symptoms were defined as the subject's hypersensitivity to cat, dog or pollen, noticed and reported by the parents. For negative answers, there was an additional question regarding parental suspicion of hypersensitivity to cat, dog or pollen. Allergic symptoms at follow-up were defined as above, but reported by the participant and only at the

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level “have you noticed hypersensitivity to cat, dog or pollen”. Incident allergic symptoms refer to no reported symptoms at baseline but at follow-up.

Asthma was defined as ever having had parental- or self-reported asthma plus having used corticosteroid asthma treatment or having wheezing or whistling in the chest (ISAAC) or having a respiratory infection that caused wheezing or whistling in the chest in the previous year (ISAAC). Wheeze was defined as having had wheezing or whistling in the chest at any time in the previous year (ISAAC). Rhinitis was defined as having had sneezing, nasal congestion or rhinorrhoea during the previous twelve months, without having a cold (ISAAC).

Asthma and rhinitis in the family were questionnaire-assessed, with separate questions regarding mother, father and siblings. Family smoking, the subject’s own current smoking habits at follow-up, and exposure to cat and dog allergens at home were also questionnaire-assessed.

Exhaled NO measurements

Measurements of FeNO were performed with the Aerocrine NO system (Aerocrine AB, Sweden), including the CLD 77 AM chemiluminescence analyser (Eco Physics AG, Dürnten, Switzerland) , as previously described [13], and in accordance with the recommendations from the European Respiratory Society [19]. Before measurement, each subject’s mouth was washed with 25 ml of 10% sodium bicarbonate for 20 s. Three exhalations during 10 s 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 way to transform the $FeNO_{0.1}$ values to obtain a rough estimate of $FeNO_{0.05}$, is to multiply them with a coefficient of approximately 1.6 [21].

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Measurements of pulmonary function

Pulmonary function measurements were performed in accordance with the criteria from the American Thoracic Society, using a Spirolab spirometer (Medical International Research, Rome, Italy). Using the Excel macro for The Global Lung Function Initiative (GLI) [22] reference values, lower limit of normal (LLN), Z-scores and percentiles for FEV_1 were calculated for each subject in the study population.

Skin-prick test

Skin-prick tests (SPTs) were performed in a sub-sample (n = 374) at baseline as previously described [9]. This sub-sample consisted of all volunteering participants from two of the schools, as well as adolescents from all 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 took part in a clinical follow-up visit within two 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, Horsholm, Denmark) together with Dermatophagoides pteronyssinus, which is uncommon in this part of Sweden. Only 3.3% were SPT positive for Dermatophagoides pteronyssinus and consequently, these data were not included in further analyses. A positive SPT 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, College Station, Texas, USA). Comparisons at the group level were done using t-tests for normally distributed continuous variables or using chi-squared tests for categorical variables. FeNO was log-transformed to achieve normal distribution before t-tests. Multiple logistic regression, with

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incident allergic symptoms as outcomes, and FeNO, along with relevant confounders identified as significant ($p<0.05$) in the univariate analyses, were used as predictors. Height, known from literature to be the best determinant of FeNO in healthy schoolchildren [24], was an additive predictor as well as FeNO measurements inside or outside the pollen season. Furthermore, a model was created where a FeNO value above different arbitrary levels (50^{th} percentile, 75^{th} percentile and 90^{th} percentile) was used as a predictor for incident allergic symptoms to cat and dog, respectively, after adjustments for confounders identified in previously described univariate analyses. A p value < 0.05 was considered statistically significant.

Ethics

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

Results

A total of 921 of the 959 subjects (96%) completed the questionnaire in SPAIS II. There were 38 (4%) non-responders, who differed only with regard to having higher FEV_1 % predicted, and having reported less wheeze at baseline compared with those who participated in SPAIS II (E-table 1). Questions concerning smoking were only addressed to the mother, father and older siblings at SPAIS I but at SPAIS II there was an additional question concerning self-reported current smoking, which was found to be 8.9%.

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

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FeNO measured inside the pollen season, between March and September, were significantly higher (p=0.03) compared to FeNO measured between October and February.

Incident allergic symptoms to cat

The subjects with self-reported incident allergic symptoms to cat had significantly higher FeNO values at baseline compared with those without parental- and self-reported symptoms to cat at either assessment. When also excluding subjects with parental-reported suspected hypersensitivity to cat at baseline (n=14), this result remained significant (p<0.001). The group with self-reported incident allergic symptoms to cat had higher FEV_1 % predicted, were shorter, and had parental- and self-reported asthma, wheeze, rhinitis and allergic symptoms to dog and pollen to a larger extent at baseline. In the incident group, fewer subjects had performed FeNO measurements inside the pollen season, March to September, compared to subjects who never reported any allergic symptoms to cat (Table 2). At the follow-up, among participants with self-reported incident allergic symptoms to cat, 30% also self-reported allergic symptoms to dog and 60% to pollen. However, the number of subjects who parental- and self-reported having a cat at home had decreased over the four years in this group (from 26% to 18%). At follow-up, but not at baseline, there was a significant difference in holding a cat at home (p=0.04), with lower prevalence in the incident group compared to those who never developed any allergic symptoms to cat.

Incident allergic symptoms to dog

Participants with self-reported incident allergic symptoms to dog had significantly higher FeNO values and parental- and self-reported asthma, wheeze, rhinitis and allergic symptoms to cat and pollen at baseline than those without allergic symptoms to dog at either time point. After also excluding subjects with parental-reported suspected hypersensitivity to dog at baseline (n=5), there was still a significant difference in FeNO (p<0.001). A family history of

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asthma was more common in the incident group, as was rhinitis reported by the mother (Table 3). Among subjects 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 subjects who parental- and self-reported having a dog at home, at baseline or follow-up.

Incident allergic symptoms to pollen

Subjects who self-reported incident allergic symptoms to pollen more often parental- and self-reported asthma, wheeze, rhinitis, allergic symptoms to cat and allergic rhinitis among siblings at baseline, when compared with subjects without parental- and self-reported allergic pollen symptoms at either time point. There was no significant difference regarding FeNO, for parental-and self-reported incident hypersensitivity to pollen (p=0.08) (E-table 2) or when subjects with suspected hypersensitivity to pollen at baseline were excluded (p=0.11). Among subjects 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

In a multiple logistic regression analysis, elevated FeNO at baseline independently related to self-reported incident allergic symptoms to cat ($p < 0.001$), after adjustments were made for variables identified as significant in univariate analysis as well height and FeNO measurements inside or outside pollen season (Table 2). Similarly, elevated FeNO values independently related to self-reported incident allergic symptoms to dog ($p<0.048$; Table 3).

Analyses based on different arbitrary FeNO cut-offs showed that having a FeNO above the 50^{th}, 75^{th} or 90^{th} percentile was related to incident allergic symptoms to cat after the same adjustments as above. This was consistent, except for FeNO $>50^{th}$ percentile which just

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showed a trend to be significant, when looking at subjects without parental- and self-reported asthma, wheeze or rhinitis at baseline (18 subjects remained with incident self-reported allergic symptoms to cat) (Table 4).

Similarly, having a FeNO above the 50th, 75th or 90th percentile was related to self-reported incident allergic symptoms to dog, after the same adjustments as above (Table 3). However, no significant relations were found when looking only at subjects without parental- and self-reported asthma, wheeze or rhinitis at baseline (9 subjects remained with incident allergic symptoms to dog) (Table 5).

SPT results from baseline were available in 374 subjects. A positive SPT for cat was present in 69 subjects, 45 of which had parental-reported allergic symptoms to cat and 24 had not. Corresponding positive SPT results for dog at baseline were 46, 25 of which had parental-reported allergic symptoms to dog and 21 had not. In the group of subjects who had developed allergic symptoms to cat in SPAIS II (n = 50), 25 subjects performed SPT at baseline and out of them 14 had a positive and 11 had a negative SPT for cat. When using the same logistic regression model as above for the subgroup with SPT results available (not excluding subjects with asthma, wheeze or rhinitis at baseline) and adding adjustment for sensitization to cat, significant relations were found for FeNO > 50th (aOR of 6.18 (1.15, 33.30)) and > 75th percentile (aOR of 4.07 (1.19, 13.94)), but not for FeNO > 90th percentile. SPT results were also available for dog in 330 subjects. Of the 33 subjects with self-reported incident allergic symptoms to dog, 22 subjects performed SPT at baseline and out of them 8 had a positive and 14 had a negative SPT for dog. When adding the adjustment for sensitization to dog in a similar logistic regression model as above, no significant relation to FeNO at baseline could be seen.

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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 four years, despite that the number of subjects reporting holding a cat at home had decreased and was unchanged for dogs. However, no relation 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 a subclinical Th2-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, indicating a higher degree of airway inflammation [9]. Less than one third of the subjects 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 subjects are sensitized to more allergens. This may explain the higher NO values, as the level of FeNO is related to the degree of IgE sensitization [11], [12], as well as exposure to allergens.

In support of the above, we have previously reported in this cohort, for a subgroup with SPT results, that dog-sensitized subjects have higher levels of FeNO than cat-sensitized subjects [9]. For subjects with incident self-reported allergic symptoms to dog or cat, FeNO at baseline was in between the levels for non-sensitized subjects and those for subjects sensitized to dog or cat. This supports the view that FeNO signals subclinical airway inflammation that precedes the development of allergic symptoms and even confirmed sensitization, and other

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studies have shown that low-grade IgE sensitization (IgE antibody concentrations < 0.35 kU_A/L) may precede symptoms [25]. Such low-grade IgE sensitization may not be detected through SPTs.

A family history of asthma and allergy is a known risk factor for developing asthma and allergic symptoms, and, in our study, a family history was seen more frequently among those who developed allergic symptoms to dogs than cats. This may possibly be explained by cat being a stronger sensitizing allergen, not dependent on family history of atopic disease to break tolerance, whereas dog sensitization 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 subjects 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.

Subjects with self-reported incident allergic symptoms to cat or dog had parental- and self-reported asthma, wheeze and rhinitis to a larger extent at baseline, conditions 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 could confirm the main findings after adjustments for asthma, wheeze or rhinitis at baseline. Furthermore, this association was found even after exclusion of subjects who parental- and self-reported asthma, wheeze or rhinitis at baseline, at least for incident allergic symptoms to cat.

Elevated FeNO at baseline did not associate with self-reported incident allergic symptoms to pollen, which is in line with 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

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September to the end of May and included the birch pollen period, but not the grass or mugwort pollen periods. Subjects who performed their FeNO measurements between March and September had significantly higher values than subjects who measured FeNO between October and February. This was 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 with the current longitudinal study of schoolchildren is the very high participation rate (96%) in the follow-up part. Another strength is the use of well-validated questions from the ISAAC questionnaire and to a great extent, the same additional questions at both time points. However, questionnaire data have the weakness of depending on how individual subjects interpret the questions and how they assess possible experienced symptoms. Furthermore, some additional bias might have been introduced by the fact that parents reported on their children's allergic symptoms at baseline, whereas the participants themselves reported their allergic symptoms at follow-up. There is a risk for report bias as the parents may not remember their child's past medical history and may not perceive the child's symptoms in an adequate way. The adolescents were judged to be too young to answer that part of the questionnaire in SPAIS I, but, four years later when they were 16-19 years, they were more appropriate responders than their parents. However, we find a bias unlikely, as baseline FeNO is an objective measure and the subjects did not have information on FeNO available at the follow-up. Furthermore, the sensitivity at baseline was increased by asking the parents if they suspected hypersensitivity in their child, making the omission of hypersensitivity less likely.

Another limitation of the study may be that we had SPT results only in a subpopulation. However, our study focused on allergic symptoms and not IgE sensitization and the SPT

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results have only been used to validate the specificity of the questions regarding parental- and self-reported allergic symptoms. Furthermore, the available data showed a poor relationship between a positive SPT and parental-reported ongoing allergic symptoms at baseline as well as incident self-reported symptoms. Moreover, we could confirm that elevated FeNO related to incident allergic symptoms to cat even when adjusting for a positive SPT to cat. Due to our generally cold and dry climate, Dermatophagoides pteronyssinus is not a major sensitizer and inducer of allergic symptoms in this part of Sweden and consequently we have chosen to not study these allergic symptoms any further. This may limit the possibilities to generalize our results to other European or world regions where mite is a major cause of allergic symptoms.

This study was performed twenty years ago with a questionnaire follow-up four years later. The data reported on the prevalence of allergic diseases are different from today but the aim of this study was mainly to evaluate the relationship between FeNO and the development of allergic symptoms in a four-year time frame.

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

Conclusions

Results from this study showed that increased levels of exhaled NO in adolescents, 12-15 years old, precede incident self-reported allergic symptoms to cat and dog within four years. These results were consistent for cat when excluding subjects with any kind of respiratory symptoms at baseline. Thus, elevated FeNO seems to indicate increased risk of the development of perennial allergies.

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Table 1. Subjects' characteristics at baseline visit, SPAIS I, and at follow-up, SPAIS II

	SPAIS I (n=921)	SPAIS II (n=921)	p value
Male sex (%)	49.5		
Age (years)	13.6 ± 0.41		
$FeNO_{0.1}$ (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	0.19
Wheeze (%)	13.7	16.4	0.04
Rhinitis (%)	25.3	31.2	0.001
Allergic symptoms to cat (%)	10.3	14.7	<0.001
Allergic symptoms to dog (%)	5.4	7.6	0.003
Allergic symptoms to pollen (%)	17.5	23.7	<0.001
Family members smoking (%)	32.5	31.8	0.59

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.

All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

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Table 2. Characteristics of subjects at baseline in relation to incident allergic symptoms to cat at SPAIS II.

	No allergic symptoms to cat at baseline or follow-up (n = 776)	Incident allergic symptoms to cat (n = 50)	p value
Male sex (%)	49.7	42	0.29
Age (years)	13.6 ± 0.41	13.6 ± 0.39	0.42
$FeNO_{0.1}$ (ppb)	4.17 (3.90, 4.46)	6.89 (4.93, 9.62)	< 0.001
FEV_1 (% predicted)	94.7 ± 10.6	98.0 ± 11.5	0.03
Height (cm)	162.5 ± 8.1	160.2 ± 6.6	0.05
Weight (kg)	52.6 ± 10.3	52.4 ± 8.8	0.89
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
Cat exposure year 1 (%)	21.3	10	0.06
Cat exposure SPAIS I (%)	30.2	26	0.53
Cat exposure 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.

All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

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Table 3. Characteristics of subjects at baseline in relation to incident allergic symptoms to dog at SPAIS II

	No allergic symptoms to dog at baseline or follow-up (n = 838)	Incident allergic symptoms to dog (n = 33)	p value
Male sex (%)	49.6	45.5	0.64
Age (years)	13.6 ± 0.41	13.6 ± 0.38	0.96
$FeNO_{0.1}$ (ppb)	4.37 (4.10, 4.67)	9.60 (7.26, 12.69)	< 0.001
FEV_1 (% predicted)	94.9 ± 10.6	94.7 ± 10.2	0.91
Height (cm)	162.3 ± 8.1	162.8 ± 8.1	0.68
Weight (kg)	52.5 ± 10.4	55.3 ± 10.0	0.14
Asthma (%)	5	24.2	< 0.001
Wheeze (%)	10.1	39.4	< 0.001
Rhinitis (%)	21.7	60.6	< 0.001
Allergic symptoms to cat (%)	4.7	39.4	< 0.001
Allergic symptoms to pollen (%)	12.9	51.5	< 0.001
Dog exposure year 1 (%)	14.7	18.2	0.58
Dog exposure SPAIS I (%)	21.4	24.2	0.69
Dog exposure SPAIS II (%)	22.8	21.2	0.83
FeNO measurements inside pollen season (%)	35.7	39.4	0.66
Ever asthma (mother) (%)	9.4	21.2	0.03
Ever asthma (father) (%)	8.5	18.2	0.05
Ever asthma (siblings) (%)	13.8	27.3	0.03
Ever allergic rhinitis (mother) (%)	25.4	45.5	0.01
Ever allergic rhinitis (father) (%)	24.1	27.3	0.68
Ever allergic rhinitis (siblings) (%)	19.9	27.3	0.30

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.

All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

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Table 4. Adjusted[†] OR for self-reported incident allergic symptoms to cat

aOR (95%CI) for incident allergic symptoms to cat	All subjects without allergic symptoms to cat at baseline (n = 826)	All subjects without allergic symptoms to cat and no asthma, wheeze or rhinitis at baseline (n = 610)
$FeNO_{0.1} > 50^{th}$ percentile[‡]	3.02 (1.50, 6.06)	2.89 (1.00, 8.37)
$FeNO_{0.1} > 75^{th}$ percentile[‡]	4.18 (2.19, 7.98)	8.56 (3.04, 24.12)
$FeNO_{0.1} > 90^{th}$ percentile[‡]	4.03 (1.90, 8.56)	10.94 (3.63, 33.02)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1,}$ fractional exhaled nitric oxide measured at 100 mL/s; OR, odds ratio.

[†] Adjusted for FEV_1 % predicted, height, asthma, wheeze, rhinitis, allergic symptoms to dog and pollen, FeNO measurements inside pollen season, at baseline and cat exposure at SPAIS II.

[‡] for incident allergic symptoms to cat, levels corresponding to the 50^{th} percentile are 4.6 ppb, 75^{th} percentile: 7.3 ppb, 90^{th} percentile: 12.1 ppb.

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Table 5. Adjusted[†] OR for self-reported incident allergic symptoms to dog

aOR (95%CI) for incident allergic symtoms to dog	All subjects without allergic symptoms to dog at baseline (n=871)	All subjects without allergic symptoms to dog and no asthma, wheeze or rhinitis at baseline (n=621)
FeNO >50th percentile[‡]	2.83 (1.07, 7.45)	2.29 (0.51, 10.22)
FeNO >75th percentile[‡]	3.32 (1.45, 7.58)	4.38 (0.96, 20.05)
FeNO >90th percentile[‡]	3.19 (1.35, 7.55)	1.13 (0.10, 12.63)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1,}$ fractional exhaled nitric oxide measured at 100 mL/s.

[†] Adjusted for asthma, wheeze, rhinitis, allergic symptoms to cat and pollen, maternal asthma and rhinitis, height and FeNO measurements inside pollen season, at baseline.

[‡] for incident allergic symptoms to dog, levels corresponding to the 50th percentile are 4.7 ppb, 75th percentile: 7.6 ppb, 90th percentile: 13 ppb.

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