

Very low birth weight and respiratory outcome: association between airway inflammation and hyperresponsiveness

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ABSTRACT

Background: The respiratory outcomes after preterm birth have changed, and it is unclear whether increased airway hyperresponsiveness (AHR) later in childhood is associated with airway inflammation.

Objective: To investigate the association between AHR and fractional exhaled nitric oxide (FeNO), including the alveolar concentration of nitric oxide, in school-age children with very low birth weight (VLBW).

Methods: Twenty-nine children with VLBW, 33 children with a history of early wheeze, and 60 healthy controls underwent a FeNO measurement and bronchial challenge test with histamine. Atopy was assessed with skin prick tests.

Results: Children with VLBW had well-preserved baseline lung function but significantly increased AHR, expressed as the dose response slope ($P < .001$). Geometric mean FeNO levels were similar between VLBW children and healthy controls, and a history of bronchopulmonary dysplasia had no effect. In the VLBW and early wheeze groups, AHR was associated with FeNO ($r = 0.47$, $P = .01$, and $r = 0.43$, $P = .013$, respectively), but in a stratified analysis, this association was significant only in atopic individuals. By using the multiple flow FeNO technique, the bronchial nitric oxide flux rather than alveolar nitric oxide concentrations were associated with AHR in both children with early wheeze and VLBW.

Conclusion: We conclude that in VLBW children AHR is related to FeNO but only in atopic individuals. Similar to children with early wheeze, this association is dependent on bronchial flux rather than alveolar nitric oxide concentration. It is likely that AHR is modified by atopic inflammation rather than by inflammatory process due to prematurity.

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Introduction

Preterm birth is often associated with long-term airflow limitation later in life. Children with a history of preterm birth, in particular those with bronchopulmonary dysplasia (BPD), may develop asthmalike symptoms in childhood, with variable response to β_2 -agonists. Despite many clinical similarities, the causal pathophysiologic mechanisms, risk factors, and natural history differ between asthma and respiratory morbidity due to prematurity. After introduction of measures to prevent and treat respiratory distress syndrome (eg, surfactant replacement) and advances in respiratory care, newborns have survived younger, and the respiratory outcomes of preterm birth have changed. The new BPD is

primarily characterized by diffusely reduced alveolar development, and airway injury is less dominant.¹

Asthma is a chronic disease with airway inflammation, which leads to airway hyperresponsiveness (AHR). Earlier studies of children with a history of preterm birth, and particularly of those with BPD, also found increased airway responsiveness,² but it is unclear whether this is associated with ongoing inflammatory processes or sequelae of lung injury. Longitudinal studies have found that airflow limitation persists until adulthood, with no evidence of catch-up growth in lung function. Recently, it was reported that BPD survivors may have a substantial accelerated decline in pulmonary function over time, suggesting ongoing remodeling of the airways.^{3,4} Whether this decline in lung function is associated with persistent airway inflammation is not known.

Increased levels of fractional exhaled nitric oxide (FeNO) have been observed in infants with BPD shortly after birth.⁵ So far, there is little evidence that airway inflammation persists in those with a history of BPD. In school-age children with a history of BPD and airflow limitation, inhaled corticosteroids may alleviate respiratory

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symptoms but do not improve lung function.⁶ and there is no association between the severity of disease and the FeNO.⁷ However, previous studies of FeNO in BPD children reflect the old form of disease, and the inflammatory sequelae of the new form are still unclear. Furthermore, the routine method to measure FeNO reflects airway inflammation in the larger airways and cannot exclude increased nitric oxide production at near alveolar level. By using the extended nitric oxide analysis, it has been possible to find that, although the airway tissue content of nitric oxide is increased in asthma, adult patients with chronic obstructive pulmonary disease have normal airway transfer rates but high alveolar levels of nitric oxide.⁸ So far, there have been no studies of very low-birth-weight (VLBW) children using the extended analysis of nitric oxide.

We hypothesized that in school-age children with a history of very preterm birth, AHR was related to airway inflammation expressed as FeNO. Therefore, we investigated school-age children with VLBW with or without BPD, children with a history of early wheeze, and healthy controls, who underwent a series of lung function tests, including the measurement of FeNO and bronchial challenge test with histamine. To differentiate between the effect of alveolar and bronchial nitric oxide production, we used the nitric oxide analysis with multiple flows. The association of airway inflammation and AHR was compared between the study groups.

Methods

From January 2005 through February 2011, 29 children with VLBW (defined as birth weight $\leq 1,500$ g), 33 children born full term but with physician-diagnosed early-onset wheezing illness, and 60 healthy controls underwent respiratory function tests and the measurement of FeNO during their clinical visits in the Skin and Allergy Hospital, Helsinki University Central Hospital. The age of the study participants at the time of the study was 8 to 10 years. The VLBW group was drawn from a cohort of 44 children born very preterm and treated consecutively at the intensive care unit of the Hospital for Children and Adolescents, Helsinki University Central Hospital, from January 1995 through August 1996. All these individuals were invited, but 14 did not want to participate (7 had moved), 1 did not show up for the scheduled appointment, and 1 could not perform lung function tests because of a neurologic condition, leaving a group of 28 VLBW children with successful respiratory function tests. The children with early wheezing disorder were also participating in a follow-up study of infant asthma.⁹ These children had been referred to a tertiary center because of recurrent lower respiratory tract symptoms of airflow obstruction, including dyspnea, cough, and wheeze, between the ages of 0.3 and 2.2 years. At the time of the study, in most children the symptoms had subsided: only 14 (42%) had used inhaled corticosteroids during the last year. The group of healthy children were selected from a control cohort prospectively followed up for a study of cow milk allergy.¹⁰ They did not have history of asthma or any other chronic disease and were not using any medication. The main characteristics of the study groups are summarized in Table 1. All the children with early wheeze and in the control group were born full term; more detailed neonatal data of the VLBW group are presented in Table 2. Twelve (41%) of the children in the VLBW group had a history of BPD, defined as a requirement for supplemental oxygen at the corrected postnatal gestational age of 36 weeks.¹¹ The neonatal data of nonparticipating VLBW children did not differ significantly from those who participated.

Only one child in the VLBW group received regular inhaled corticosteroid treatment during the last year. Before the lung function assessment, regular asthma medication, including inhaled corticosteroids, was withheld for 4 weeks in all children. At the time of lung function testing, all the children were free of respiratory tract infections during the preceding 2 weeks, and short-acting β_2 -agonists were withheld for at least the previous 12 hours.

Table 1

Characteristics of the study groups

Characteristic	VLBW group (n = 28)	Wheeze group (n = 33)	Control group (n = 60)
Male, No. (%)	13 (46)	22 (67)	30 (50)
Age, mean (range), y	9.7 (9.1–10.3) ^a	8.2 (7.6–9.0) ^a	8.6 (8.1–9.3)
Height, mean (range), cm	135 (121–157)	129 (121–147)	133 (122–150)
Weight, mean (range), kg	31 (20–53)	30 (22–43)	31 (21–53)
FEV ₁ , mean (SD), L	2.0 (0.45)	1.6 (0.28) ^a	1.9 (1.4–2.6)
FEV ₁ , mean (SD), % predicted	100 (14)	88 (13) ^a	98 (76–131)
Prick positive, No. (%)	9 (32)	13 (39)	20 (33)
Rhinitis, No. (%)	4 (14)	11 (33)	13 (22)
Eczema, No. (%)	4 (14)	13 (39)	14 (23)
Regular asthma medication last year, No. (%)	1 (4)	14 (42) ^a	0 (0)
Parental smoking, No. (%)	12 (43)	7 (21)	16 (27)
Parental asthma, No. (%)	3 (11)	14 (42) ^a	5 (8)

Abbreviations: VLBW, very low birth weight; FEV₁, forced expiratory volume in 1 second

^aP < .05 compared with controls (analysis of variance/Dunnett test for continuous variables; χ^2 test for dichotomous variables).

Informed consent was obtained from the children and from one or both parents, and the Ethics Committee of Diseases of Children and Adolescents, Helsinki University Central Hospital, approved the study protocol.

Lung Function Tests

FeNO was measured using computerized equipment with a chemiluminescence analyzer (Niox; Aerocrine AB, Solna, Sweden), according to the European Respiratory Society/American Thoracic Society recommendations.^{12,13} The system was calibrated with a certified nitric oxide calibration gas mixture according to the manufacturer's instructions. Exhalations that did not meet the American Thoracic Society requirements were rejected by the system, and the child was asked to perform new exhalation maneuvers, until 3 reproducible FeNO values within 10 % were obtained. The mean FeNO was recorded.

To assess the flow-independent nitric oxide parameters that reflect alveolar and bronchial compartments, FeNO was measured at 4 flow rates (10, 50, 100, and 200 mL/s)¹⁴ in the children with a history of VLBW and early wheeze. By using flow specific resistors (Niox Flex; Aerocrine) at least 2 acceptable measurements were performed at each flow rate. The results were fitted according to a linear method¹⁵ using a 2-compartment model, where at each expiratory flow between 50 and 200 mL/s, nitric oxide output can be calculated as a product of FeNO and flow:

$$V_{NO} = C_{alv} \times V + J_{NO,Br}$$

Nitric oxide output was plotted against exhalation flow rate, and a linear regression was set by the least square method. The slope and the intercept of this regression line are estimates of alveolar nitric oxide concentration (C_{alv}) and the bronchial nitric oxide flux

Table 2

Perinatal data of the 28 individuals in the VLBW group

Variable	Finding
Gestational age, mean (range), wk	28 (25–31)
Birth weight, mean (range), g	991 (520–1453)
Supplemental oxygen, mean (range), d	74 (1–185)
Ventilatory treatment, mean (range), d	15 (0–52)
Surfactant therapy, No. (%)	16 (57)
Antenatal steroids, No. (%)	16 (57)
Postnatal steroids, No. (%)	6 (21)
RDS, No. (%)	19 (68)
BPD, No. (%)	12 (43)

Abbreviations: VLBW, very low birth weight; RDS, respiratory distress syndrome; BPD, bronchiopulmonary dysplasia.

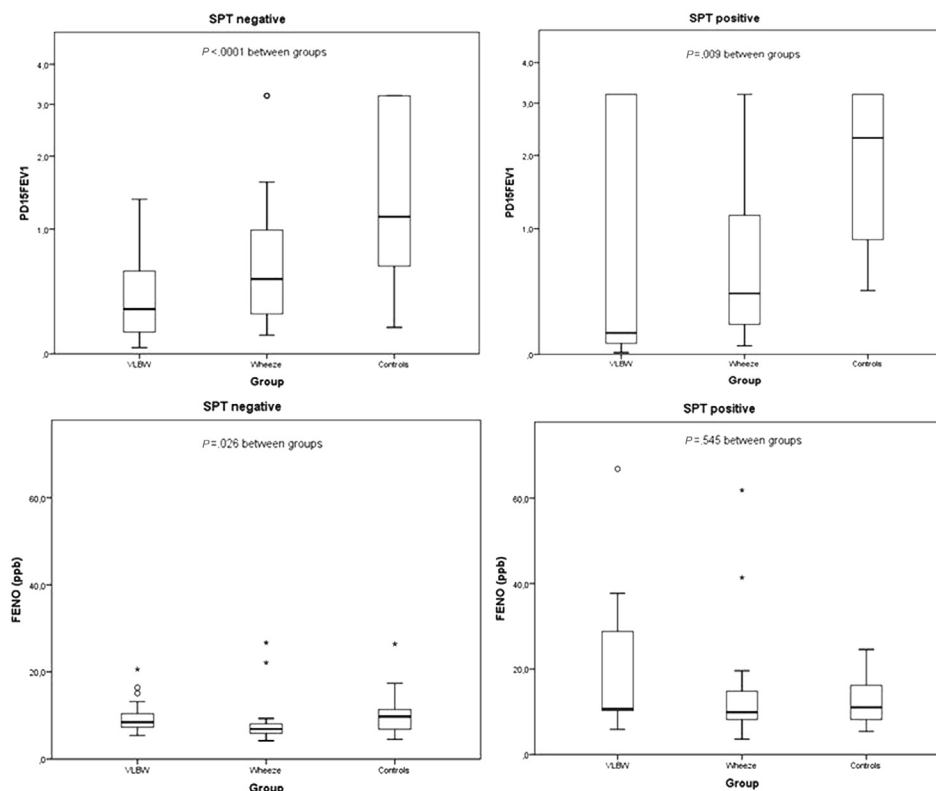


Figure 1. Airway hyperresponsiveness and levels of fractional exhaled nitric oxide (FeNO) at a flow of 50 mL/s in skin prick test (SPT)—positive and SPT-negative children with a history of very low birth weight (VLBW), early wheezing illness (wheeze), and healthy controls. Abbreviation: PD15FEV1, provocative dose of histamine causing a decrease in forced expiratory volume in 1 second (FEV₁) of 15 %.

(JNO_{Br}), respectively. Linearity was tested individually by calculating correlation coefficient of the model, and only tests with $r^2 > 0.90$ were accepted for analysis.

The lung function was measured with a pneumotachograph-based flow-volume spirometer (Masterscreen Pneumo; Jaeger GmbH, Wurzburg, Germany). At least 3 acceptable forced expirations were performed, and the highest forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were recorded. National reference values for spirometric tests in children, adjusted by height and sex, were applied.¹⁶

AHR was evaluated with a dosimetric histamine challenge test as described previously.^{17,18} An inhalation synchronized dosimeter with controlled tidal breathing (Spira Elektro 2; Respiratory Health Care Centre, Hämeenlinna, Finland) was used to nebulize increasing inhaled doses of 0.025, 0.1, 0.4, and 1.6 mg of buffered histamine diphosphate. By using the dose-response curve, the provocative dose of inhaled histamine producing a decrease of 15% in FEV₁ was determined. The bronchial responsiveness to histamine was also expressed in terms of the slope of the dose response curve (DRS), as a continuous and log-normally distributed index,¹⁹ by dividing the percentage decrease in FEV₁ after the last dose by the last dose of histamine.

Skin prick testing (SPT) was performed with 9 common inhalant allergens (ALK, Horsholm, Denmark), including birch, timothy, *Festuca pratensis*, mugwort, cat, dog, horse, cow, dust mite, 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 (histamine dihydrochloride, 10 mg/mL, as a positive control and solvent as a negative control).

Statistical Analyses

Nonparametric tests were used for group comparisons (Kruskal-Wallis or Mann-Whitney) and for correlation analyses (Spearman). Before analysis, DRS and FeNO data were transformed into natural

logarithms (LOGDRS and LOGFeNO, respectively) to improve the symmetry of the distribution, whenever analysis of variance or linear regression methods were used. Statistical analyses were performed using the SPSS software package (version 19.0 for Windows; SPSS Inc, Chicago, Illinois).

Results

The baseline lung function in children with a history of early wheeze was lower than in controls, but VLBW children, including those with a history of BPD, had similar lung function compared with healthy controls (Table 1). However, airway responsiveness to histamine was significantly increased in VLBW children (Fig 1) compared with controls ($P < .001$). Expressed as LOGDRS, the children with a history of BPD had more severe AHR (mean, 2.03; 95% CI, 1.75–2.32) than non-BPD VLBW children (mean, 1.66; 95% CI, 1.43–1.90; $P = .04$). Atopy defined by at least one positive reaction in SPTs had no significant effect on baseline lung function or AHR in the VLBW group.

The geometric means (interquartile ranges [IQRs]) of FeNO levels between the VLBW group (mean, 10.7 ppb; IQR, 7.6–12.5 ppb) and healthy controls (mean, 9.9 ppb; IQR, 7.6–12.1 ppb) were similar ($P = .96$). The history of BPD had no effect. Compared with controls, the children born full term but with a history of early wheeze had slightly lower FeNO levels with a geometric mean of 8.9 ppb (IQR, 6.1–7.4 ppb), but after adjusting for the height difference between the groups, the levels were statistically similar ($P = .14$). The SPT-positive individuals had higher FeNO than nonatopic individuals in the VLBW ($P = .02$) and early wheeze ($P = .01$) groups, and the difference was almost significant in controls ($P = .06$). Therefore, the analyses of FeNO levels were also stratified by atopy (Figure 1). SPT-positive VLBW children had similar levels of FeNO than SPT-positive controls ($P = .46$); accordingly, SPT-negative VLBW children had similar levels of FeNO than SPT-negative controls ($P = .59$).

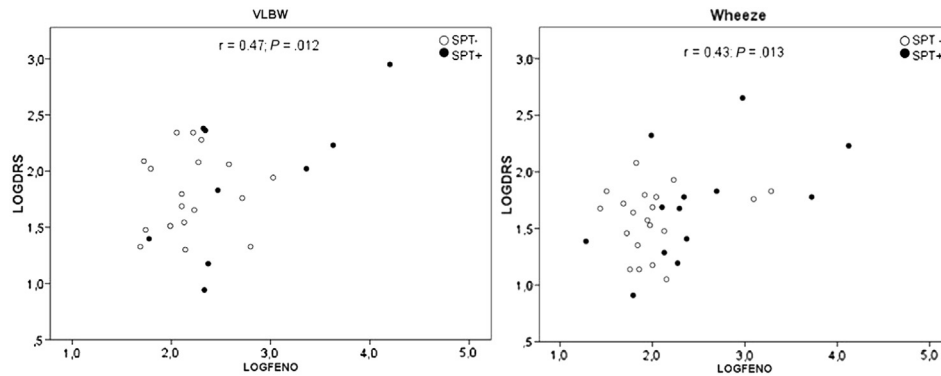


Figure 2. The association between airway hyperresponsiveness and levels of fractional exhaled nitric oxide (FeNO) at a flow of 50 mL/s in children with a history of very low birth weight (VLBW) ($n = 28$) and history of early wheezing illness (wheeze) ($n = 33$). Closed dots indicate skin prick test–positive individuals and open dots indicate skin prick test–negative individuals. Abbreviations: LOGFENO, natural logarithm of FeNO; LOGDRS, natural logarithm of dose response slope.

Twenty-four VLBW and 28 full-term children with early wheeze had successful FeNO tests with multiple flows of 50 to 200 mL/s. The geometric mean C_{alv} was 2.58 ppb (IQR, 2.1–3.3 ppb) in VLBW children and 2.56 ppb (IQR, 1.8–3.5 ppb) in children with early wheeze, with no statistical difference ($P = .67$). Similarly, there were no statistical differences between VLBW and early wheeze regarding $V_{NO,Br}$ with geometric means of 0.39 nL/s (IQR, 0.22–0.36 nL/s) and 0.28 nL/s (IQR, 0.16–0.32 nL/s), respectively ($P = .08$).

AHR, expressed as DRS, was related to FeNO in children with VLBW ($r = 0.47$, $P = .01$) and early wheeze ($r = 0.43$, $P = .01$) but not in controls ($r = 0.058$, $P = .66$) (Fig 2). In stratified analysis by atopy, this association was statistically significant in SPT-positive children with VLBW and early wheeze but not in SPT-negative individuals (Table 3). By using the extended analysis of FeNO, the association between FeNO and AHR was explained by bronchial nitric oxide output in children with VLBW and early wheeze, and alveolar concentration had no effect on AHR (Table 3).

General linear methods were used to assess independent predictors of AHR (LOGDRS) in the data set ($n = 121$). After introducing possible confounding factors, including age, parental smoking, and rhinitis as covariates, the following factors of AHR were entered in the final model (adjusted $r^2 = 0.49$; $P = .004$): history of VLBW ($P < .001$), history of early wheeze ($P = .02$), and LOGFeNO ($P = .02$) were independently associated with AHR, whereas skin prick positivity had no effect ($P = .77$). A significant interaction was observed between FeNO and atopy relating to the association with AHR ($P = .03$).

Discussion

Despite clearly increased AHR, we could not find any differences in FeNO levels between school-age children with VLBW (or BPD) and healthy controls. In VLBW children, however, AHR was related to FeNO levels, suggesting an inflammatory mechanism. Even after adjusting for several confounders in the data set, history of VLBW and FeNO, as well as history of early wheeze, were independent

predictors of AHR. Because this relationship between FeNO and AHR was limited to atopic individuals, it is likely that enhanced AHR in VLBW children is modified by atopic inflammation rather than being a reflection of persistent inflammatory process due to prematurity. These findings may have implications for the treatment of AHR in VLBW children.

Prior studies on the inflammatory characteristics of BPD have focused on the early neonatal period: increasing FeNO levels have been observed in established BPD, with increasing severity.⁵ Evidence that airway inflammation persists in patients with a history of BPD is still lacking. In an earlier sample of 7- to 9-year-old, prematurely born children with or without history of BPD, we found similar FeNO levels as in their controls.⁷ In agreement with our results, a recent follow-up study of extremely preterm children at 11 years of age found normal levels of FeNO, despite widely reduced lung function.²⁰ In contrast, Baraldi et al²¹ reported low FeNO levels in school-aged children with BPD and airflow limitation, which they proposed to result from impaired endothelial release or reduced diffusion of nitric oxide.

Considering the large variation in AHR, the sample size of participating VLBW children was small and limits the strength of the results, particularly in the stratified analyses. Because of different growth patterns, a completely matched control group is difficult to obtain for prematurely born children. In our sample, the VLBW children were slightly older, but the body size in terms of height and weight was matched with the control group, which should enable comparison of the lung function results. One limitation of the study is that controls did not perform FeNO measurements with multiple flows. In VLBW children, we found slightly higher levels of alveolar nitric oxide concentration than Sepponen et al¹⁴ found in healthy children of similar age, but the levels of bronchial flux were comparable. Whether this difference is related to methods or suggests a persistent inflammatory process at the lung periphery in VLBW children remains unclear. So far, we are not aware of any controlled study using the extended analysis of FeNO in children with a history of BPD.

Table 3
Association of exhaled nitric oxide with airway hyperresponsiveness (dose response slope), stratified by atopy^a

Variable	VLBW group		Wheeze group		Control group	
	SPT positive	SPT negative	SPT positive	SPT negative	SPT positive	SPT negative
FeNO, ppb	0.67 (<.05)	0.17 (.48)	0.56 (<.05)	0.09 (.69)	0.14 (.57)	0.12 (.44)
C_{alv} , ppb	0.31 (.46)	0.25 (.37)	0.19 (.60)	0.15 (.54)	NA	NA
$J_{NO,Br}$, nL/s	0.71 (.04)	0.01 (.98)	0.87 (.01)	0.25 (.32)	NA	NA

Abbreviations: C_{alv} , alveolar nitric oxide concentration; FeNO, fractional exhaled nitric oxide; $J_{NO,Br}$, bronchial nitric oxide flux; NA, not applicable; SPT, skin prick test; VLBW, very low birth weight.

^aResults expressed as Spearman r (P value).

Despite clinical similarities between asthma and respiratory outcomes due to prematurity, FeNO levels have been found to distinguish the patients, with higher values being more characteristic of asthmatic children.²² Our group of children with early-onset wheezing illness did not represent children with active asthma, but they all had significant troublesome lung symptoms before the age of 3 years, which may be associated with asthma later.²³ However, most of these children were asymptomatic at the time of the study, which may explain the similar levels of FeNO compared with controls. However, this group was characterized by reduced lung function and increased AHR, the latter being associated with FeNO in atopic individuals. The similar relationship between AHR and bronchial nitric oxide flux in children with early wheezing illness and VLBW suggests similar interactions between airway inflammation and AHR. Interestingly, in a cohort of 6-year-old children with maternal asthma, FeNO and AHR to cold air hyperventilation were associated and continuous traits independent of asthma.²⁴ We found concurring results, which extend the concept even to children with history of prematurity.

The current results indicate that despite well-preserved lung function, children with a history of VLBW should be monitored for asthmalike symptoms due to increased AHR. So far, there is little evidence that prematurely born children with respiratory symptoms would benefit from inhaled corticosteroids later in childhood. In school-aged children born preterm, inhaled corticosteroids did not improve lung function, but symptoms and diurnal variation of peak expiratory flow decreased.⁶ This finding may indicate that AHR in some children with premature birth is partly reversible with anti-inflammatory treatment. The results of the present study warrant such an intervention to be studied at least in VLBW survivors who are atopic. Furthermore, no studies have evaluated the efficacy of long-term use of anti-inflammatory treatment to prevent airway remodeling in survivors of very preterm birth.

Since the introduction of antenatal corticosteroids and neonatal surfactant replacement, combined with a more conservative approach to respiratory care in the early 1990s, BPD is now restricted to more immature infants. Longitudinal studies have found that prematurity itself has an important independent influence on the respiratory outcome.¹ So far, there is no evidence that the advances in the neonatal respiratory care of prematurely born children have resulted in better lung function at school age. Although increased AHR was a characteristic feature, our sample of VLBW (and BPD) children had preserved their baseline lung function remarkably well, which contrasts the results of our previous study from an older cohort² and of a similar cohort from Tampere in the surfactant era.²⁵ A selection bias is possible because not all the invited participants were assessed; however, based on the neonatal data, those VLBW children who could not participate did not differ from those who participated.

In particularly, children with extremely low birth weight (<1,000 g) have significantly abnormal lung function at school age.^{26,27} Clearly, reduced lung function was observed by Lum et al.²⁰ in a study of extremely preterm children (born at ≤25 weeks of gestation) at 11 years of age. Their study demonstrated the extent of lung function abnormalities in the smallest survivors, characterized by airway obstruction, ventilation inhomogeneity, gas trapping, and AHR. The authors conclude that this new form of chronic lung disease is probably due to small airway changes, secondary to disruption of the interstitial collagen network, and disruption of alveolar development and angiogenesis. It is likely that this loss of elastic support in the lung periphery contributes to AHR, but so far the precise mechanisms of increased airway lability in children born very preterm are unclear.

Previous studies have suggested that preterm birth reduces the incidence of atopy later in childhood and adulthood.^{28,29} However, atopic predisposition was found to be associated with persistence

of wheezing.²⁹ The current study is the first to show that airway inflammation may modify AHR in children with VLBW. The association was significant only in atopic individuals, and although atopy was not an independent determinant for AHR, there was a significant interaction with FeNO. This means that atopic predisposition by itself is not important, but the consequence (ie, the degree of T_H2-type inflammation of which FeNO is a surrogate marker) is the modifier of AHR.³⁰

In conclusion, similar FeNO levels between children with VLBW or BPD and healthy controls were observed, and in VLBW children AHR was associated with FeNO only in atopic individuals. Similar to children with early-onset wheezing illness, bronchial nitric oxide flux rather than alveolar nitric oxide production is associated with AHR. It is likely that enhanced AHR in VLBW children is modified by atopic inflammation rather than by persistent inflammatory process due to prematurity. Whether AHR in atopic VLBW children is reversible by anti-inflammatory treatment remains to be studied.

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