

Exhaled nitric oxide and exercise-induced bronchoconstriction in young wheezy children – interactions with atopy

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The association between exercise-induced bronchoconstriction (EIB) and exhaled nitric oxide (FE_{NO}) has not been investigated in young children with atopic or non-atopic wheeze, two different phenotypes of asthma in the early childhood. Steroid naïve 3- to 7-yr-old children with recent wheeze ($n = 84$) and age-matched control subjects without respiratory symptoms ($n = 71$) underwent exercise challenge test, measurement of FE_{NO} and skin prick testing (SPT). EIB was assessed by using impulse oscillometry, and FE_{NO} by standard online technique. Although FE_{NO} levels were highest in atopic patients with EIB, both atopic and non-atopic wheezy children with EIB showed higher FE_{NO} than atopic and non-atopic control subjects, respectively. In atopic wheezy children, a significant relationship between FE_{NO} and the severity of EIB was found ($r = 0.44$, $p = 0.0004$), and FE_{NO} was significantly predictive of EIB. No clear association between FE_{NO} and EIB or predictive value was found in non-atopic wheezy children. Both atopic and non-atopic young wheezy children with EIB show increased FE_{NO} levels. However, the association between the severity of EIB and FE_{NO} is present and FE_{NO} significantly predictive of EIB only in atopic subjects, suggesting different interaction between bronchial responsiveness and airway inflammation in non-atopic wheeze.

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Nitric oxide (NO) has been proposed as a marker of airway inflammation in asthma, based on the findings that its fractional concentration in exhaled air (FE_{NO}) is increased in adults and children with asthma and correlates with measures of eosinophilic activity in the airway mucosa (1). In children, FE_{NO} seems to be a useful clinical tool in assessing airway inflammation in asthma, with the advantage that the measurement is non-invasive and the results are immediately available (2). However, there is evidence of different phenotypes of childhood asthma, which differ by their relationship with FE_{NO} (3). The distinction of different wheezing

phenotypes may be one clinically important advantage of FE_{NO} measurements (4).

Exercise is a common cause of acute airway obstruction in asthmatic children, and exercise-induced bronchoconstriction (EIB) is a specific sign of active asthma (5). A close relationship between EIB and FE_{NO} has been reported in asthmatic adults (6–8) and school-aged children (9, 10). In children, FE_{NO} has been proposed to be a simple, time- and resource-efficient tool to screen and predict EIB (10). So far, the predictive value of FE_{NO} for EIB has not been confirmed in young children, and there is limited data how the relationship between FE_{NO} and EIB is affected by

the atopic status of the child. Non-atopic wheeze appears to be a distinct phenotype of asthma in the childhood, presenting with unique features as regards the risk factors, immunopathology, clinical presentation, and natural course (11). Therefore, we hypothesized that atopic and non-atopic children with wheeze may differ by their relationship between FE_{NO} and EIB.

This study was aimed to clarify the association between FE_{NO} and EIB, and the value of FE_{NO} in predicting EIB in young wheezy children, with special reference to the atopic status of the child. Therefore, we measured FE_{NO} and performed an exercise test in a sample of 3- to 7-yr-old wheezy children and age-matched controls, and determined their atopic status by SPT. The severity of EIB was measured by using impulse oscillometry (12, 13).

Material and methods

Subjects

During a period from January 2003 to June 2005, 84 preschool children referred to Division of Allergy, Helsinki University Central Hospital because of suspected asthma were included. The children were eligible for inclusion if they had a history of recent wheeze (within 12 months), did not use regular antiasthmatic medication at the time of testing and had successful FE_{NO} and lung function measurements. Doctor-diagnosed concomitant diseases like eczema or allergic rhinitis were recorded based on hospital records, and symptom duration, family history of atopy, asthma, or smoking were assessed with a standardized questionnaire. An age-matched control group was collected from a cohort of children taking part in a prospective outcome study of adenoidectomy ($n = 217$). Three years before the present study, these children had been randomized to different treatment strategies because of recurrent otitis media or chronic otitis media with effusion (14). From this cohort, a sample of children considered healthy at the time of testing ($n = 71$), was chosen by using the following criteria: (i) born at term (≥ 36 gestational weeks), (ii) no history of asthma or allergic rhinitis, (iii) no history of wheeze, (iv) no other current or past chronic disease, (v) successful FE_{NO} and lung function measurements. Baseline characteristics of the patients and control subjects are shown in Table 1.

At the time of testing, none of the children had experienced a respiratory tract infection in the preceding 2 wk. Short-acting β_2 -agonists were

Table 1. Baseline characteristics of the study groups

	Wheeze group	Control group
n	84	71
Male gender, n	62	37
Age, mean (range), yr	5.6 (3.8–7.8)	5.2 (3.9–7.2)
Height, mean (range), cm	115 (99–132)	113 (96–128)
Skin prick test positive, n (%)	63 (75)	11 (15)
Parental smoking, %	54	45
Rrs5, mean (s.d.), kPas ⁻¹ L	0.88 (0.20)	0.86 (0.16)
FE_{NO} , median (interquartile range), p.p.b.	23.6 (22.0)	7.4 (3.9)

withheld for at least 12 h preceding the test. The children were also free from inhaled corticosteroids or other antiasthmatic medication. The study was approved by the institutional pediatric ethics committee of Helsinki University Central Hospital, and informed consent was obtained from the parents.

Methods

Exhaled nitric oxide was measured using a computerized equipment with a chemiluminescence analyzer (Niox, Aerocrine AB, Sweden), according to the recommendations of American Thoracic Society (ATS). Children were seated, without a nose clip, and were asked to fill their lungs completely with NO-free air, and thereafter to exhale with a mean and instantaneous flow of 50 ± 5 ml/s for at least 6 s. Exhalations not meeting the ATS requirements were automatically rejected by the system, and the maneuvers repeated until two to three reproducible FE_{NO} values within 10% were obtained. The mean FE_{NO} was recorded. Age-specific height-adjusted reference values were used (16), and the concentration was regarded as high when increased by two standard deviations from predicted (z -score $\geq +2$). The measurement of FE_{NO} always preceded challenge test to avoid the confounding effect of exercise (17).

Exercise challenge was performed as an outdoor running test according to a validated protocol (18), as described earlier (13). Lung function was measured by using impulse oscillometry (IOS, Jaeger GmbH, Würzburg, Germany) at the baseline, and repeated after 2, 5, and 10 min after the exercise. The maximum change in respiratory resistance (Rrs5) was recorded, and an increase at least 40% was regarded as indicative of EIB (12, 13).

The SPT was carried out with 10 common inhalant allergens (SQ, ALK, Horsholm, Denmark) including birch, timothy, *Festuca*

pratensis, mugworth, cat, dog, horse, cow, dust mite, latex, and *Cladosporium herbarum*. The reaction was regarded as positive, if the wheal diameter was 3 mm or greater and the control solutions gave expected results.

In both study groups, FE_{NO} levels were not normally distributed and logarithmic transformation was used for statistical analyses. ANOVA was applied for comparison of FE_{NO} levels in wheezy children with and without significant EIB and control subjects. Regression analysis was used to estimate association between FE_{NO} and EIB. Stratified analyses were made in SPT positive and negative patients and subjects. Logistic regression analysis was used to estimate interactions between EIB and SPT positivity by their relationship with FE_{NO} . The effects were given as odds ratios (OR) with 95% confidence intervals (CI). The discriminatory property of FE_{NO} to identify children with EIB was evaluated by constructing receiver operating characteristics (ROC) curves. For comparison between SPT positive and negative children, the area under curve (AUC) with 95% CI was determined. Statistical analyses were done using STATVIEW and SPSS software packages (Version 11.0 for Windows, Chicago, IL, USA).

Results

Altogether 84 children fulfilled the inclusion criteria with a history of recent wheeze. Of these children, 63 showed positive SPT, and 54 showed a significant increase ($\geq 40\%$) in Rrs5 after exercise challenge indicating EIB. None of the control subjects responded significantly to exercise. Within the study population, children with significant EIB were characterized more often with SPT positivity ($p = 0.01$), allergic rhinitis ($p = 0.02$), and eczema ($p = 0.02$), but did not significantly differ from those without

significant EIB by duration of symptoms, family history of atopy, asthma, or smoking.

Exercise-induced bronchoconstriction positive wheezy children showed higher FE_{NO} levels than EIB negative wheezy children ($p = 0.0002$) and controls ($p < 0.0001$). The difference between wheezy EIB positive children and controls was significant in a stratified analysis of both SPT positive and negative patients and subjects (Fig. 1). The results in the corresponding groups are presented in Table 2. In wheezy children, there was a significant relationship between the change in Rrs5 after exercise and FE_{NO} ($r = 0.42$, $p < 0.0001$). Fig. 2 shows this association in SPT positive and negative wheezy children. Although there was a highly significant correlation in the SPT positive subgroup ($r = 0.44$, $p = 0.0004$), no association was observed in the SPT negative children ($p = 0.32$). In univariate analyses, both atopy (OR 2.87; 95% CI 1.04–7.904; $p = 0.04$) and EIB (OR 2.71; 95% CI 1.08–6.85; $p = 0.03$) were significantly associated with increased FE_{NO} , and a significant interaction was found between EIB and atopy by their relationship with FE_{NO} ($p = 0.004$).

The discriminative property of FE_{NO} to predict significant EIB in wheezy SPT positive and negative children has been illustrated in the ROC analysis graph (Fig. 3). FE_{NO} showed significant predictive power in the SPT positive children (AUC = 0.69; 95% CI 0.55–0.82; $p = 0.019$), but not in the SPT negative children (AUC = 0.57; 95% CI 0.32–0.82; $p = 0.61$). In the present series of atopic wheezy young children, FE_{NO} level of at least 28 p.p.b. (or z-score + 3.3) predicted significant EIB with a probability (positive predictive value) of 90%, and showed specificity of 89% and sensitivity of 41%. At the cutoff level of 24 p.p.b., the corresponding figures were: PPV 83%, specificity 79%, sensitivity 50%.

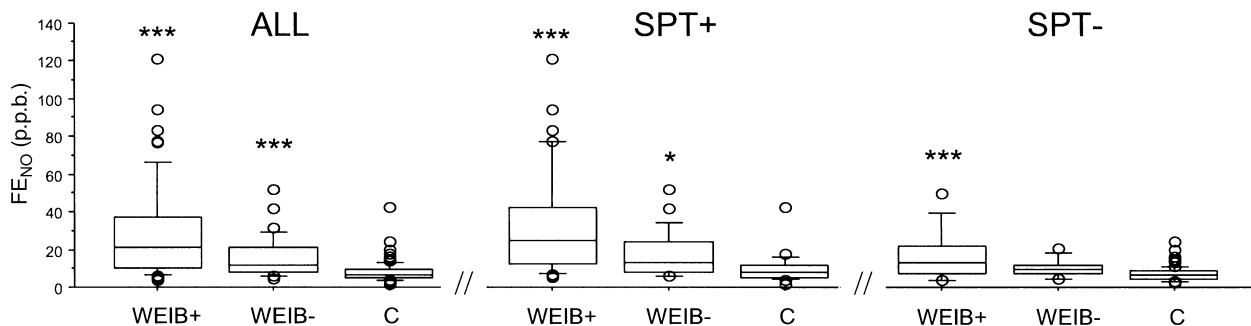


Fig. 1. The box plot presentations of exhaled nitric oxide (FE_{NO}) in the wheezy children with (WEIB+) and without (WEIB-) exercise induced bronchoconstriction, and control subjects without respiratory symptoms (C). SPT+ and SPT- = skin prick test positive and negative, respectively; *** $p < 0.0001$ and * $p < 0.05$ compared with control subjects (ANOVA of the log transformed FE_{NO}).

Table 2. Median (interquartile range) FE_{NO} and the mean (s.d.) change in Rrs5 after exercise challenge ($\Delta Rrs5$) in study groups stratified by exercise-induced bronchoconstriction (EIB) and atopy

	Wheezers with EIB		Wheezers without EIB		Control group	
	SPT+	SPT–	SPT+	SPT–	SPT+	SPT–
n	41	13	22	8	11	60
$\Delta Rrs5$ (%)	69.1 (25.1) ^{†§}	58.4 (25.0) ^{†§}	30.1 (9.1) ^{*¶}	15.6 (14.8)	14.4 (14.4)	11.0 (9.8)
FE_{NO} (p.p.b.)	24.6 (30.3) ^{†*¶}	13.3 (14.5) ^{†‡}	13.1 (16.4) [*]	9.7 (4.6)	8.3 (6.2)	6.6 (4.3)

SPT+ and SPT– = skin prick test positive and negative, respectively.

* $p < 0.05$; [†] $p < 0.001$ compared with control subjects with corresponding SPT result.

[‡] $p < 0.05$; [§] $p < 0.001$ compared with wheezers without significant EIB with corresponding SPT result.

[¶] $p < 0.05$ compared with SPT negative wheezers with corresponding EIB response.

p values refer to anova (log transformed FE_{NO} values compared).

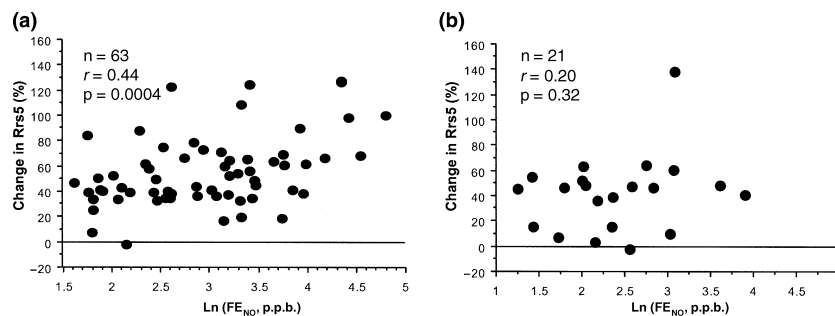


Fig. 2. The relationship of exhaled nitric oxide (FE_{NO}) with change in respiratory resistance (Rrs5) after exercise challenge in skin prick test positive (a) and negative (b) young children with a history of recent wheeze.

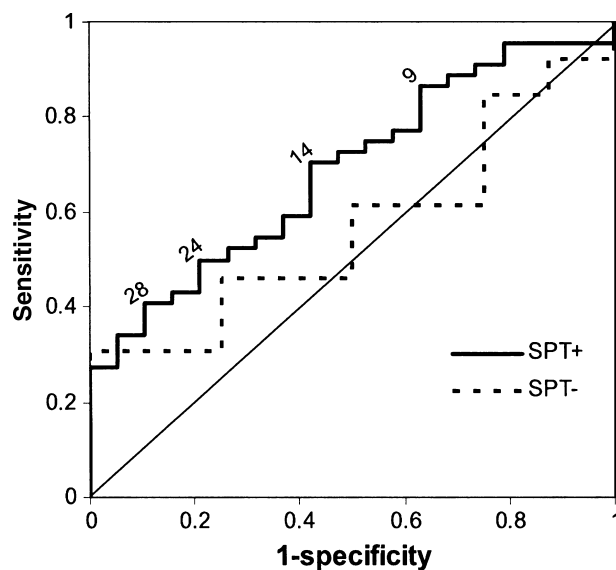


Fig. 3. Receiver operating characteristics (ROC) analysis graph illustrating the discriminative property of FE_{NO} to predict significant EIB (increase of Rrs5 of at least 40% after exercise) in wheezy SPT positive (SPT+) and negative (SPT–) children. Beside the ROC curve of wheezy SPT+ children, examples of corresponding cutoff levels (FE_{NO} , p.p.b.) are indicated.

Because of high prevalence of EIB positive children among atopic wheezers, the negative predictive values were low.

Discussion

In this study, a significant association between EIB and FE_{NO} levels was found in young 3- to 7-yr-old children with a history of recent wheeze. Although both atopic and non-atopic wheezy children with EIB showed increased mean levels of FE_{NO} compared with control subjects, EIB was significantly predicted by FE_{NO} only in the atopic children. Therefore, the relationship between EIB and FE_{NO} was affected by the atopic status of the wheezing child.

The present data emphasize the clinical value of EIB as a sign of active asthma, as baseline lung function (respiratory resistance) was not sensitive enough to discriminate between wheezy children and control subjects. EIB is thought to initiate from high rate of water loss from the airways during increased ventilation, with bronchoconstrictor mediator release in response to increase in ion concentration of the periciliary fluid involving inflammatory, mainly mast cells (19). The immunopathology of asthma with EIB is characterized by increased concentrations of columnar epithelial cells and eosinophils (20). It is therefore not surprising that FE_{NO} , being a surrogate for airway eosinophilia (1), has been found to be increased in asthmatic patients with EIB.

Scollo et al. (9) were the first to report a significant relationship between exhaled nitric oxide and EIB in 24 school-aged children with asthma. Subsequently, similar results have been reproduced in adult asthmatics (6–8). Buchvald et al. (10) studied a large group of school-aged children with asthma, and concluded that FE_{NO} may be used for pre-screening of asthmatic children with exercise symptoms, and to optimize the resources for exercise testing in pediatric asthma monitoring. The current study is in agreement with the previous reports on the relationship between FE_{NO} and EIB, but included significantly younger children (age 3–7 yr). Therefore, similar immunopathology of EIB, which is present in older children and adults seems to involve also young children with a history of wheeze. In young children who are less cooperative, the assessment of EIB requires even more expertise and time resources, and a simple screening tool would be very useful. As young children are usually not capable of reliable spirometric measurements, we assessed EIB by measuring changes in respiratory resistance with the impulse oscillometry as described previously (13). A recently published ATS/ERS statement on preschool lung function testing proposes that an increase of 35–40% in respiratory resistance is indicative of significant bronchoconstriction during bronchial challenge tests (12).

Atopy is a main determinant of FE_{NO} in population-based studies (21–23). In steroid naïve patients with current symptoms, FE_{NO} is more strongly associated with asthma as we have demonstrated in preschool children (16). The current authors found that in symptomatic children with wheeze, even non-atopic children may show increased FE_{NO} levels.

In the community, FE_{NO} levels seem to correlate with symptoms and bronchial hyper-responsiveness in atopic asthmatics, but less in non-atopic patients (24, 25). Only one study by Rouhos et al. (8) including adult male conscripts has investigated the effect of atopy on the relationship between FE_{NO} and EIB. They found a significant association only in atopic subjects, which is in agreement with the present results. In the studies performed in children with asthma (9, 10), most of the study subjects had history of allergy. Moreover, neither was IgE-mediated atopy objectively assessed nor its effect investigated in these previous reports. The present study suggests that the atopic status and EIB interact by their relationship with FE_{NO} , and in non-atopic subjects, the severity of EIB cannot be accurately predicted by FE_{NO} . This finding may have important clinical impli-

cations when FE_{NO} measurements are used to prescreen children with exercise-induced symptoms, as suggested (10).

Non-atopic asthma of adulthood is characterized by locally enhanced expression of Th2-type cytokines (26). In contrast, the immunopathology of non-atopic asthma in the early childhood is poorly known. The non-atopic wheezers are thought to represent an asthma phenotype, which starts out with normal lung function at infancy, but end up with lower lung function and enhanced airway reactivity later in childhood (11). Many of these children have a history of lower respiratory tract infection-related wheeze. Whether their increased airway reactivity is present from the birth, or the consequence of virus-induced immune responses, especially caused by respiratory syncytial virus (27), is currently not known. The present data with young 3- to 7-yr-old children suggest airway inflammation as reflected by raised FE_{NO} levels even in non-atopic wheezers with current symptoms, but show a lack of association between this inflammatory process and EIB. Therefore, different mechanisms of bronchial responsiveness are likely to involve EIB in atopic and non-atopic wheeze. Either inflammatory changes responsible for EIB in non-atopic children are not accurately reflected by FE_{NO} , mechanisms leading to EIB are more heterogeneous or EIB in non-atopic children is mainly dependent on other mechanisms, such as vascular phenomena during exercise induced mucosal cooling and dehydration (19). Little is known about the barrier functions on bronchial epithelium, but it is tempting to speculate that atopic subjects may have a biased barrier in the bronchi, potentially explaining the findings of this study. For example, mutations in the gene coding for filaggrin, an important component of the epidermal surface, have been shown to be associated not only with atopic dermatitis, but with atopy and severity of asthma (28).

In conclusion, both atopic and non-atopic young wheezy children with EIB show increased FE_{NO} levels. The association between the severity of EIB and FE_{NO} is present and FE_{NO} predictive of EIB only in SPT positive subjects, suggesting different interaction between bronchial responsiveness and airway inflammation in non-atopic wheeze.

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References

1. TAYLOR DR, PIJNENBURG MW, SMITH AD, DE JONGSTE JC. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006; 61: 817–27.
2. MALMBERG LP. Exhaled nitric oxide in childhood asthma – time to use inflammometry rather than spirometry? *J Asthma* 2004; 41: 511–20.
3. PAYNE DNR, WILSON NM, JAMES A, HABLAS H, AGRAFIOTI C, BUSH A. Evidence for different subgroups of difficult asthma in children. *Thorax* 2001; 56: 345–50.
4. BACHARIER LB. Does exhaled nitric oxide measurement help distinguish between wheezing phenotypes in preschool children? *J Allergy Clin Immunol* 2008; 171: 710–1.
5. AMERICAN THORACIC SOCIETY. Guidelines for methacholine and exercise testing – 1999. *Am J Respir Crit Care Med* 2000; 161: 309–29.
6. EL HALAWANI S, LY N, MAHON R, AMUNDSON D. Exhaled nitric oxide as a predictor of exercise-induced bronchoconstriction. *Chest* 2003; 124: 639–43.
7. BERKMAN N, AVITAL A, BREUER R, BARDACH E, SPRINGER C, GODFREY S. Exhaled nitric oxide in the diagnosis of asthma: comparison with bronchial provocation tests. *Thorax* 2005; 60: 383–8.
8. ROUHOS A, EKROOS H, KARJALAINEN J, SARNA S, SOVIÄRVI ARA. Exhaled nitric oxide and exercise-induced bronchoconstriction in young male conscripts: association only in atopics. *Allergy* 2005; 60: 1493–8.
9. SCOLLO M, ZANCONATO S, ONGARO R, ZARAMELLA C, ZACCHELLO F, BARALDI E. Exhaled nitric oxide and exercise-induced bronchoconstriction in asthmatic children. *Am J Respir Crit Care Med* 2000; 161: 1047–50.
10. BUCHVALD F, HERMANSEN M, NIELSEN KG, BISGAARD H. Exhaled nitric oxide predicts exercise-induced bronchoconstriction in asthmatic children. *Chest* 2005; 128: 1964–7.
11. STEIN RT, MARTINEZ FD. Asthma phenotypes in childhood: lessons from an epidemiological approach. *Paediatr Respir Rev* 2004; 5: 155–61.
12. BEYDON N, DAVIS SD, LOMBARDI E, et al. An Official ATS/ERS Statement. Pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007; 175: 1304–45.
13. MALMBERG LP, MÄKELÄ MJ, MATTILA PS, HAMMAREN-MALMI S, PELKONEN AS. Exercise-induced changes in respiratory impedance in young wheezy children and nonatopic controls. *Pediatr Pulmonol* 2008; 43: 538–44.
14. HAMMAREN-MALMI S, SAXEN H, TARKKANEN J, MATTILA P. Adenoidectomy does not significantly reduce the incidence of otitis media in conjunction with the insertion of tympanostomy tubes in children who are younger than 4 years: a randomized trial. *Pediatrics* 2005; 116: 185–9.
15. AMERICAN THORACIC SOCIETY. Recommendations for standardized procedures for online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. *Am J Respir Crit Care Med* 1999; 160: 2104–17.
16. MALMBERG LP, PELKONEN A, HAAHTELA T, TURPEINEN M. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. *Thorax* 2003; 58: 494–9.
17. GABRIELE C, PIJNENBURG MW, MONTI F, HOP WC, BAKKER ME, DE JONGSTE JC. The effect of spirometry and exercise on exhaled nitric oxide in asthmatic children. *Pediatr Allergy Immunol* 2005; 16: 243–7.
18. HABY MM, ANDERSON SD, PEAT JK, MELLIS CM, TOELLE BG, WOOLCOCK AJ. An exercise challenge protocol for epidemiological studies of childhood asthma. *Eur Respir J* 1995; 8: 729–36.
19. VAN SCHOOR J, JOOS GF, PAUWALS RA. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. *Eur Respir J* 2000; 16: 514–33.
20. HALLSTRAND TS, MOODY MW, AITHEN ML, HENDERSON WR. Airway immunopathology of asthma with exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 2005; 116: 586–93.
21. FRANK TL, ADISESH A, PICKERING AC, et al. Relationship between exhaled nitric oxide and childhood asthma. *Am J Respir Crit Care Med* 1998; 158: 1032–6.
22. BARRETO M, VILLA MP, MONTI F, et al. Additive effect of eosinophilia and atopy on exhaled nitric oxide levels in children with or without a history of respiratory symptoms. *Pediatr Allergy Immunol* 2005; 16: 52–8.
23. MALMBERG LP, PETÄYS T, HAAHTELA T, et al. Exhaled nitric oxide in healthy nonatopic school-aged children – determinants and height-adjusted reference values. *Pediatr Pulmonol* 2006; 41: 635–42.
24. FRANKLIN PJ, TURNER S, LE SOUEF PN, STICK S. Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. *Thorax* 2003; 58: 1048–52.
25. STEERENBERG PA, JANSSEN NAH, DE MEER G, et al. Relationship between exhaled NO, respiratory symptoms, lung function, bronchial hyperresponsiveness, and blood eosinophilia in school children. *Thorax* 2003; 58: 242–5.
26. HUMBERT M, DURHAM SR, YING S, et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against “intrinsic” asthma being a distinct immunopathological entity. *Am J Respir Crit Care Med* 1996; 154: 1497–504.
27. STEIN RT, SHERRIL D, MORGAN WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; 353: 541–5.
28. PALMER CN, ISMAIL T, LEE SP, et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. *J Allergy Clin Immunol* 2007; 120: 64–8.