

Increased exhaled nitric oxide predicts new-onset rhinitis and persistent rhinitis in adolescents without allergic symptoms

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Summary

Background The fraction of nitric oxide in exhaled air (FE_{NO}) is increased in rhinitis and asthma. We have previously suggested that elevated FE_{NO} levels in the absence of asthma symptoms may be a sign of 'early asthma'. In the present study, we hypothesize that elevated exhaled NO levels may also precede rhinitis symptoms.

Objective To investigate in a cohort of adolescents whether or not increased exhaled NO levels at the age of 13–14 years predicted new-onset or persistent rhinitis within a 4-year period.

Methods A total of 959 randomly selected adolescents (13–14 years) completed a questionnaire on respiratory symptoms at baseline and follow-up, 4 years later. Exhaled NO was measured at baseline. After exclusion of subjects with asthma diagnosis or asthma symptoms at baseline, 657 participants were eligible for the present study.

Results Higher FE_{NO} levels at baseline were associated with increased risk for new-onset ($P = 0.009$) and persistent rhinitis ($P = 0.03$) within a 4-year period. The risk of new-onset rhinitis was 2.32 (1.23, 4.37) [OR (95% CI)] times higher if FE_{NO} > 90th percentile of the group without rhinitis at baseline. This increased risk for new-onset rhinitis was significant [2.49 (1.24, 5.01)] after excluding subjects with allergic symptoms. The risk of persistent rhinitis was 5.11 (1.34, 19.57) times higher if FE_{NO} > 90th percentile of the group without rhinitis at baseline.

Conclusion Elevated exhaled nitric oxide levels predicted incident and persistent rhinitis in this population-based study of adolescents. Moreover, these findings were consistent after excluding subjects with allergic symptoms. Thus, it appears that elevation of exhaled NO precedes airway symptoms and predicts development of rhinitis in subjects without allergic symptoms or family history of allergic disease.

Keywords exhaled nitric oxide, population-based study, predictor, rhinitis

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Background

Rhinitis is one of the most common chronic conditions in childhood [1], and the prevalence is increasing, especially in children and young adults [2, 3]. Exhaled nitric oxide (NO) is a well-validated marker of steroid-sensitive inflammation in the airways [4] with more than 2000 published articles on this topic. The presence of rhinitis has been associated with increased exhaled NO levels [5], probably reflecting the extension of inflammation all through the airways, a feature well in line with the concept of the 'united airway' [6]. We have previously reported association of upper airways symptoms, such as rhinitis or rhinoconjunctivitis, with

increased exhaled NO in the present cohort [7]. Rhinitis, both allergic and non-allergic, is an important risk factor for the development of asthma [8, 9].

Increased exhaled NO levels in subjects without acknowledged respiratory symptoms have previously been reported both in children and adults [7, 10, 11]. Similarly, increased bronchial responsiveness in the absence of clear asthma symptoms is not uncommon [12].

Apparently, no research has been done concerning the predictive value of increased exhaled NO for the development of upper airways symptoms. Recent research has focused on the relation between degree of IgE sensitization and the risk of developing allergic

disease [13], particularly asthma [14], rhinitis and rhinoconjunctivitis [15, 16]. Since rhinitis is the disease entity among allergic diseases that most strongly relates to atopy, and levels of exhaled NO relate to the degree of IgE sensitization [17, 18], it is plausible that elevated exhaled NO may precede onset of rhinitis symptoms.

We have previously suggested that increased exhaled NO levels without asthma symptoms may indicate sub-clinical airway inflammation and that this could be a sign of 'early asthma' [7], a hypothesis supported by a recent study [11]. In the present study, we have extended that thought and hypothesize that elevated exhaled NO levels may also precede rhinitis symptoms.

Thus, in our cohort of children we investigated whether or not increased exhaled NO levels at the age of 13–14 years predicted new-onset rhinitis within 4 years among subjects without respiratory symptoms at baseline. Moreover, we studied the relation between increased exhaled NO levels and persistent rhinitis symptoms among subjects who had upper airway symptoms at baseline.

Methods

Study subjects

This population-based study in schoolchildren has been previously described in detail [7]. The investigation was performed in randomized community schools in Uppsala, Sweden, from October 1998 to December 1999 (stage I). At that stage, 959 randomly selected schoolchildren aged 13–14 years answered a questionnaire and performed lung function tests and exhaled NO measurements.

A total of 928 (96.8%) participated in stage II, 4 years later (2002–2003), which consisted only of answering an identical questionnaire as in the baseline investigation. Adolescents with self-reported physician-diagnosed asthma, asthma symptoms or use of inhaled corticosteroids during the year previous to the inclusion in Stage I were excluded and a total of 657 adolescents aged 17–18 years were included in the present analysis.

Exhaled NO measurement

Measurement of the fraction of NO in exhaled air (FE_{NO}) is described in detail in a previous publication [7]. Briefly, measurements were done according to the current recommendations for on-line NO measurements at that time [19]. The target exhalation flow rate was 0.1 L/s and the exhalation time was 10 s. The coefficient of variation for the flow rate is 5 mL/s [20]. More details are presented in the Online Supplement.

Questionnaire

A validated questionnaire, previously used as part of the worldwide ISAAC study [21], was used. Cases with asthma diagnosis [22] or asthma symptoms as defined by the ISAAC questions A2 (wheezing last year), A7 (exercise-induced wheezing last year), A8 (dry cough at night last year) were excluded. Current rhinitis was defined as a positive answer to ISAAC question N2 (rhinitis last 12 months).

Allergic symptoms were defined as parent-observed allergic symptoms when the children were exposed to cats, dogs or pollens (see Online Supplement). These questions were validated in a subgroup of this material where skin prick tests (SPT) were performed and there was a good negative predictive value of these questions for negative SPT (see Table S1 Online Supplement). The overall negative predictive value of the parent-observed allergic symptoms for positive SPT was of 86.9% (see Table S1 Online Supplement).

The questions regarding ever use of corticosteroid treatment for respiratory problems, exposure to tobacco smoke and family history of allergic disease are presented in the Online Supplement. Active smoking was reported by only five subjects, representing 0.7% of the study participants.

Measurements performed in March–September were considered as performed in pollen season whereas measurements performed in October–February were considered outside season.

Statistics

Statistical analyses were performed using STATA 10 (Stata Corp., College Station, TX, USA). A P -value < 0.05 was considered as statistically significant.

Unpaired t -test of log-transformed FE_{NO} levels was used for comparisons between different groups. FE_{NO} levels are presented as geometric mean (95% confidence interval).

The value of FE_{NO} to predict new-onset or persistent rhinitis was assessed by means of logistic regression models. FE_{NO} was used in these models both as a quantitative and qualitative variable, using different percentiles of FE_{NO} in subjects without rhinitis at baseline as cut-off values. Fitted predicted probability curves for the risk of rhinitis according to FE_{NO} levels were plotted using the results from the logistic regression. Multiple logistic regression models were used also to adjust for the effect of covariates, as gender, BMI, exposure to tobacco smoke and inclusion in study during the pollen season (Tables 2 and 3). Additional covariates were included in Supplementary Tables S2 and S4 and these were presence of allergic symptoms, ever use of corticosteroids and family history of allergic disease. Height

and weight, separately, were used instead of BMI to confirm the main results presented in Tables 2, 3, S2 and S4 as height is one of the major determinants of exhaled NO in children [23].

Results

The participants were categorized into four groups based on their answers to the questionnaires at stage I and II: (i) no rhinitis at either time point ($n = 460$), (ii) new-onset rhinitis (rhinitis at stage II but not stage I) ($n = 92$), (iii) remission of rhinitis (rhinitis at stage I but not stage II) ($n = 40$) and (iv) persistent rhinitis (rhinitis at both stage I and II) ($n = 65$). The characteristics of the subjects are presented in Table 1.

New-onset rhinitis

Subjects with new-onset rhinitis showed a tendency towards higher FE_{NO} levels compared to subjects who did not develop rhinitis ($P = 0.06$) and the only observed differences were regarding increased prevalence of self-reported allergy ($P < 0.001$), increased BMI ($P = 0.003$) and a trend towards increased prevalence of family allergic disease ($P = 0.07$) (Table 1).

The probability of new-onset rhinitis increased with increasing FE_{NO} levels in a logistic regression model ($P = 0.009$) (Figure 1). The probability of new-onset rhinitis increased with increasing FE_{NO} both after excluding subjects with allergic symptoms at baseline ($n = 41$, $P = 0.01$) and after excluding subjects who developed allergic symptoms at follow-up ($n = 48$, $P = 0.01$). The probability of new-onset rhinitis increased with increasing FE_{NO} levels ($P = 0.03$) after adjusting for gender, BMI, exposure to tobacco smoke, allergic symptoms, ever use of corticosteroids for respi-

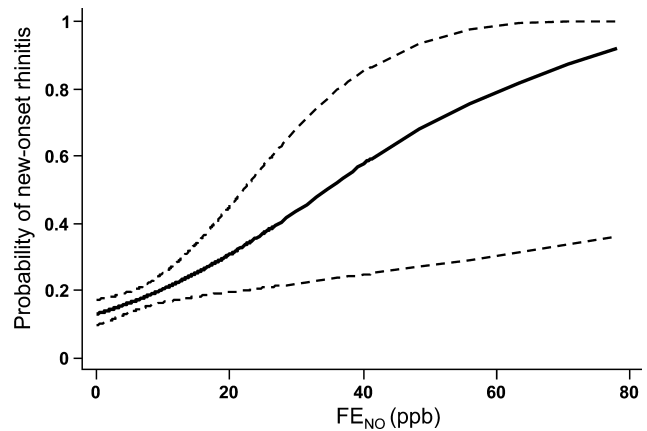


Fig. 1. Fitted predicted probability curves (continuous line) and 95% CI (dotted lines) for new-onset rhinitis at a given FE_{NO} level, as derived from the logistic regression analysis.

ratory problems, family history of allergic disease, season of FE_{NO} measurements (Online Supplement Table S2). The results were consistent when looking in the non-allergic stratum ($P = 0.01$) and also after further excluding subjects who developed allergic symptoms ($P = 0.02$). A trend towards an association of increased risk of new-onset rhinitis with increased FE_{NO} levels was found in a similar model as the one described in Online Supplement Table S2, when height and weight were used separately instead of BMI ($P = 0.052$). This association became significant after excluding subjects with allergic symptoms at baseline ($P = 0.03$).

In a multiple logistic regression, an increased FE_{NO} , defined as either 75th, 80th, 85th or 90th percentile of FE_{NO} values among the group who did not have any rhinitis symptoms at baseline, was a predictor of new-onset rhinitis (Table 2). The association with increased risk for onset of rhinitis for FE_{NO} levels over 75th per-

Table 1. Demographic characteristics, BMI and lung function parameters (FEV_1 , FEV_1/FVC) of study participants ($n = 657$) (mean $\pm$ SD or $n(\%)$) at baseline (1998–1999). The four groups are based on presence/absence of rhinitis at baseline and at follow-up 4 years later

	No rhinitis at both time points ($n = 460$)	New-onset rhinitis ($n = 92$)	Remission of rhinitis ($n = 40$)	Persistent rhinitis ($n = 65$)
Age (years)	13.6 $\pm$ 0.4	13.7 $\pm$ 0.4	13.6 $\pm$ 0.3	13.6 $\pm$ 0.4
Male sex [$n(\%)$]	242 (52.6%)	42 (45.7%)	23 (57.5%)	34 (52.3%)
Height (cm)	162.5 $\pm$ 8.0	163.4 $\pm$ 8.5	162.8 $\pm$ 8.7	162.4 $\pm$ 8.4
BMI (kg/m ²)	19.57 $\pm$ 2.69	20.55 $\pm$ 3.73	19.87 $\pm$ 3.04	20.09 $\pm$ 3.28
FE_{NO} (ppb)	4.0 (3.6, 4.3)	4.8 (4.0, 5.9)	3.6 (2.6, 4.8)	6.1 (4.8, 7.7)
FEV_1 (%predicted)	97.3 $\pm$ 10.8	99.7 $\pm$ 10.3	95.8 $\pm$ 13.9	99.9 $\pm$ 11.8
FEV_1/FVC (%predicted)	106.5 $\pm$ 6.5	107.6 $\pm$ 5.7	108.0 $\pm$ 5.4	107.0 $\pm$ 6.9
Exposure to tobacco smoke	49 (10.7%)	8 (8.7%)	5 (12.5%)	8 (12.3%)
Allergic symptoms	22 (4.8%)	19 (20.7%)	9 (22.5%)	42 (64.6%)
Ever use of corticosteroids	27 (5.9%)	8 (8.7%)	3 (7.5%)	10 (15.4%)
Family history of rhinitis	208 (45.2%)	50 (54.4%)	19 (47.5%)	40 (61.5%)
Family history of allergic disease*	274 (59.6%)	65 (70.7%)	25 (62.5%)	49 (75.4%)

*asthma, rhinitis or eczema

Table 2. Risk of new-onset rhinitis if FE_{NO} levels over 75th, 80th, 85th or 90th percentile of FE_{NO} values, after adjustments for gender, BMI, exposure to tobacco smoke and season of FE_{NO} measurements

	Risk for new-onset rhinitis		
	All subjects (<i>n</i> = 552)	Subjects without allergic symptoms (<i>n</i> = 511)*	Subjects with allergic symptoms (<i>n</i> = 38)*
FE _{NO} > 75th percentile [#]	1.99 (1.22, 3.23)	2.13 (1.24, 3.67)	1.18 (0.29, 4.76)
FE _{NO} > 80th percentile	2.06 (1.23, 3.44)	2.31 (1.31, 4.07)	1.39 (0.28, 6.81)
FE _{NO} > 85th percentile	2.32 (1.33, 4.04)	2.67 (1.45, 4.93)	1.05 (0.20, 5.48)
FE _{NO} > 90th percentile	2.32 (1.23, 4.37)	2.49 (1.24, 5.01)	7.61 (0.49, 118.9)

*allergic symptoms missing in three subjects

[#]levels corresponding to the 75th percentile – 7.0 ppb, 80th percentile – 7.6 ppb, 85th percentile – 9.1 ppb, 90th percentile – 10.6 ppb.

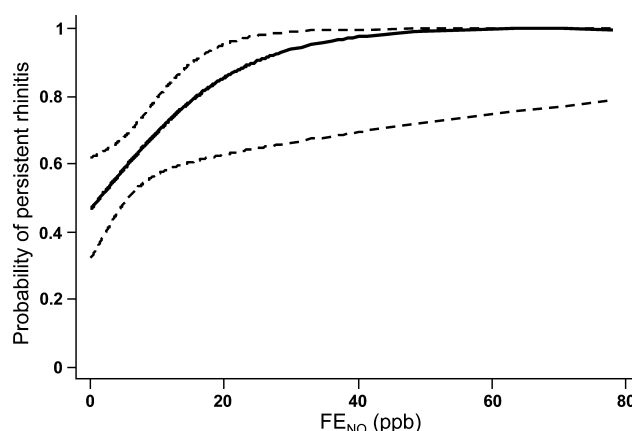
centile was statistically significant in the stratum without reported allergic symptoms (Table 2). No significant relationships were found in the stratum with reported allergic symptoms. The association with increased risk for onset of rhinitis for FE_{NO} levels over the cut-offs given in Table 2 was consistent when height and weight were used separately instead of BMI.

These results remained statistically significant after excluding subjects who have ever used corticosteroids for respiratory problems, had reported allergic symptoms, or had any family history of allergic rhinitis or any family history of allergic disease (Online Supplement Table S3).

Persistent rhinitis

Subjects with rhinitis remission had significantly lower levels of FE_{NO} than subjects with persistent rhinitis (*P* = 0.04) (Table 1). Subjects with persistent rhinitis had a higher prevalence of self-reported allergy than subjects with remission of symptoms (*P* < 0.001) (Table 1). No other differences between these two groups were found regarding the other variables listed in Table 1.

The probability of persistence of rhinitis was increased with increasing FE_{NO} levels in a logistic regression model (*P* = 0.03) (Figure 2). There was a trend (*P* = 0.057) towards a significant association between increased FE_{NO} and persistent rhinitis in logistic regression model after adjustments for gender, BMI,

**Fig. 2.** Fitted predicted probability curves (continuous line) and 95% CI (dotted lines) for persistent rhinitis at a given FE_{NO} level, as derived from the logistic regression analysis.

exposure to tobacco smoke, allergic symptoms, ever use of corticosteroids for respiratory problems, family history of allergic disease, season of FE_{NO} measurements (Online Supplement Table S4). This association was significant in the non-allergic stratum (*P* = 0.04), but not in the allergic stratum (*P* = 0.42). A significant association between increased FE_{NO} and persistent rhinitis (*P* = 0.048) was found in a similar model with the one described in Online Supplement Table S4 where weight and height were used instead of BMI.

In a multiple logistic regression, an increased FE_{NO}, defined as levels either > 75th, 80th, 85th or 90th per-

Table 3. Risk of persistent rhinitis if FE_{NO} levels over 75th, 80th, 85th or 90th percentile of FE_{NO} values, after adjustments for gender, BMI, exposure to tobacco smoke and season of FE_{NO} measurements

	Risk for persistent rhinitis		
	All subjects (<i>n</i> = 105)	Subjects without allergic symptoms (<i>n</i> = 54)	Subjects with allergic symptoms (<i>n</i> = 51)
FE _{NO} > 75th percentile [#]	3.71 (1.39, 9.90)	4.34 (1.08, 17.5)	2.95 (0.45, 19.5)
FE _{NO} > 80th percentile	3.33 (1.24, 8.95)	4.34 (1.08, 17.5)	2.02 (0.31, 13.0)
FE _{NO} > 85th percentile	3.33 (1.11, 9.96)	3.07 (0.63, 15.0)	2.97 (0.40, 22.1)
FE _{NO} > 90th percentile	5.11 (1.34, 19.57)	8.17 (0.76, 88.0)	2.31 (0.29, 18.6)

[#]levels corresponding to the 75th percentile – 7.0 ppb, 80th percentile – 7.6 ppb, 85th percentile – 9.1 ppb, 90th percentile – 10.6 ppb

centile of FE_{NO} values among the group who did have rhinitis symptoms at baseline, was a predictor of persistent rhinitis (Table 3). The relationship remained statistically significant after excluding subjects who ever used corticosteroids for any respiratory symptoms (Online Supplement Table S5). The association with increased risk for persistent rhinitis for FE_{NO} levels over 75th or 80th percentile was statistically significant in the stratum without allergic symptoms (Table 3). No significant relationships were found in the stratum with allergic symptoms. The association with increased risk for persistent rhinitis for FE_{NO} levels over the cut-offs given in Table 3 was consistent when height and weight were used separately instead of BMI.

The relationship between increased FE_{NO} levels (defined as > 75th or 90th percentile) and increased risk for persistent rhinitis remained statistically significant after excluding subjects who ever used corticosteroids for any respiratory symptoms (Online Supplement Table S5).

Discussion

This is the first population-based study analysing relationships between elevated exhaled NO levels and incidence and persistence of symptoms of rhinitis. The major and novel finding of the present study is that increased levels of exhaled NO in adolescents predict onset of rhinitis within 4 years in subjects without rhinitis at baseline. Furthermore, this indicator of risk for new-onset rhinitis was found in adolescents without known risk factors as reported allergic symptoms or family history of allergic disease. Rhinitis tended to persist among those who had both rhinitis and elevated NO levels and this finding was also consistent in subjects without any reported allergic symptoms.

One strength of this epidemiological study is the use of well-validated questions, previously used in studies reporting an association between degree of IgE sensitization and onset or persistence of rhinitis [15, 16]. Another strength is that the main findings were consistent when FE_{NO} was used as a continuous measure, instead of setting an arbitrary cut-off, to predict increased risk of new-onset of rhinitis.

The finding that the risk of new-onset of rhinitis increased with increasing exhaled NO levels is novel and in line with the very recent findings showing increased exhaled NO levels as a predictor of later onset of asthma or asthma symptoms in children [24] and adults [11]. The results are not as conclusive in infants, as one study reported a predictive value of increased exhaled NO for later development of respiratory symptoms, although only in infants with atopic and/or smoking mothers [25], and another study reported that only transient wheeze, but not persistent wheeze was associated with neonatal increased exhaled NO [26]. A possible mechanistic

explanation might be an enhanced Th2 cytokine-driven response in these subjects. In analogy, several studies have demonstrated a relationship between IgE sensitization and the risk for development of allergic disease [13, 14, 16], a relationship that is probably mainly explained by an enhanced Th2 lymphocyte response in subjects with a high degree of IgE sensitization [14, 27]. Th2 cells mainly secrete IL-4, IL-5 and IL-13. It has previously been demonstrated that IL-13 induces production of NO in the bronchial epithelium through induction of inducible nitric oxide synthase (iNOS) [28], and bronchial epithelial iNOS appears to be the main determinant of exhaled NO [29]. Furthermore, medications that block IL-4/IL-13 have been reported to reduce exhaled NO [30]. Thus, a high level of exhaled NO probably signals increased Th2-driven inflammatory activity in the airways [31]. Importantly, it appears that these inflammatory mechanisms may, in an early phase, be active without producing any symptoms of asthma or rhinitis. Thus, such symptoms manifest themselves later than elevation of exhaled NO.

Remission of rhinitis was associated with lower FE_{NO} levels at baseline, suggesting that subjects with normal levels of exhaled NO, but symptoms of rhinitis at 13–14 years, have a transient symptomatology and different type of underlying pathology. No studies focusing on airways inflammation in relation to rhinitis remission exist so this is only a plausible explanation.

We could demonstrate the predictive role of increased exhaled NO for incident or persistent rhinitis in the subgroup without allergic symptoms. As there was a very good negative predictive value for the questionnaire-assessed allergic symptoms, there is little risk that some of the subjects not reporting allergic symptoms were IgE-sensitized to cat, dog or pollen, the most common aeroallergens in Swedish children [32]. A significant proportion of the subjects with new-onset or persistent rhinitis appeared not to be IgE-sensitized, similarly with the subgroup of adolescents with asthma symptoms from this population [22]. Increased FE_{NO} has been reported in non-allergic asthma [33–35], although to a lower extent than the increase in allergic asthma, and a recent study reported that the predictive role of exhaled NO for new-onset asthma in schoolchildren was not affected by respiratory allergy status [24]. Both allergic and non-allergic rhinitis are risk factors for developing asthma [36]. Non-allergic rhinitis appears to be associated with local IgE production [37] so a local Th2 response with induction of NO formation is still possible even though atopy cannot be confirmed with conventional tests. A practical implication of the increased risk signalled for by exhaled NO for incident or persistent rhinitis in subjects without allergic symptoms at baseline might be to test these subjects more extensively for IgE sensitization and to regularly conduct their follow-up.

The lack of predictive value of increased exhaled NO for incident/persistent rhinitis in adolescents with allergic symptoms might be due to higher baseline levels of exhaled NO which might weaken the signal regarding risk for incident/persistent rhinitis. However, due to a smaller number of subjects in these groups, lack of statistical power for this kind of subanalysis prevents a definite conclusion on these aspects.

It should be emphasized that the present study is epidemiological and therefore rhinitis in the present study refers to self-reported rhinitis symptoms. Further, it cannot be excluded that a few of the patients classified as non-rhinitis might have developed rhinitis shortly after the FE_{NO} measurements. Even if the questionnaire-assessed allergic symptoms have a good negative predictive value for negative skin prick tests, there is still a possibility that a minority of subjects could be sensitized to other allergens than the ones assessed by the questionnaire.

The FE_{NO} was measured at a flow rate of 100 mL/s in the present material. Theoretically, data collected at a higher flow rate than the standard 50 mL/s would over-emphasize the effects of processes in peripheral lung; however, the percentual contribution from the peripheral airways to FE_{NO 0.1} is still very low. Moreover, allergic sensitization and rhinitis are not associated with any increase in the peripheral airways' contribution to exhaled NO [17]. There is good agreement between measurements at 50 and 100 mL/s [20] and therefore it is plausible that similar results would have been obtained for FE_{NO 0.05}. Thus, the results expressed with help of different cut-offs defined as 75th, 80th, 85th or 90th percentile could be extrapolated to other paediatric populations for which there are published reference values of FE_{NO} [23, 38]. Another way of extrapolating the present results could be to multiply the present FE_{NO} values with a coefficient of approximately 1.6 to obtain FE_{NO 0.05} values. This coefficient is retrieved from a publication where a similar device was used in children of similar age as in the present study and where FE_{NO} was measured at both 50 and 100 mL/s [20]. Height and atopy, respectively, are the major determinants of exhaled NO in healthy children, with an increase of FE_{NO} with height [23]. In our material, the main results on increased FE_{NO} as predictor for

new-onset and persistent rhinitis were consistent even after adjusting for height.

In conclusion, increased levels of exhaled NO were predictors of rhinitis in this population-based study of schoolchildren. Thus, it appears that elevation of exhaled NO precedes airway symptoms and predicts development of rhinitis in subjects without allergic symptoms or family history of allergic disease. Adolescents with increased exhaled NO levels could benefit from follow-up which includes objective testing of allergic sensitization.

Authors' contributions

KA, CJ and LN designed the study. Data were collected by PKS. The statistical analysis and data interpretation were performed by AM. The first draft of manuscript was prepared by AM. All authors contributed to and approved the final version of the manuscript.

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Conflict of interest: KA is an employee and minority shareholder of Aerocrine AB (which manufactures devices to measure exhaled NO) and has received funds for research from the same company. LN has received a fee for speaking from Aerocrine AB and funding for research from AstraZeneca. None of the other authors have any conflict of interest to declare.

Ethics

The study was approved by the Ethics Committee of the Medical Faculty of Uppsala University (decision 243/1999).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials.

Data S2. Results.

Table S1. The negative predictive value of questionnaire-assessed allergic symptoms for relevant IgE sensitization.

Table S2. Multiple logistic regression of different risk factors for new-onset rhinitis, adjusted for the other risk factors in the table.

Table S3. The relative risk for new-onset rhinitis associated with an increased FE_{NO} (defined either as FE_{NO} over 75th or 90th percentile among the whole non-rhinitis group at baseline). Results adjusted for gender, BMI, exposure to tobacco smoke, season of FE_{NO} measurements.

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A Malinowski¹, K Alving², P Kalm-Stephens², C Janson³, L Nordvall²

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Allergic symptoms were defined as a positive answer from the parents to one of the following questions: “Have you observed allergic symptoms when your child has been exposed to cats?”, “Have you observed allergic symptoms when your child has been exposed to dogs?”, “Have you observed allergic symptoms when your child has been exposed to pollen?”.

Skin-prick tests were performed in 374 subjects and enrichment with subjects reporting symptoms of asthma from the same study material was performed as previously described [3]. Skin prick testing was performed with extracts of the commonest air borne allergens in the area, *Dermatophagoides pteronyssinus*, cat, dog, birch pollen, timothy pollen. IgE-sensitisation was defined as a positive prick test with a mean diameter of at least 3 mm.

The negative predictive values (NPV) of the questions on allergic symptoms in response to exposure to cat, dog, pollen or any of these allergens for respective positive skin prick tests were over 85% (see Table E1). The positive predictive values (PPV) were more modest with the best PPV for reported allergic symptoms to any of the above allergens for any positive skin prick test to corresponding allergens of 73.9%.

Results

Description of cut-off levels for different percentiles

The cut-off levels for FE_{NO} according to different percentiles among subjects with no rhinitis symptoms at baseline (n=552) and presented in Tables 2, 3, E3 and E5 are the following: 7.0 ppb for 75th percentile, 7.4 ppb for 80th percentile, 9.1 ppb for 85th percentile and 10.6 ppb for 90th percentile.

New-onset rhinitis

The levels of exhaled NO in subjects without allergic symptoms were higher in subjects with new-onset rhinitis (4.6 ppb (3.6, 5.9), n=73) than subjects with no rhinitis at both timepoints (3.9 ppb (3.6, 4.2), n=438), but this was not significant (p=0.14). No differences were found between subjects with new-onset rhinitis (5.7 ppb (4.3, 7.6), n=19) and no rhinitis at both timepoints (6.2 (4.9, 7.8), n=22) in the group of subjects with allergic symptoms (p=0.64).

Increased levels of exhaled NO, expressed as a continuous variable, predicted new-onset rhinitis, independently of BMI and the presence of allergic symptoms in a multiple logistic regression model (Table E2). A trend towards an association of increased risk of new-onset rhinitis with increased FE_{NO} levels was found in a similar model as the one described in Online Supplement Table E2, when height and weight were used separately instead of BMI (p=0.052). This association become significant after excluding subjects with allergic symptoms at baseline (p=0.03).

The relationship between increased FENO levels (defined as >75th or 90th percentile) and increased risk for new-onset rhinitis remained statistically significant after excluding subjects who ever used

corticosteroids for any respiratory symptoms, had allergic symptoms, had any family history of allergic rhinitis and any family history of allergic disease (Table E3).

Persistent rhinitis

The levels of exhaled NO in subjects without allergic symptoms were higher in subjects with persistent rhinitis (5.7 ppb (4.1, 7.9), n=23) than subjects with remission of rhinitis (3.0 ppb (2.1, 4.3), n=31) ($p=0.01$). No differences were found between subjects with persistent rhinitis (6.3 ppb (4.6, 8.7), n=42) and remission of rhinitis (6.1 (3.3, 11.1), n=9) in the group of subjects with allergic symptoms ($p=0.93$)

In a similar model as the one described for new-onset rhinitis, there was a trend towards an association of increased risk of persistent rhinitis with increased exhaled NO levels ($p=0.06$) after adjusting for all other confounders of interest in all adolescents with rhinitis (n=105) (Table E4). A significant association between increased FE_{NO} and persistent rhinitis ($p=0.048$) was found in a similar model with the one described in Online Supplement Table E4 where weight and height were used instead of BMI.

The relationship between increased FENO levels (defined as >75th or 90th percentile) and increased risk for persistent rhinitis remained statistically significant after excluding subjects who ever used corticosteroids for any respiratory symptoms (Table E5).

References

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No allergic symptoms to any of above (n=259)	225	34	86.9%

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Table E2. Multiple logistic regression of different risk factors for new-onset rhinitis, adjusted for the other risk factors in the table.

<i>Risk factor</i>	<i>Odds ratios (95% CI)</i>	<i>p-value</i>
FE _{NO} (per 5 units)	1.28 (1.03, 1.59)	0.03
Female sex	1.38 (0.86, 2.21)	0.18
BMI (per 5 units)	1.80 (1.24, 2.61)	0.002
Exposure to tobacco smoke	0.81 (0.36, 1.83)	0.61
Allergic symptoms	5.00 (2.50, 9.99)	<0.001
Ever use of corticosteroids for respiratory problems	1.15 (0.47, 2.82)	0.77
Family history of allergic disease	1.53 (0.92, 2.53)	0.10
FENO measurements in pollen season	0.99 (0.60, 1.64)	0.98

Table E3. The relative risk for new-onset rhinitis associated with an increased FE_{NO} (defined either as FE_{NO} over 75th or 90th percentile among the whole non-rhinitis group at baseline). Results adjusted for gender, BMI, exposure to tobacco smoke, season of FE_{NO} measurements.

	Risk of new-onset rhinitis if FE _{NO} >75 th percentile	Risk of new-onset rhinitis if FE _{NO} >90 th percentile	Subjects with new-onset rhinitis (n)
All subjects (n=552)	1.99 (1.22, 3.23)	2.32 (1.23, 4.37)	92
After excluding subjects who ever used corticosteroids for respiratory problems (n=517)	2.12 (1.28, 3.50)	2.40 (1.25, 4.61)	84
After further excluding subjects with self-reported allergy (n=483)	2.06 (1.19, 3.58)	2.37 (1.16, 4.87)	71
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Increased levels of exhaled NO, expressed as a continuous variable, predicted new-onset rhinitis, independently of BMI and the presence of allergic symptoms in a multiple logistic regression model (Table E2). A trend towards an association of increased risk of new-onset rhinitis with increased FE_{NO} levels was found in a similar model as the one described in Online Supplement Table E2, when height and weight were used separately instead of BMI (p=0.052). This association become significant after excluding subjects with allergic symptoms at baseline (p=0.03).

The relationship between increased FENO levels (defined as >75th or 90th percentile) and increased risk for new-onset rhinitis remained statistically significant after excluding subjects who ever used

corticosteroids for any respiratory symptoms, had allergic symptoms, had any family history of allergic rhinitis and any family history of allergic disease (Table E3).

Persistent rhinitis

The levels of exhaled NO in subjects without allergic symptoms were higher in subjects with persistent rhinitis (5.7 ppb (4.1, 7.9), n=23) than subjects with remission of rhinitis (3.0 ppb (2.1, 4.3), n=31) ($p=0.01$). No differences were found between subjects with persistent rhinitis (6.3 ppb (4.6, 8.7), n=42) and remission of rhinitis (6.1 (3.3, 11.1), n=9) in the group of subjects with allergic symptoms ($p=0.93$)

In a similar model as the one described for new-onset rhinitis, there was a trend towards an association of increased risk of persistent rhinitis with increased exhaled NO levels ($p=0.06$) after adjusting for all other confounders of interest in all adolescents with rhinitis (n=105) (Table E4). A significant association between increased FE_{NO} and persistent rhinitis ($p=0.048$) was found in a similar model with the one described in Online Supplement Table E4 where weight and height were used instead of BMI.

The relationship between increased FENO levels (defined as >75th or 90th percentile) and increased risk for persistent rhinitis remained statistically significant after excluding subjects who ever used corticosteroids for any respiratory symptoms (Table E5).

References

1. ATS statement--Snowbird workshop on standardization of spirometry. *Am Rev Respir Dis* 1979;119: 831-8.
2. Knudson RJ, Slatin RC, Lebowitz MD, Burrows B, The maximal expiratory flow-volume curve. Normal standards, variability, and effects of age. *Am Rev Respir Dis* 1976;113: 587-600.
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Table E1. The negative predictive value of questionnaire-assessed allergic symptoms for relevant IgE sensitization.

	Negative SPT	Positive SPT	NPV
No allergic symptoms to cat (n=305)	281	24	92.1%
No allergic symptoms to dog (n=337)	316	21	93.8%
No allergic symptoms to pollen* (n=283)	246	37	86.9%
No allergic symptoms to any of above (n=259)	225	34	86.9%

* was compared to skin prick tests to birch and timothy pollen

Table E2. Multiple logistic regression of different risk factors for new-onset rhinitis, adjusted for the other risk factors in the table.

<i>Risk factor</i>	<i>Odds ratios (95% CI)</i>	<i>p-value</i>
FE _{NO} (per 5 units)	1.28 (1.03, 1.59)	0.03
Female sex	1.38 (0.86, 2.21)	0.18
BMI (per 5 units)	1.80 (1.24, 2.61)	0.002
Exposure to tobacco smoke	0.81 (0.36, 1.83)	0.61
Allergic symptoms	5.00 (2.50, 9.99)	<0.001
Ever use of corticosteroids for respiratory problems	1.15 (0.47, 2.82)	0.77
Family history of allergic disease	1.53 (0.92, 2.53)	0.10
FENO measurements in pollen season	0.99 (0.60, 1.64)	0.98

Table E3. The relative risk for new-onset rhinitis associated with an increased FE_{NO} (defined either as FE_{NO} over 75th or 90th percentile among the whole non-rhinitis group at baseline). Results adjusted for gender, BMI, exposure to tobacco smoke, season of FE_{NO} measurements.

	Risk of new-onset rhinitis if FE _{NO} >75 th percentile	Risk of new-onset rhinitis if FE _{NO} >90 th percentile	Subjects with new-onset rhinitis (n)
All subjects (n=552)	1.99 (1.22, 3.23)	2.32 (1.23, 4.37)	92
After excluding subjects who ever used corticosteroids for respiratory problems (n=517)	2.12 (1.28, 3.50)	2.40 (1.25, 4.61)	84
After further excluding subjects with self-reported allergy (n=483)	2.06 (1.19, 3.58)	2.37 (1.16, 4.87)	71
After further excluding subjects with family history of rhinitis (n=270)	4.21 (1.98, 8.95)	3.31 (1.28, 8.58)	36
After further excluding subjects with family history of any allergic disease* (n=197)	5.63 (2.14, 14.84)	2.93 (0.96, 8.97)	24

*astma, rhinitis or eczema

Table E4. Multiple logistic regression of different risk factors for persistent rhinitis, adjusted for the other risk factors in the table.

<i>Risk factor</i>	<i>Odds ratios (95% CI)</i>	<i>p-value</i>
FE _{NO} (per 5 units)	1.54 (0.99, 2.41)	0.06
Female sex	1.48 (0.58, 3.82)	0.41
BMI (per 5 units)	1.07 (0.52, 2.19)	0.85
Exposure to tobacco smoke	0.90 (0.22, 3.69)	0.88
Allergic symptoms	6.06 (2.24, 16.34)	<0.001
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Family history of allergic disease	1.02 (0.37, 2.82)	0.96
FENO measurements in pollen season	0.38 (0.14, 1.03)	0.06

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	Risk of persistent rhinitis if FE _{NO} >75 th percentile	Risk of persistent rhinitis if FE _{NO} >90 th percentile	Subjects with persistent rhinitis (n)
All subjects (n=105)	3.71 (1.39, 9.90)	5.11 (1.34, 19.6)	65
After excluding subjects who ever used corticosteroids for respiratory problems (n=92)	3.68 (1.32, 10.2)	5.08 (1.29, 19.9)	55
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*asthma, rhinitis or eczema

Increased exhaled nitric oxide predicts new-onset rhinitis and persistent rhinitis in adolescents without allergic symptoms

A Malinowski¹, K Alving², P Kalm-Stephens², C Janson³, L Nordvall²

Methods

Population

A total of 928 subjects participated in Stage II. In the present analysis, 267 adolescents were excluded as they reported physician-diagnosed asthma or asthma symptoms (wheeze, exercise-induced wheeze or dry cough at night) during the year previous to study inclusion at stage I. Four subjects reported use of inhaled corticosteroids the year previous to the study and were therefore excluded. Therefore, 657 adolescents aged 17-18 years were included in the present analysis.

Exhaled NO measurement

Subjects rinsed the mouth with sodium bicarbonate mouthwash prior to measurements. NO was measured with an Aerocrine NO system (Aerocrine AB, Stockholm, Sweden), including the CLD 77 AM chemiluminescence analyzer (Eco Physics AG, Dürnten, Switzerland) for on-line NO measurements and monitoring of flow and pressure. Measurements were done in triplicate and mean values are presented.

Lung function measurements

Pulmonary function was evaluated according to ATS criteria,[1] using a Spirolab spirometer (Medical International Research, Rome, Italy), by an experienced nurse. The best of three maneuvers was automatically chosen by the software. FEV₁ was measured and the values are reported as % of predicted, according to Knudson's normal reference values.[2]

Questionnaire

Ever use of corticosteroid treatment for respiratory problems was recorded with the question “Have your child ever received corticosteroid treatment for any respiratory problems?”.

Subjects were considered as exposed to tobacco smoke if they reported being exposed to at least one cigarette/day inside their home.

A history of allergic disease in the parents or siblings was obtained with the following questions regarding mother, father and siblings respectively: “Have you ever had asthma?”, “Have you ever had hay fever?” or “Have you ever had atopic eczema?”.

Validation of the questionnaire-assessed allergic symptoms

Allergic symptoms were defined as a positive answer from the parents to one of the following questions: “Have you observed allergic symptoms when your child has been exposed to cats?”, “Have you observed allergic symptoms when your child has been exposed to dogs?”, “Have you observed allergic symptoms when your child has been exposed to pollen?”.

Skin-prick tests were performed in 374 subjects and enrichment with subjects reporting symptoms of asthma from the same study material was performed as previously described [3]. Skin prick testing was performed with extracts of the commonest air borne allergens in the area, *Dermatophagoides pteronyssinus*, cat, dog, birch pollen, timothy pollen. IgE-sensitisation was defined as a positive prick test with a mean diameter of at least 3 mm.

The negative predictive values (NPV) of the questions on allergic symptoms in response to exposure to cat, dog, pollen or any of these allergens for respective positive skin prick tests were over 85% (see Table E1). The positive predictive values (PPV) were more modest with the best PPV for reported allergic symptoms to any of the above allergens for any positive skin prick test to corresponding allergens of 73.9%.

Results

Description of cut-off levels for different percentiles

The cut-off levels for FE_{NO} according to different percentiles among subjects with no rhinitis symptoms at baseline (n=552) and presented in Tables 2, 3, E3 and E5 are the following: 7.0 ppb for 75th percentile, 7.4 ppb for 80th percentile, 9.1 ppb for 85th percentile and 10.6 ppb for 90th percentile.

New-onset rhinitis

The levels of exhaled NO in subjects without allergic symptoms were higher in subjects with new-onset rhinitis (4.6 ppb (3.6, 5.9), n=73) than subjects with no rhinitis at both timepoints (3.9 ppb (3.6, 4.2), n=438), but this was not significant (p=0.14). No differences were found between subjects with new-onset rhinitis (5.7 ppb (4.3, 7.6), n=19) and no rhinitis at both timepoints (6.2 (4.9, 7.8), n=22) in the group of subjects with allergic symptoms (p=0.64).

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The relationship between increased FENO levels (defined as >75th or 90th percentile) and increased risk for new-onset rhinitis remained statistically significant after excluding subjects who ever used

corticosteroids for any respiratory symptoms, had allergic symptoms, had any family history of allergic rhinitis and any family history of allergic disease (Table E3).

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The levels of exhaled NO in subjects without allergic symptoms were higher in subjects with persistent rhinitis (5.7 ppb (4.1, 7.9), n=23) than subjects with remission of rhinitis (3.0 ppb (2.1, 4.3), n=31) ($p=0.01$). No differences were found between subjects with persistent rhinitis (6.3 ppb (4.6, 8.7), n=42) and remission of rhinitis (6.1 (3.3, 11.1), n=9) in the group of subjects with allergic symptoms ($p=0.93$)

In a similar model as the one described for new-onset rhinitis, there was a trend towards an association of increased risk of persistent rhinitis with increased exhaled NO levels ($p=0.06$) after adjusting for all other confounders of interest in all adolescents with rhinitis (n=105) (Table E4). A significant association between increased FE_{NO} and persistent rhinitis ($p=0.048$) was found in a similar model with the one described in Online Supplement Table E4 where weight and height were used instead of BMI.

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References

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Family history of allergic disease	1.53 (0.92, 2.53)	0.10
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Table E3. The relative risk for new-onset rhinitis associated with an increased FE_{NO} (defined either as FE_{NO} over 75th or 90th percentile among the whole non-rhinitis group at baseline). Results adjusted for gender, BMI, exposure to tobacco smoke, season of FE_{NO} measurements.

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	Risk of new-onset rhinitis if FE _{NO} >75 th percentile	Risk of new-onset rhinitis if FE _{NO} >90 th percentile	Subjects with new-onset rhinitis (n)
All subjects (n=552)	1.99 (1.22, 3.23)	2.32 (1.23, 4.37)	92
After excluding subjects who ever used corticosteroids for respiratory problems (n=517)	2.12 (1.28, 3.50)	2.40 (1.25, 4.61)	84
After further excluding subjects with self-reported allergy (n=483)	2.06 (1.19, 3.58)	2.37 (1.16, 4.87)	71
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After excluding subjects who ever used corticosteroids for respiratory problems (n=92)	3.68 (1.32, 10.2)	5.08 (1.29, 19.9)	55
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Exhaled NO measurement

Subjects rinsed the mouth with sodium bicarbonate mouthwash prior to measurements. NO was measured with an Aerocrine NO system (Aerocrine AB, Stockholm, Sweden), including the CLD 77 AM chemiluminescence analyzer (Eco Physics AG, Dürnten, Switzerland) for on-line NO measurements and monitoring of flow and pressure. Measurements were done in triplicate and mean values are presented.

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Subjects were considered as exposed to tobacco smoke if they reported being exposed to at least one cigarette/day inside their home.

A history of allergic disease in the parents or siblings was obtained with the following questions regarding mother, father and siblings respectively: “Have you ever had asthma?”, “Have you ever had hay fever?” or “Have you ever had atopic eczema?”.

Validation of the questionnaire-assessed allergic symptoms

Allergic symptoms were defined as a positive answer from the parents to one of the following questions: “Have you observed allergic symptoms when your child has been exposed to cats?”, “Have you observed allergic symptoms when your child has been exposed to dogs?”, “Have you observed allergic symptoms when your child has been exposed to pollen?”.

Skin-prick tests were performed in 374 subjects and enrichment with subjects reporting symptoms of asthma from the same study material was performed as previously described [3]. Skin prick testing was performed with extracts of the commonest air borne allergens in the area, *Dermatophagoides pteronyssinus*, cat, dog, birch pollen, timothy pollen. IgE-sensitisation was defined as a positive prick test with a mean diameter of at least 3 mm.

The negative predictive values (NPV) of the questions on allergic symptoms in response to exposure to cat, dog, pollen or any of these allergens for respective positive skin prick tests were over 85% (see Table E1). The positive predictive values (PPV) were more modest with the best PPV for reported allergic symptoms to any of the above allergens for any positive skin prick test to corresponding allergens of 73.9%.

Results

Description of cut-off levels for different percentiles

The cut-off levels for FE_{NO} according to different percentiles among subjects with no rhinitis symptoms at baseline (n=552) and presented in Tables 2, 3, E3 and E5 are the following: 7.0 ppb for 75th percentile, 7.4 ppb for 80th percentile, 9.1 ppb for 85th percentile and 10.6 ppb for 90th percentile.

New-onset rhinitis

The levels of exhaled NO in subjects without allergic symptoms were higher in subjects with new-onset rhinitis (4.6 ppb (3.6, 5.9), n=73) than subjects with no rhinitis at both timepoints (3.9 ppb (3.6, 4.2), n=438), but this was not significant (p=0.14). No differences were found between subjects with new-onset rhinitis (5.7 ppb (4.3, 7.6), n=19) and no rhinitis at both timepoints (6.2 (4.9, 7.8), n=22) in the group of subjects with allergic symptoms (p=0.64).

Increased levels of exhaled NO, expressed as a continuous variable, predicted new-onset rhinitis, independently of BMI and the presence of allergic symptoms in a multiple logistic regression model (Table E2). A trend towards an association of increased risk of new-onset rhinitis with increased FE_{NO} levels was found in a similar model as the one described in Online Supplement Table E2, when height and weight were used separately instead of BMI (p=0.052). This association become significant after excluding subjects with allergic symptoms at baseline (p=0.03).

The relationship between increased FENO levels (defined as >75th or 90th percentile) and increased risk for new-onset rhinitis remained statistically significant after excluding subjects who ever used

corticosteroids for any respiratory symptoms, had allergic symptoms, had any family history of allergic rhinitis and any family history of allergic disease (Table E3).

Persistent rhinitis

The levels of exhaled NO in subjects without allergic symptoms were higher in subjects with persistent rhinitis (5.7 ppb (4.1, 7.9), n=23) than subjects with remission of rhinitis (3.0 ppb (2.1, 4.3), n=31) ($p=0.01$). No differences were found between subjects with persistent rhinitis (6.3 ppb (4.6, 8.7), n=42) and remission of rhinitis (6.1 (3.3, 11.1), n=9) in the group of subjects with allergic symptoms ($p=0.93$)

In a similar model as the one described for new-onset rhinitis, there was a trend towards an association of increased risk of persistent rhinitis with increased exhaled NO levels ($p=0.06$) after adjusting for all other confounders of interest in all adolescents with rhinitis (n=105) (Table E4). A significant association between increased FE_{NO} and persistent rhinitis ($p=0.048$) was found in a similar model with the one described in Online Supplement Table E4 where weight and height were used instead of BMI.

The relationship between increased FENO levels (defined as >75th or 90th percentile) and increased risk for persistent rhinitis remained statistically significant after excluding subjects who ever used corticosteroids for any respiratory symptoms (Table E5).

References

1. ATS statement--Snowbird workshop on standardization of spirometry. *Am Rev Respir Dis* 1979;119: 831-8.
2. Knudson RJ, Slatin RC, Lebowitz MD, Burrows B, The maximal expiratory flow-volume curve. Normal standards, variability, and effects of age. *Am Rev Respir Dis* 1976;113: 587-600.
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Table E1. The negative predictive value of questionnaire-assessed allergic symptoms for relevant IgE sensitization.

	Negative SPT	Positive SPT	NPV
No allergic symptoms to cat (n=305)	281	24	92.1%
No allergic symptoms to dog (n=337)	316	21	93.8%
No allergic symptoms to pollen* (n=283)	246	37	86.9%
No allergic symptoms to any of above (n=259)	225	34	86.9%

* was compared to skin prick tests to birch and timothy pollen

Table E2. Multiple logistic regression of different risk factors for new-onset rhinitis, adjusted for the other risk factors in the table.

<i>Risk factor</i>	<i>Odds ratios (95% CI)</i>	<i>p-value</i>
FE _{NO} (per 5 units)	1.28 (1.03, 1.59)	0.03
Female sex	1.38 (0.86, 2.21)	0.18
BMI (per 5 units)	1.80 (1.24, 2.61)	0.002
Exposure to tobacco smoke	0.81 (0.36, 1.83)	0.61
Allergic symptoms	5.00 (2.50, 9.99)	<0.001
Ever use of corticosteroids for respiratory problems	1.15 (0.47, 2.82)	0.77
Family history of allergic disease	1.53 (0.92, 2.53)	0.10
FENO measurements in pollen season	0.99 (0.60, 1.64)	0.98

Table E3. The relative risk for new-onset rhinitis associated with an increased FE_{NO} (defined either as FE_{NO} over 75th or 90th percentile among the whole non-rhinitis group at baseline). Results adjusted for gender, BMI, exposure to tobacco smoke, season of FE_{NO} measurements.

	Risk of new-onset rhinitis if FE _{NO} >75 th percentile	Risk of new-onset rhinitis if FE _{NO} >90 th percentile	Subjects with new-onset rhinitis (n)
All subjects (n=552)	1.99 (1.22, 3.23)	2.32 (1.23, 4.37)	92
After excluding subjects who ever used corticosteroids for respiratory problems (n=517)	2.12 (1.28, 3.50)	2.40 (1.25, 4.61)	84
After further excluding subjects with self-reported allergy (n=483)	2.06 (1.19, 3.58)	2.37 (1.16, 4.87)	71
After further excluding subjects with family history of rhinitis (n=270)	4.21 (1.98, 8.95)	3.31 (1.28, 8.58)	36
After further excluding subjects with family history of any allergic disease* (n=197)	5.63 (2.14, 14.84)	2.93 (0.96, 8.97)	24

*astma, rhinitis or eczema

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Increased exhaled nitric oxide predicts new-onset rhinitis and persistent rhinitis in adolescents without allergic symptoms

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