

ORIGINAL ARTICLE

Relation of bronchial and alveolar nitric oxide to exercise-induced bronchoconstriction in atopic children and adolescents

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Keywords

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Abstract

Background and objective: Exercise challenge test is widely used in diagnostics and follow-up of childhood asthma, but the method is complex, time consuming, and expensive. In this study, we aimed to find out whether flow-independent nitric oxide (NO) parameters (bronchial NO flux [J'_{awNO}] and alveolar NO concentration [CA_{NO}]) predict exercise-induced bronchoconstriction (EIB) in atopic children and adolescents with asthma-like symptoms. Also, the respective NO parameters corrected for axial backward diffusion ($J'_{awNO}[TMAD]$ and $CA_{NO}[TMAD]$) were calculated and included in the analysis.

Methods: Thirty patients (6–19 yr old) with confirmed atopy (positive skin prick tests or allergen-specific IgE) and asthma-like respiratory symptoms were included in the study. Before the current investigations, none of the patients had been diagnosed to have asthma and none were on inhaled corticosteroids. Exhaled NO was measured at multiple exhalation flow rates, and exercise challenge test was carried out. Bronchial NO flux and alveolar NO concentration were calculated according to the linear method with and without correction for axial backward diffusion. Sixty-six healthy school children served as controls.

Results: The patients were divided into two groups according to EIB. Patients with EIB (EIB+ group, $n = 18$) had enhanced bronchial NO output as compared to patients without EIB (EIB– group, $n = 12$); but the EIB– group did not differ from healthy controls. EIB+ group had also higher alveolar NO concentration than EIB– group and healthy controls, but EIB– group did not differ from healthy controls. When bronchial NO flux and alveolar NO concentration were corrected for axial diffusion, $J'_{awNO}(TMAD)$ had equal difference as J'_{awNO} between the groups as expected. However, only EIB+ had higher $CA_{NO}(TMAD)$ than healthy controls, and the patient groups did not differ from each other. In patients, bronchial NO output correlated with the magnitude of exercise-induced change in PEF ($r_s = -0.388$, $p = 0.034$), FEV_1 ($r_s = -0.395$, $p = 0.031$), and $FEF_{50\%}$ ($r_s = -0.431$, $p = 0.020$), i.e., the higher the bronchial NO output, the larger the decrease in $PEF/FEV_1/FEF_{50\%}$. Alveolar NO concentrations correlated with the change in FEV_1 ($r_s = -0.439$, $p = 0.015$), $FEF_{50\%}$ ($r_s = -0.454$, $p = 0.013$), $FEF_{75\%}$ ($r_s = -0.447$, $p = 0.017$), and FVC ($r_s = -0.375$, $p = 0.045$). For $J'_{awNO}(TMAD)$, the correlations and p-values were equal to those of J'_{awNO} , but, interestingly, $CA_{NO}(TMAD)$ had no significant correlations with any of the exercise-induced changes in lung function.

Conclusion: The results showed that in atopic children and adolescents, increased bronchial NO output as well as $J'_{awNO}(TMAD)$ were associated with EIB, while alveolar NO concentration (but not $CA_{NO}[TMAD]$) correlated with the degree of obstruction in smaller airways induced by exercise challenge.

Asthma is the most common chronic disease in childhood, and it is diagnosed in 4–7% of children. Bronchial inflammation causes bronchial hyper-reactivity, which is one of the key features of the most frequent phenotype of childhood asthma. Exercise is a common cause of acute airway obstruction in asthmatic children with hyperreactive airways, and exercise-induced bronchoconstriction (EIB) is one of the specific signs of active asthma (1). Children with asthma suffer from EIB more often than adult asthma patients, and EIB has been shown to correlate with the degree of bronchial eosinophilic inflammation (2). The diagnosis of EIB is based on the demonstration of exercise-induced decrease of at least 12% in forced expiratory volume in 1 s (FEV_1) as compared to the value in resting conditions, according to the guidelines of the European Respiratory Society and the American Thoracic Society (1, 3, 4). EIB is usually investigated by a treadmill or free running test, but the test is complex, time consuming, and expensive.

Nitric oxide (NO) is an important endogenous signaling molecule in inflammation (5), and increased NO levels in exhaled breath are associated with allergic asthma and correlate with measures of eosinophilic inflammation in the airway mucosa (6, 7). A clear association between increased exhaled NO concentration and EIB has been reported in asthmatic adults (8, 9) and children (10–13) when measured with the conventional single exhalation flow rate method. In that method, exhaled NO is usually measured at the exhalation flow rate of 50 ml/s (14), and it mainly reflects NO production and inflammation in larger airways. Measurement of exhaled NO at multiple exhalation flow rates is an extension of the method and allows the assessment of alveolar/peripheral and bronchial/central NO output separately, and the method is more sensitive to peripheral inflammation than the single exhalation flow rate method (14–16). The multiple exhalation flow rate method is based on a mathematical model, which divides the lung into two compartments, namely the alveolar/peripheral and bronchial/central compartments, and allows the calculation of flow-independent NO parameters bronchial NO flux (J'_{awNO}) and alveolar NO concentration (CA_{NO}) (17). We and others have shown that the multiple exhalation flow rate method can be used to successfully differentiate between bronchial and peripheral inflammation in asthma, chronic obstructive pulmonary disease (COPD), asbestosis, and alveolitis (18–22). A more recent development of the multiple flow rate method is to include calculations of axial backward diffusion from bronchial compartment to alveoli to further characterize central and peripheral NO output in the lungs (23).

The aim of this study was to investigate whether central or peripheral NO output is associated with EIB in larger or smaller airways in atopic children and adolescents with asthma-like symptoms.

Methods

Patients

We recruited 30 children (10 girls and 20 boys) who were referred to a pediatric allergist because of asthma-like symp-

toms (Table 1). They all had positive reaction in skin prick tests (commercially available allergens of birch, cat, timothy, wheat, oat, barley, and rye; ALK, Allergologisk Laboratorium A/S, Hørsholm, Denmark) or had increased levels of allergen-specific IgE against common aeroallergens (ImmunoCAP-phadiatop-examination; Phadia AB, Uppsala, Sweden). Three children had been treated with inhaled corticosteroids seasonally, but none of the children had used corticosteroids during the last month before the investigation. The protocol was approved by the Ethics Committee of Tampere University Hospital. The children and their parents gave written informed consent before inclusion.

As a control group, we used previously collected data of 66 healthy children (32 boys and 34 girls) with normal spirometry and no history or symptoms of asthma, allergy, atopy, or other diseases (24).

Protocol

Exhaled NO concentrations were measured before exercise challenge test at multiple exhalation flows (see below). Spirometry values were measured at baseline and 4, 10, and 15 min after exercise and again after salbutamol inhalation (0.3 mg if <10 yr old and 0.4 mg if >10 yr old), which was given 20 min after exercise. The lung function values were compared with normal values in Finnish children (25).

Exhaled NO measurements

Nitric oxide concentrations in exhaled air were measured with Sievers NOA 280 analyzer (Sievers Instruments, Boulder, CO, USA) with a sensitivity of <1 ppb. The analyzer was calibrated daily with a known NO concentration (103.00 ppm; AGA, Lidingö, Sweden) and before every subject with filtered NO-free air (zero-air filter; Sievers Instruments, Boulder, CO, USA). Exhaled NO concentration was measured at four exhalation flow rates (50, 100, 200 and 300 ml/s). Exhalation flow rates were achieved by using a computer-driven servo system designed to maintain flow rate at a pre-determined level (NOFLA device developed at the University of Tampere, Tampere, Finland) (24). NO output (product of exhaled NO concentration and exhalation flow rate) was calculated, and NO output was plotted against exhalation flow rate at flow rates 100–300 ml/s, and a regres-

Table 1 Clinical data of the patients with (EIB+) or without (EIB–) exercise-induced bronchoconstriction and of healthy controls

	EIB+ (n = 18)	EIB– (n = 12)	Control (n = 66)
Boys/girls	12/6	8/4	32/34
Height (cm, mean $\pm$ s.e.m.)	142.8 $\pm$ 3.1	136.0 $\pm$ 3.3	139.3 $\pm$ 1.3
Age [yr, mean (range)]	10.7 (8–19)	9.6 (6–13)	9.7 (7–13)
Total IgE (U/l, mean $\pm$ s.e.m.)	1531 $\pm$ 496	1019 $\pm$ 319	n.d.

n.d. = not determined

sion line was set between these variables. All subjects had a correlation coefficient >0.95 in the regression analysis. Alveolar NO concentration and bronchial NO flux are the slope and intercept of the regression line, respectively (17). Alveolar NO concentration (CA_{NO}) reflects NO dynamics in the peripheral lung (from respiratory bronchioles to alveoli), and bronchial NO flux ($J'aw_{NO}$) represents NO dynamics in central conducting airways.

Axial backward diffusion of NO from bronchial compartment to alveoli may cause falsely high CA_{NO} and falsely low $J'aw_{NO}$ especially in subjects with increased $J'aw_{NO}$. CA_{NO} and $J'aw_{NO}$ adjusted for trumpet-shaped airways and axial diffusion ($CA_{NO}[TMAD]$ and $J'aw_{NO}[TMAD]$) were calculated according to the following equations as described by Condorelli et al. (23).

$$CA_{NO}(TMAD) [ppb] = CA_{NO} [ppb] - J'aw_{NO} [nl/s] / 0.86 [1/s]$$

$$J'aw_{NO}(TMAD) [nl/s] = J'aw_{NO} [nl/s] \times 1.7$$

The difference between CA_{NO} and $CA_{NO}(TMAD)$ is dependent on individual $J'aw_{NO}$ values, and there may therefore be differences in result profiles between CA_{NO} and $CA_{NO}(TMAD)$. On the contrary, $J'aw_{NO}(TMAD)$ is precisely 1.7 times higher than $J'aw_{NO}$ in every subject, and therefore, all the correlations to other markers or treatment changes in $J'aw_{NO}$ and $J'aw_{NO}(TMAD)$ are similar, but the absolute values are 1.7 times higher in $J'aw_{NO}(TMAD)$.

Exercise challenge test

Exercise challenge test was carried out as a free running test with wireless heart rate monitor. The heart rate goal was at least 80% of the maximal heart rate calculated according to the patient's age. Spirometry was measured using V_{max} Series Spirometry (Sensormedics, Yorba Linda, CA, USA). The maximal change (in %) in different parameters (Q in the example) was calculated 4, 10 and 15 min after exercise and after salbutamol inhalation, which was given 20 min after exercise:

Maximal change in Q = (lowest Q after exercise – Q before exercise) / Q before exercise $\times 100$.

Exercise-induced bronchoconstriction was considered positive if maximal decrease in FEV_1 was 12% or higher (1, 4).

Statistics

SPSS 16.1 (SPSS Inc., Chicago, IL, USA) program was used in the statistical analysis. Because of skew distributions of the data (Kolmogorov–Smirnov test), Mann–Whitney U -test, Kruskal–Wallis test, and Spearman's rank correlations were applied. Performance characteristics of $J'aw_{NO}$ and $FENO_{0.05}$ at different cut points to predict exercise-induced broncho-spasm were analyzed by constructing receiver operator characteristic (ROC) curves. p -values < 0.05 were considered significant.

Results

Thirty children and adolescents with asthma-like symptoms were examined. The patients were divided into EIB+ group (patients who developed EIB, $n = 18$) and EIB– group (patients who did not develop EIB, $n = 12$). The demographic data of patients and healthy controls ($n = 66$) are shown in Table 1. The patient groups did not differ from each other by height, age, or total IgE (Table 1). Also, the pre-exercise spirometry values were similar in EIB+ and EIB– groups: ($FEV_{1\%}$ pred. 97 ± 2 vs. 96 ± 3 , $p = 0.723$, FVC % pred. 99 ± 3 vs. 97 ± 3 , $p = 0.312$, PEF % pred. 109 ± 5 vs. 110 ± 5 , $p = 0.977$, and $FEF_{50\%}$ % pred. 90 ± 4 vs. 93 ± 4 , $p = 0.643$).

Patients with EIB had higher bronchial NO output ($J'aw_{NO}$) as compared to patients who did not develop EIB or to healthy controls, while the EIB– group did not differ from healthy controls (Fig. 1a). Also, alveolar NO concentrations (CA_{NO}) were higher in EIB+ than in EIB– group and, accordingly, only EIB+ group but not EIB– group had higher values than healthy controls (Fig. 1b). The NO concentrations at exhalation flow rates of 50, 100, 200, and 300 ml/s are given in the Table 2. The area under the ROC curve for $J'aw_{NO}$ and $FENO_{0.05}$ to predict EIB were 0.757 and 0.782, respectively, and were significantly different from 0.5 ($p = 0.019$ and $p = 0.01$). The performance characteristics of $J'aw_{NO}$ and $FENO_{0.05}$ to predict EIB at different cut points are given in Table 3.

When correction for axial backward diffusion was included in the calculations to obtain $J'aw_{NO}(TMAD)$ and $CA_{NO}(TMAD)$, $J'aw_{NO}(TMAD)$ had equal difference as $J'aw_{NO}$ between the patient groups (Fig. 1c), but $CA_{NO}(TMAD)$ did not differ between EIB+ and EIB– groups (Fig. 1d). EIB+ group had higher $J'aw_{NO}(TMAD)$ (Fig. 1c) and $CA_{NO}(TMAD)$ (Fig. 1d) than healthy controls, but EIB– group did not differ from healthy controls in either of these values (Fig. 1c,d).

In these atopic children, bronchial NO output ($J'aw_{NO}$) correlated with the exercise-induced changes in PEF ($r_s = -0.388$, $p = 0.034$), FEV_1 ($r_s = -0.395$, $p = 0.031$; Fig. 2a), and $FEF_{50\%}$ ($r_s = -0.431$, $p = 0.020$), i.e., the higher the bronchial NO output, the larger the decrease in PEF , FEV_1 or $FEF_{50\%}$. Alveolar NO concentrations (CA_{NO}) correlated with the changes in FEV_1 ($r_s = -0.439$, $p = 0.015$), $FEF_{50\%}$ ($r_s = -0.454$, $p = 0.013$), $FEF_{75\%}$ ($r_s = -0.447$, $p = 0.017$; Fig. 2b), and FVC ($r_s = -0.375$, $p = 0.045$). The correlations of $J'aw_{NO}(TMAD)$ to the exercise-induced decrease in spirometry values were equal to those of $J'aw_{NO}$ (Table 4, Fig. 2c), but $CA_{NO}(TMAD)$ had no statistically significant correlations with the measured exercise-induced changes in lung function. (Table 4, Fig. 2d). No correlations were found between NO parameters and pre-exercise spirometry values.

Discussion

In the present study, we assessed central and peripheral airway inflammation in atopic children with asthma-like

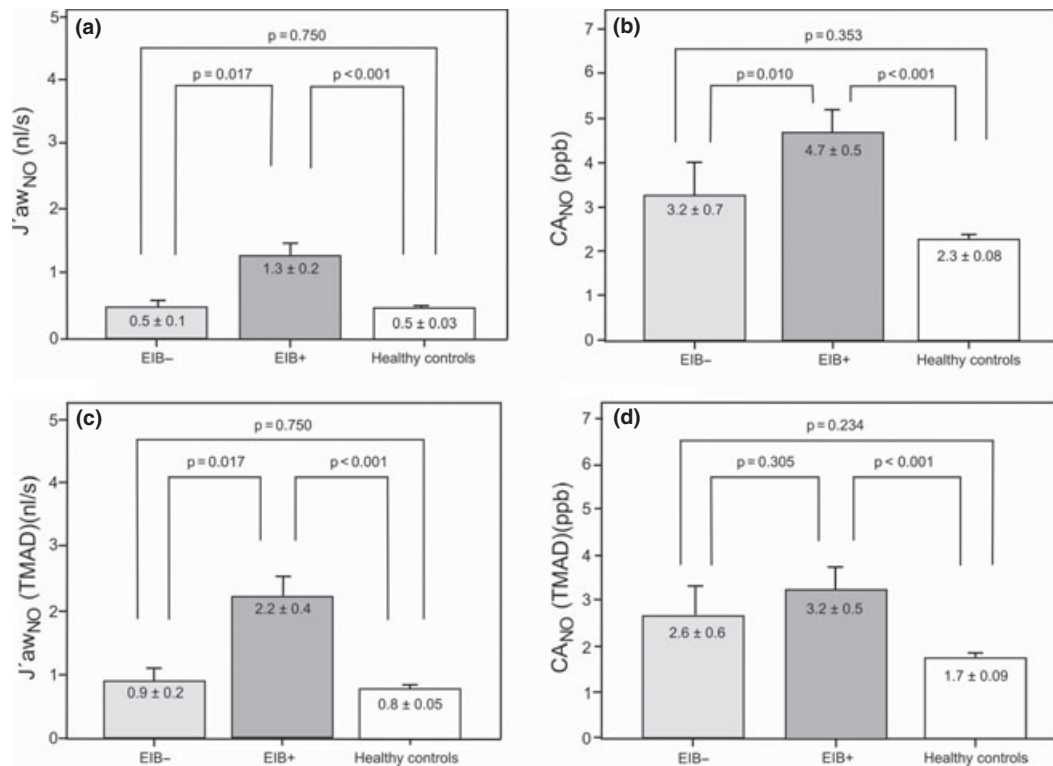


Figure 1 Bronchial nitric oxide (NO) flux ($J'aw_{NO}$) (a), alveolar NO concentration (CA_{NO}) (b), bronchial NO flux corrected for axial backward diffusion ($J'aw_{NO}$ (TMAD)) (c) and alveolar NO concentration corrected for axial backward diffusion (CA_{NO} (TMAD)) (d) in atopic

children/adolescents with (EIB+, $n = 18$) or without (EIB-, $n = 12$) exercise-induced bronchoconstriction and in healthy controls ($n = 66$) (mean $\pm$ s.e.m.).

Table 2 NO concentrations (ppb) at different flow rates in patients with (EIB+) or without (EIB-) exercise-induced bronchoconstriction and in healthy controls

Flow (ml/s)	EIB+ ($n = 18$)	EIB- ($n = 12$)	Controls ($n = 66$)	p-value EIB+ vs. EIB-	p-value EIB+ vs. controls	p-value EIB- vs. controls
50	31.3 $\pm$ 4.1	15.6 $\pm$ 3.6	11.7 $\pm$ 1.4	<0.01	<0.001	>0.05
100	17.7 $\pm$ 2.2	8.4 $\pm$ 1.9	6.7 $\pm$ 0.8	<0.01	<0.001	>0.05
200	11.0 $\pm$ 1.2	5.6 $\pm$ 1.1	4.4 $\pm$ 0.5	<0.01	<0.001	>0.05
300	9.0 $\pm$ 0.9	4.5 $\pm$ 0.7	3.8 $\pm$ 0.5	<0.01	<0.001	>0.05

NO, nitric oxide.

Mean $\pm$ s.e.m.

symptoms by non-invasive exhaled NO measurement with multiple exhalation flow rates. Linear method (17) was used to calculate bronchial NO flux and alveolar NO concentration, and a more recent modification of the method to consider axial backward diffusion (23) was also included in the analysis. Patients with EIB had higher bronchial NO output at baseline than patients who did not develop EIB, and both patients groups had higher values than healthy controls. Moreover, bronchial NO output correlated positively with the severity of EIB, i.e., the higher the bronchial NO output at baseline, the bigger the decrease in PEF or FEV₁. Also,

alveolar NO concentration was higher in EIB+ than in EIB- group, but the difference disappeared when the values were corrected for axial backward diffusion. These results suggest that increased bronchial NO output ($J'aw_{NO}$) is associated with the risk of EIB in atopic children and may be used as a predictive biomarker.

Exhaled NO has usually been measured at a single exhalation flow rate of 50 ml/s (14). The multiple exhalation flow rate method is a promising extension of exhaled NO measurement, as it allows separate assessment of NO output in large central airways (bronchial NO flux) and in the periph-

Table 3 Performance characteristics of $J'aw_{NO}$ (nl/s) (a) and $FENO_{0.05}$ (ppb) (b) at different cut points to predict exercise-induced bronchospasm (decrease in $FEV_1 \geq 12\%$)

	Sensitivity (%)	Specificity (%)	LR+	LR–	PPV (%)	NPV (%)
(a)						
0.4	72	42	1.2	0.7	65	50
0.8	72	83	4.3	0.3	87	67
1.0	56	92	6.7	0.5	91	58
1.5	44	92	5.3	0.6	89	52
2.0	28	100	*	0.7	100	48
(b)						
10	89	33	1.3	0.3	67	67
20	72	83	4.3	0.3	87	67
30	50	92	6.0	0.5	90	55
40	39	92	4.7	0.7	88	50
50	11	92	1.3	0.9	67	41

$J'aw_{NO}$, bronchial NO flux; $FENO_{0.05}$, exhaled NO concentration at exhalation flow rate of 50 ml/s; LR+, likelihood ratio positive; LR–, likelihood ratio negative; PPV, positive predictive value; NPV, negative predictive value; NO, nitric oxide.

*Cannot be calculated as specificity is 100%.

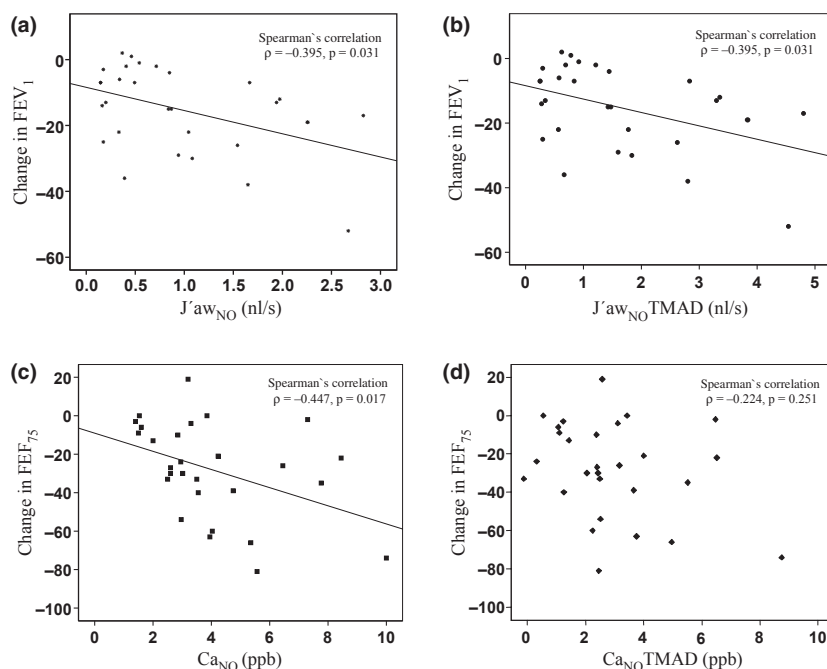


Figure 2 In exercise-challenge test, baseline bronchial nitric oxide (NO) flux ($J'aw_{NO}$) (a) as well as bronchial NO flux corrected for axial backward diffusion ($J'aw_{NO-TMAD}$) (b) correlated negatively with maximal change in FEV_1 , while baseline alveolar NO concentration (Ca_{NO}) correlated negatively with maximal change in $FEF_{75\%}$

in 30 atopic children and adolescents. (c). No statistically significant correlation was found between alveolar NO concentration corrected for axial backward diffusion ($Ca_{NO-TMAD}$) and $FEF_{75\%}$ in the same patient group (d)

eral small airways and pulmonary parenchyma (alveolar NO concentration) (17, 18, 26). This method has been shown to be suitable for measuring central and peripheral inflammation in airway and parenchymal diseases, respectively (18, 19, 21, 22), but just a few studies have applied the method in pediatric patients (24, 27, 28). A further extension of the

method to consider the axial backward diffusion of NO from the bronchial compartment to the alveoli was recently described (23).

Previous studies using a single exhalation flow rate method have shown that children with EIB have higher exhaled NO concentrations than patients who do not develop EIB (10–

Table 4 Correlations between $J'aw_{NO}$, $J'aw_{NO}(TMAD)$, CA_{NO} , and $CA_{NO}(TMAD)$ and the maximal exercise-induced change (%) in spirometry values

	$J'aw_{NO}$ and $J'aw_{NO}(TMAD)$	CA_{NO}	$CA_{NO}(TMAD)$
ΔPEF	$r_s = -0.388, p = 0.034$	$r_s = -0.293, p = 0.117$	$r_s = -0.074, p = 0.698$
ΔFEV_1	$r_s = -0.395, p = 0.031$	$r_s = -0.439, p = 0.015$	$r_s = -0.211, p = 0.262$
$\Delta FEF_{50\%}$	$r_s = -0.431, p = 0.020$	$r_s = -0.454, p = 0.013$	$r_s = -0.216, p = 0.260$
$\Delta FEF_{75\%}$	$r_s = -0.316, p = 0.101$	$r_s = -0.447, p = 0.017$	$r_s = -0.224, p = 0.251$
ΔFVC	$r_s = -0.282, p = 0.138$	$r_s = -0.375, p = 0.045$	$r_s = -0.279, p = 0.142$

NO, nitric oxide.

13). Recently, Shin et al. applied the multiple flow rate breath-hold method to measure exhaled NO in adult steroid-naïve asthmatics with EIB. They found that pre-exercise bronchial NO flux and airway diffusing capacity of NO were higher in asthmatics with EIB than in healthy subjects, whereas alveolar NO concentration was normal (29). Our present results showing higher bronchial NO output (independent of correction for axial backward diffusion) in atopic children with EIB as compared to healthy controls are in line with those by Shin et al. Interestingly, we also found a correlation between bronchial NO output measured before exercise challenge test, and the magnitude of the EIB measured as a change in PEF, FEV₁, or FEF_{50%}.

When alveolar NO concentration was calculated using the linear method without correction for axial diffusion, the patients with EIB had higher levels than those without EIB. A similar (but not statistically significant) trend was found in adult asthmatics with EIB by Shin et al. (29). In the present study, alveolar NO concentration also correlated with the decreases in FEF_{75%}, FEF_{50%}, and FEV₁ i.e., the higher the CA_{NO} , the bigger the decrease in FEF_{75%}, FEF_{50%}, and FEV₁. However, when alveolar NO concentration was corrected for axial backward diffusion, no significant difference between EIB+ and EIB- groups was detected, and no correlations between $CA_{NO}(TMAD)$ and the magnitude of bronchoconstriction in larger or smaller airways were found. This difference between the results of the two models is interesting and suggests that the TMAD-corrected values figure 'real' alveolar NO concentration excluding NO produced by small/peripheral airways. The finding also suggests that the method described by Condorelli et al. (23) is effective in correcting for the falsely increased alveolar NO concentration because of axial backward diffusion in patients with increased bronchial NO flux. The production of NO is likely to be increased

in the inflamed mucosa, and it is those areas in which the inflammatory process also enhances bronchial hyper-reactivity and predisposes to EIB. If NO production is significantly increased also in smaller airways (whose constriction is reflected as decreases in FEF_{50%} and FEF_{75%}), the axial backward diffusion of NO is likely to be bigger than in the case where NO production is increased primarily in the larger airways. The trumpet-shaped airway model accounting for axial diffusion has been successfully applied also in some other recent studies involving exhaled NO methodology (20, 30).

$J'aw_{NO}$ and FENO_{0.05} were able to predict EIB with a reasonable reliability. However, the relatively small number of subjects (n = 30) tested is a limitation of the study to fully estimate the sensitivity and specificity of exhaled NO measurement. In addition, other factors affecting exhaled NO levels (like atopic sensitization to house dust mite, age, height, and population) (31, 32) may interfere with the applicability and generalizability of the results and further studies are thus needed.

In conclusion, increased bronchial NO output ($J'aw_{NO}$) was found to be associated with the risk and magnitude of EIB in atopic children and may be used as a predictive biomarker.

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