ORIGINAL ARTICLE

Exhaled nitric oxide levels in atopic children: relation to specific allergic sensitisation, AHR, and respiratory symptoms

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Background: Exhaled nitric oxide (eNO), which has been proposed as a measure of airway inflammation, is increased in atopic subjects. This raises the question of whether eNO provides any additional information about airway inflammation in asthmatic subjects, other than as a marker for atopy. A study was undertaken to determine whether eNO levels in a population of atopic children are associated with sensitisation or natural exposure to specific allergens, and to examine the relationship between eNO, airway responsiveness, and current respiratory symptoms.
Methods: Exhaled NO and airway responsiveness to histamine were measured in winter and in summer in 235 children aged 8–14 years who had been classified as atopic by skin prick testing. Current respiratory symptoms, defined as wheeze or cough during the month preceding the test, were measured by a parent completed questionnaire. Airway hyperresponsiveness (AHR) was defined as a dose response ratio (DRR) of >8.1 (% fall in forced expiratory volume in 1 second (FEV_1)/μmol + 3).
Results: Sensitisation to house dust mite was associated with raised eNO levels in winter while sensitisation to *Cladosporium* was associated with raised eNO levels in both winter and summer. Grass pollen sensitisation was not associated with raised eNO levels in either season. Exhaled NO correlated significantly with DRR histamine (r=0.43, p<0.001) independently of whether the children had current symptoms or not. In children with current wheeze, those with AHR had eNO levels 1.53 (95% CI 1.41 to 1.66) times higher than those without AHR (p=0.006). Neither DRR (p=1.0) nor eNO levels (p=0.92) differed significantly between children with or without persistent dry cough in the absence of wheeze.
Conclusions: In atopic children, raised eNO levels are associated with sensitisation to perennial allergens, but not to seasonal allergens such as grass pollen. In this population, an increase in eNO is associated with AHR and current wheezing, suggesting that eNO is more than just a marker for atopy.

The measurement of exhaled nitric oxide (eNO) may be used to monitor airway inflammation in asthma[1] and other lung diseases.[2] Higher levels of eNO have been found in adults and children with allergy and asthma than in the rest of the population.[3 4] Lower levels have been found in patients taking inhaled corticosteroids.[5–7]

Exhaled nitric oxide is also increased in atopic individuals and higher levels are found in atopic than in non-atopic individuals among asymptomatic subjects[8] and those with rhinitis[9] and asthma.[10] This suggests that eNO may simply be a marker for atopy rather than providing information about airway inflammation. Alternatively, it may reflect the presence of subclinical airway inflammation, as has been observed previously in asymptomatic atopic subjects[11] and those with atopic rhinitis.[12]

Sensitisation to specific allergens such as house dust mite, mould spores, or cat may be more likely to cause airway inflammation since these allergens are more closely associated than others with airway hyperresponsiveness (AHR)[13] and symptoms of asthma.[14] This is supported by evidence that sensitisation to some allergens is more strongly related to eNO than sensitisation to others. If so, the strength of the association between eNO and airway inflammation might be expected to vary by season if allergen exposure varied by season.

A relationship between eNO and both AHR and wheeze symptoms has been observed in previous studies.[3] The presence of both AHR and recent symptoms of wheeze has been proposed as a definition for current asthma,[15] and eNO levels are increased in subjects meeting this criterion.[16] However, in children, more than 90% of those with recent wheeze and AHR are also atopic compared with less than 50% of the remainder of the population.[17] It is not established whether there is an association between eNO levels and either AHR or wheezing that is independent of the presence of atopy.

Chronic cough is a common respiratory symptom in children that may be associated with asthma.[18] Two thirds of children with cough cannot be allocated to any commonly used paediatric diagnostic category[19] and do not have airway responsiveness, but would be diagnosed as having asthma by many physicians.[20] There is some evidence that chronic cough is associated with airway inflammation.[21] It has been suggested that eNO could be useful in evaluating the importance of chronic cough as an indicator of asthma.[22]

The aims of this study were, firstly, to test the hypothesis that eNO levels vary with change in natural exposure to environmental allergens by examining seasonal differences in the relation between eNO and specific allergen sensitisation. Secondly, we wanted to test the hypothesis that eNO reflects clinically relevant features of allergic airway disease, not just the presence of atopy, by determining if, in a population of atopic children, eNO differs in those with and without asthma symptoms and AHR. Finally, we wanted to test the hypothesis that cough without wheeze is not associated with increased eNO levels, which would suggest that it has a different pathogenesis from wheezing illness in these children.

METHODS

Study population and study design

The study population was selected from primary school children aged 7–12 years who participated in a cross sectional

survey of the prevalence of atopy and asthma in children in two towns in rural NSW Australia—Wagga Wagga (population ~54 000) and Moree (population ~9000)—in 1997. Skin prick tests for allergic sensitisation were performed during July 1997 (winter) in Wagga Wagga and September 1997 (spring) in Moree. From this population we recruited 399 atopic children for a prospective study of the effects of sensitisation to *Alternaria*. For the present study we analysed data from children in whom both eNO levels and airway responsiveness to histamine were measured during summer 1998. To ensure that our study sample was representative of the population of atopic children with respect to the distribution of individual allergen sensitisation, as measured in 1997, 18 children with allergic sensitisation to *Alternaria* were randomly withdrawn from the selected sample. This gave a total sample of 235 children with data collected in summer 1998. Testing was also undertaken during the winter of 1998 (in July in Wagga and August in Moree), and data on eNO levels, airway responsiveness, and recent respiratory symptoms were obtained from 213 of the sample children.

All tests were undertaken at the schools and the study was approved by the human ethics committee of the University of Sydney, NSW Department of School Education, the Catholic Education Office, and each school principal. Parents had given written consent for their child's participation in the study.

Skin prick tests

Sensitisation to eight allergens was measured by reactions to skin prick tests on the forearm.[23] The allergens tested were the house dust mite *Dermatophagoides pteronyssinus*, rye grass pollen *Lolium perenne*, cat dander, *Alternaria alternata (tenuis)*, *Cladosporium cladosporoides*, wheat wholegrain, grain mill dust, and cotton lint. All allergens were purchased from Hollister-Stier (Bayer Corporation, Spokane, WA, USA). Histamine (10 mg/ml) was used as a positive control and a normal saline/glycerol solution as a negative control. Skin wheal size was recorded 15 minutes after skin prick as the mean of the length of the long axis to the wheal and the length of its perpendicular. Skin prick test data with a positive response to the negative control were not included in the analysis. A mean wheal size of 3 mm or more was defined as a positive response. Children were classified as atopic if they had a positive response to one or more of the allergens tested.

Questionnaires

The questionnaire administered to parents was a slightly modified version of one developed and validated by Usherwood *et al*.[24] It included the following questions: "Over the past one month has your child coughed during the day?", "Over the past one month has your child coughed at night?", "Over the past one month has your child been wheezy during the day?" to which parents were given the following options for response: "not at all", "a few days", "some days", "most days" or "every day". We also added a question about dry cough: "Over the past one month has your child had a dry cough not associated with a cold or flu?".[20]

Nitric oxide measurement

The concentration of NO in the exhaled breath of each child was measured before lung function and airway responsiveness to histamine using a similar protocol to that reported previously.[3 5] The exhaled breath was collected with the child standing, without wearing a noseclip. The child was asked to take a deep breath and then to blow hard until residual volume was reached through a mouthpiece connected to an impermeable polyethylene bag (Scholle Industries, Elizabeth West, Australia). The exhaled flow was measured by a rotameter (Dwyer Flowmeter Model VFASS-25; AMBIT Instruments, Parramatta, Australia). The mouth pressure was >20 cm H_2O for a flow of 10 l/min. The exhaled gas was analysed within 2 hours using a chemiluminescent analyser (Thermo Environmental Instruments Model 42C) which has a lower limit of detection of 1 ppb. Ambient NO was measured at the time of testing.

Lung function and airway hyperresponsiveness

Lung function was recorded by dry rolling seal spirometers (Mijnhardt BV, Bunnik, Holland) connected to a computer (IBM-PC running Scientific and Medical software). Forced expiratory manoeuvres were repeated until two readings of forced expiratory volume in 1 second (FEV_1) within 100 ml were obtained, the largest of which was used in analyses. Values for FEV_1 and forced vital capacity (FVC) were recorded as a percentage of the predicted values of Knudson *et al*.[25] A bronchial challenge test with histamine was administered to all children using the rapid method.[26] Histamine diphosphate (ICN Pharmaceuticals, Costa Mesa, USA) was administered using DeVilbiss No 45 handheld nebulisers (DeVilbiss Health Care, Somerset, PA, USA) in doubling doses from 0.03 to 3.9 μmol. The test was stopped if the FEV_1 fell by 20% or more and salbutamol aerosol was administered at the end if the fall in FEV_1 was greater than 9%. The dose of histamine that provoked a 20% fall in FEV_1 ($PD_{20}FEV_1$) was estimated by interpolation. Dose response ratio (DRR) was calculated for all subjects as the percentage fall in FEV_1 at the last dose divided by the total dose administered.[27 28] Airway hyperresponsiveness (AHR) was defined as DRR >8.1% fall/μmol + 3, corresponding to a $PD_{20}FEV_1$ of ≤3.9 μmol histamine.

Asthma medication use

The respiratory symptom questionnaires included questions about medications the child might currently be taking for asthma and had taken in the past. Children were also asked about current asthma medication use when they were tested for exhaled NO and airway responsiveness. A second appointment was made for children who had taken short acting β agonists within 6 hours or long acting β agonists within 24 hours of presenting for the tests and they were asked to withhold medication before the second test. Their parents were also telephoned the day before the second test to request that medication be withheld. If the children had taken inhaled corticosteroids within 2 weeks of the tests, the name and daily dose of the drug were recorded.

Data analysis

Both eNO and DRR to histamine were log transformed before analysis. A constant of 3 was added to all DRR values to obtain a positive normally distributed value for logarithmic conversion, and the values are reported in units of percentage fall in FEV_1/μmol + 3.[27 28] Differences between eNO and DRR between symptom categories were tested by analysis of variance and Bonferroni multicomparison tests. Fold change in eNO levels by sensitisation to individual allergens, by presence or absence of AHR, and fold change in eNO and DRR by presence or absence of symptoms were measured by *t* tests. Multiple linear regression was undertaken to assess the relation between eNO and sensitisation to individual allergens while adjusting for ambient NO and use of inhaled corticosteroids. The analyses were undertaken using STATA (Stata Statistical Software Release 6.0, Stata Corporation, Texas, USA). Significance was accepted at the 5% level.

RESULTS

Table 1 shows the characteristics of the study population of atopic children. The proportion of children who reported symptoms in the previous 4 weeks, who were currently using inhaled corticosteroids, or who had AHR was higher than might be expected in a general population sample and reflects the fact that all the children were atopic. The median number of breaths and the flow rates recorded during the collection of

Table 1 Characteristics of study population measured in summer 1998 (n=235)

Mean (SD) age (years)	10.8 (1.1)
No (%) with wheeze in the last 4 weeks	77/230 (33.5%)
No (%) with dry cough only in the 4 weeks	31/228 (13.5%)
No (%) with AHR	98/235 (41.7%)
No (%) taking inhaled corticosteroids	27/235 (11.5%)
Median (range) no of breaths collected	2 (1–7)
Median (range) flow rate (l/min)	9 (2–18)
Mean (95% CI) ambient NO (ppb)	5.8 (5.5 to 6.0)

Table 2 Distribution of sensitisation to individual allergens in study sample and the prevalence measured in the population of atopic children from which the study children were selected. Values are percentage (95% CI) of samples with positive skin prick tests to each allergen

	Source population*	Study sample*
No of atopic children	647	235
Cladosporium	25.4 (22.0 to 28.9)	25.1 (19.7 to 31.2)
D pteronyssinus	66.6 (62.8 to 70.2)	60.0 (53.4 to 66.3)
Rye grass pollen	57.0 (53.1 to 60.9)	54.0 (47.4 to 60.5)
Cat dander	19.3 (16.3 to22.6)	18.7 (13.9 to 24.3)
Alternaria	43.0 (39.1 to 46.9)	43.8 (37.4 to 50.4)
Grain mill dust	22.3 (19.1 to 25.7)	18.3 (13.6 to 23.8)
Wheat whole grain	9.3 (7.2 to 11.8)	3.8 (1.7 to 7.1)
Cotton lint	1.6 (0.7 to 2.8)	1.3 (0.3 to 3.7)

*Allergic sensitisation measured during the cross sectional survey of prevalence of atopy and asthma in 1997.

Table 3 Geometric mean (SD) eNO levels (ppb) measured in summer (n=235) and winter (n=213) in the study population of atopic children by sensitisation to individual allergens

Allergen	Not atopic	Atopic	Fold difference (95% CI)	p value
Summer:				
Cladosporium	13.5 (1.9)	18.8 (2.0)	1.40 (1.30 to 1.49)	0.001
D pteronyssinus	13.9 (2.0)	15.1 (1.9)	1.09 (1.01 to 1.17)	0.36
Rye grass pollen	14.5 (2.0)	14.8 (2.0)	1.02 (0.94 to 1.09)	0.83
Alternaria	13.6 (2.0)	16.1 (2.0)	1.18 (1.11 to 1.26)	0.05
Cat dander	14.2 (2.0)	16.6 (2.0)	1.16 (1.07 to 1.26)	0.18
Grain mill dust	14.5 (2.0)	15.4 (2.1)	1.06 (0.96 to 1.17)	0.60
Wheat whole grain	14.8 (2.0)	11.4 (2.1)	0.77 (0.56 to 0.98)	0.26
Cotton lint	14.6 (2.0)	13.9 (3.0)	0.95 (0.41 to 1.48)	0.9
Winter:				
Cladosporium	13.5 (1.6)	18.5 (1.6)	1.37 (1.31 to 1.43)	<0.001
D pteronyssinus	13.6 (1.5)	15.5 (1.7)	1.14 (1.08 to 1.19)	0.06
Rye grass pollen	13.7 (1.7)	15.6 (1.6)	1.14 (1.08 to 1.20)	0.05
Alternaria	13.6 (1.7)	16.1 (1.6)	1.18 (1.13 to 1.23)	0.01
Cat dander	14.2 (1.6)	16.8 (1.6)	1.18 (1.11 to 1.25)	0.05
Grain mill dust	14.5 (1.6)	15.6 (1.6)	1.07 (1.00 to 1.15)	0.41
Wheat whole grain	14.7 (1.6)	16.0 (1.5)	1.09 (0.97 to 1.22)	0.61
Cotton lint	14.7 (1.6)	17.6 (1.2)	1.19 (1.09 to 1.31)	0.53

expired breaths for measurement of eNO and the mean ambient NO levels are also shown. The distribution of sensitisation to specific allergens in the study population was similar to that in the population sample of atopic children from which they were drawn in 1997 (table 2).

The factors that affected eNO levels were determined using data collected in the summer. The mean level of eNO did not differ significantly between children who were using inhaled corticosteroids and those who were not (15.3 ppb (95% CI 11.4 to 20.4) versus 14.6 ppb (95% CI 13.3 to 16.0), p=0.74). The findings were similar when the analysis was restricted to children with symptoms of wheeze during the previous 4 weeks (17.4 ppb (95% CI 12.3 to 24.7) versus 18.7 ppb (95% CI 15.6 to 22.3), p=0.72). There was also no relation between eNO level and number of breaths collected (p=0.73) or flow rate (p=0.81). Level of eNO was related to the level of NO in ambient air (r=0.45, p<0.001). However, ambient NO levels were not related to DRR (r=0.08, p=0.19) and did not affect the relation between eNO and DRR.

The level of eNO was significantly correlated with the number of positive skin prick reactions (r=0.17, p<0.006) but not with maximum wheal size (r=–0.02, p=0.73). Children with positive skin prick tests to *Cladosporium* had eNO levels that were 1.40 fold (95% CI 1.30 to 1.49) higher than those

Table 4 Effect of sensitisation to specific allergens on eNO levels in winter and summer, adjusted for the effect of sensitisation to other allergens, for ambient NO levels and for the use of inhaled corticosteroids (ICS)

	Winter (n=213)		Summer (n=235)	
	Fold difference (95% CI) in eNO associated with sensitisation	p value	Fold difference (95% CI) in eNO associated with sensitisation	p value
Ambient NO	1.003 (1.001 to 1.005)	0.008	1.14 (1.09 to 1.18)	<0.001
ICS	1.12 (0.91 to 1.39)	0.29	0.96 (0.74 to 1.23)	0.74
Sensitisation to:				
Cladosporium	1.39 (1.15 to 1.68)	0.001	1.37 (1.07 to 1.75)	0.01
D pteronyssinus	1.21 (1.05 to 1.38)	0.009	1.10 (0.93 to 1.31)	0.26
Rye grass pollen	1.10 (0.96 to 1.25)	0.17	1.04 (0.88 to 1.24)	0.62
Alternaria	1.08 (0.92 to 1.27)	0.33	1.05 (0.86 to 1.29)	0.63
Cat	1.03 (0.87 to 1.23)	0.70	1.14 (0.92 to 1.41)	0.24
Grain mill dust	0.83 (0.69 to 1.002)	0.06	0.85 (0.67 to 1.08)	0.18
Whole wheat grain	1.08 (0.77 to 1.53)	0.64	0.77 (0.51 to 1.18)	0.24
Cotton lint	1.15 (0.67 to 1.95)	0.62	0.90 (0.45 to 1.82)	0.77

Table 5 Exhaled NO levels and dose response ratio (DRR) to histamine in relation to respiratory symptoms in atopic children in the month prior to testing

	No symptoms	Wheeze ± dry cough	Dry cough only	p value
No (%)	122 (53%)	77 (33%)	31 (13%)	
eNO (ppb)	12.9 (11.5 to 14.5)	18.6 (15.8 to 21.4)	13.5 (10.7 to 17.4)	0.001
DRR (% fall in FEV_1/µmol + 3)	6.9 (6.0 to 7.9)	15.9 (12.0 to 20.1)	6.9 (5.4 to 8.9)	<0.001

Values are mean (95% confidence interval).

with negative tests to *Cladosporium* (p=0.001) in summer (table 3) and this difference was of similar magnitude in winter (table 3). However, no other significant effects on eNO of sensitisation to individual allergens were seen in the univariate analyses either in summer or in winter (table 3).

The results of multiple linear regression, which assessed the effect of sensitisation to specific allergens on eNO after adjusting for the effect of other allergens, are shown in table 4. Sensitisation to *Cladosporium* was associated with higher eNO levels in both summer and winter. Sensitisation to house dust mite, assessed by the presence of a positive skin prick test to *D pteronyssinus*, was associated with higher levels of eNO during the winter but not during summer. The results from analyses of the summer data were similar even when they were restricted to those children who had eNO measured both in summer and winter. There was a significant relation between eNO levels and sensitisation to *Cladosporium* (p=0.01) and no relation between eNO levels and sensitisation to house dust mite (p=0.10).

The level of sensitisation to *Cladosporium*, assessed by the size of the skin prick test wheal, was independently associated with eNO levels in both winter and summer in multiple linear regressions. An increase of 1 mm in mean wheal size to *Cladosporium* was associated with a 1.10 fold increase in eNO in winter (95% CI 1.02 to 1.17) and a 1.08 fold increase in eNO (95% CI 1.01 to 1.16) in summer. There was no association between the level of sensitisation to any other allergen and eNO levels in either summer or winter.

Differences in eNO and AHR between children with and without symptoms were examined using data from the summer measurements. Children who had wheezed (with or without cough) in the 4 weeks prior to testing had levels of eNO that were 1.43 fold higher (95% CI 1.34 to 1.51) (p<0.001) and DRR values that were 2.24 fold higher (95% CI 2.11 to 2.37) (p<0.001) than children without current symptoms (table 5). There was no difference in either eNO or DRR between children with cough only and asymptomatic children. This was true irrespective of whether cough was defined as dry cough, cough during the day, cough during the night, or cough on most or every day or night during the preceding month. The findings were similar in the winter data.

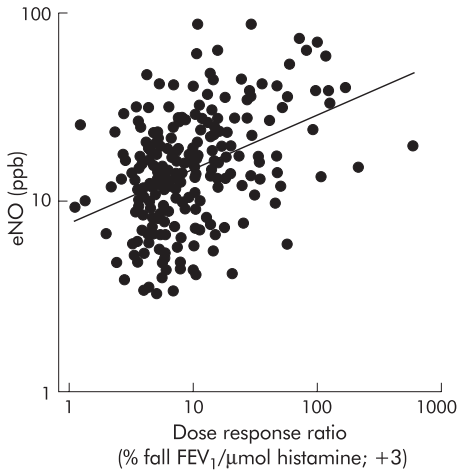

Figure 1 Relation between exhaled NO and dose response ratio to histamine in 235 atopic children.

Airway hyperresponsiveness was associated with increased eNO levels. Exhaled NO was significantly correlated with the DRR to histamine (r=0.43, p<0.001; fig 1). Airway hyperresponsiveness was present in 35% of children without current symptoms, 58% of those with current wheeze, and 26% of those with cough only. Mean eNO levels were 1.58 fold higher (95% CI 1.50 to 1.65) in children with AHR than in children without AHR (p < 0.001). The differences were significant both in subjects without current symptoms and in those with current wheeze (fig 2). Among the children with dry cough only there was only a small number of children with AHR, and

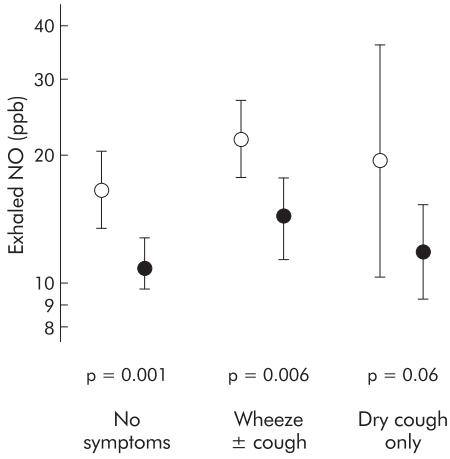

Figure 2 Geometric mean (95% confidence intervals) exhaled NO levels in children with or without symptoms in the last month and with (open circles) or without (closed circles) AHR. p values (Bonferroni) are for the differences between children with and without AHR within each of the symptom categories.

the difference in eNO levels between these children and other children with dry cough failed to reach statistical significance. In children without AHR, those with wheeze had eNO levels 1.25 fold higher (95% CI 1.14 to 1.35) than those without wheeze, but the difference was not quite significant (p=0.07).

DISCUSSION

In this study of a population of atopic children we found, firstly, that exhaled NO levels were associated with sensitisation to house dust mite and to *Cladosporium* but not with grass pollen or cat dander. Sensitisation to house dust mite was associated with increased eNO levels in winter, while sensitisation to *Cladosporium* was associated with increased eNO levels in both winter and summer. Secondly, we found that eNO was related both to the presence of symptoms of wheeze and to the presence and severity of airway hyperresponsiveness. Finally, we found that cough, in the absence of wheeze, was not associated with increased eNO levels.

Standard methods were used to measure atopy, eNO, AHR, and respiratory symptoms. The study population was selected from a population sample of schoolchildren on the basis of a positive skin prick test to a battery of common aeroallergens. The results of the study are applicable to a general population of atopic children. The offline collection method for eNO has been described previously and has good repeatability.[3] For most subjects the flow rates were high enough to minimise the flow dependency of eNO measurements, which may be an important consideration for studies of children in an epidemiological setting where expiratory flow cannot be strictly controlled. We found that the flow rates were not an important determinant of eNO in this setting. The geometric mean value for eNO in the normal group (no respiratory symptoms in the last month) is comparable with values found in other populations of asymptomatic atopic subjects,[29] but is higher than has been reported in other mixed populations of atopic and non-atopic asymptomatic children.[30 31]

Exhaled NO was associated with sensitisation to house dust mite and the fungus *Cladosporium*, but not to grass pollen or cat dander. To our knowledge, this is the first study to show an association between sensitisation to *Cladosporium* and increased eNO levels. The univariate analyses showed an association between eNO and the number of positive skin prick reactions. However, multiple linear regression showed that only sensitisation to house dust mite and *Cladosporium* were significant predictors of eNO. Our findings are similar to those of Moody *et al*[32] who found that increased eNO was associated with house dust mite sensitivity in asymptomatic Pacific Islanders. House dust mite is a perennial allergen but exposure may be highest during winter when people spend more time inside their homes. *Cladosporium* is the most ubiquitous fungi worldwide[33] and grows both inside and outside people's homes. We hypothesise that exposure to perennial allergens like house dust mite or *Cladosporium* are more likely to cause airway inflammation than exposure to seasonal allergens such as grass pollen and *Alternaria*. An Italian study showed an increase in eNO levels during natural allergen exposure in mainly grass pollen allergic children.[34] We did not find a relation between eNO levels and grass pollen in our study, but we did not measure eNO during spring when natural exposure to grass pollen concentrations would be at its highest. Cat dander is a perennial allergen and has been associated with AHR and asthma symptoms in other populations.[35] However, we did not find an association with eNO, possibly because the proportion of children sensitised to cat dander was relatively low in our population.

In these atopic children increased eNO levels were associated with both current wheezing symptoms and airway hyperresponsiveness. Children who experienced wheeze in the last month had significantly higher levels of eNO and more severe AHR—indicated by higher DRR to histamine—than those without respiratory symptoms. The presence of both symptoms and AHR has been associated with increased eNO levels in several previous studies. Salome *et al*[3] found that eNO levels were higher in subjects with AHR plus wheeze in the last 12 months than in subjects with wheeze but no AHR. In a large population based survey in adolescents, eNO levels were increased in atopic subjects with seasonal asthma symptoms and AHR when compared with atopic asymptomatic subjects with AHR.[10] On the other hand, a study in steroid naive asthmatic children found that, although eNO levels were increased compared with healthy controls, this increase was independent of the degree of AHR.[36] However, this study had a relatively small sample size and may not have had sufficient power to detect an association between eNO and AHR.

We also found that eNO was higher in children with AHR than in those without AHR, irrespective of recent wheezing or dry cough. Among children without AHR the difference between those with and without symptoms failed to reach significance. These findings suggest that there may be a closer association between eNO levels and airway responsiveness than between eNO levels and symptoms, even when the symptoms have occurred as recently as in the last month. There was a significant correlation between eNO levels and the DRR to histamine, where the level of eNO increased with increasing severity of AHR. Similar correlations between airway responsiveness and eNO levels have been found in a population sample of young adults,[3] in steroid naive adult asthmatic patients,[16] and in asthmatic children.[37] Our finding that eNO is increased in a population of atopic children with AHR irrespective of respiratory symptoms suggests that eNO may reflect airway abnormalities that are characteristic of asthma.

There was no evidence for differences in eNO levels or AHR between children with dry cough, in the absence of wheezing, and children without respiratory symptoms. The presence of dry cough has been shown to be a reliable and valid measure of persistent cough.[38] Although cough alone has been described as a feature of asthma,[39] recent epidemiological evidence has shown clear differences between recurrent or nocturnal cough alone and wheeze[20 40] which suggests that cough reflects an illness with a different aetiology to wheeze. There is also some evidence that the cough based diagnosis of asthma could explain the increased prevalence of doctor diagnosed asthma[41] and could be a cause of possible overtreatment.[20] Our findings support the view that the presence of cough without associated wheeze is not sufficient to establish the diagnosis of asthma.

In conclusion, this study of atopic children shows that an increased level of eNO is associated with sensitisation to house dust mite and *Cladosporium*, which may relate either to the nature of exposure to these allergens or to the part they play in the origin of airways inflammation and asthma. Exhaled NO was related to airway responsiveness and tended to be higher in children with AHR than in those without AHR, irrespective of history of respiratory symptoms. Exhaled NO and DRR histamine tended to be higher in children with wheeze than in those without current respiratory symptoms or with dry cough only, whereas there was no evidence of a difference between those with dry cough and the asymptomatic group. These findings suggest that an increased level of eNO is not just a marker of atopy, but reflects airway abnormalities that are characteristic of asthma. Increased eNO levels may be more closely associated with airway hyperresponsiveness than with symptoms. The findings also suggest that perennial allergens such as house dust mite and *Cladosporium* could have an important role in the airway inflammation that underlies asthma.

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